Evidence-Based Evolutionary Medicine

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Contents

Preface

The authors of this book, Drs Torday, Blackstone, and Rehan, come from different academic backgrounds in developmental biology, medicine, and evolutionary biology, respectively. Together, we have perceived evolutionary medicine much as the parable of the blind men and the elephant. Our goal in writing this book is to provide a more unified view of evolution and medicine in lieu of the fragmented, siloed way in which this information is presently provided. While we may not have entirely succeeded in this goal, the need is clear. As basic science, the lack of an appreciation of a central theory of biology has negatively impacted medicine. As a result, we see more and more medical technology, and the concomitant erosion of the quality of health care – increasing infant mortality, maternal mortality, ventilator-induced mortality, over-medication, treatments that merely eliminate symptoms without addressing the ultimate cause of disease.

Much of this failure of medicine is due to the antiquated view that health is the absence of disease, and disease is the absence of health, which derives from the descriptive view of biology as a machine, the whole being equal to the sum of its parts. In contrast to that, the mechanistic evolutionary approach explicated in this book is that health and disease are a mechanistic continuum, offering the opportunity to intervene anywhere along that line of identity both diagnostically and therapeutically, even before the patient is symptomatic, as true preventive medicine, reducing morbidity and mortality. And it should be pointed out that this approach is in contradistinction to the molecular biologic

approach currently being implemented, eradicating the cellular communication principles that have facilitated vertebrate evolution, lumping the genetic elements together without consideration of their functional biologic context. That overly reductionist approach has culminated in a *reductio ad absurdum*. This situation must be rectified in order for medicine to become predictive, not just correlative and associative, other than in the case of infectious diseases, surgery, and trauma. Having completed the book, we hope that the reader will come to share this viewpoint.

1

A Brief History of Evolutionary Thinking

Summary

Evolution is as close to a general theory of biology as we have. Remarkably, the central tenets of the theory can be traced back to the nineteenth-century work of Charles Darwin. Darwin was influenced by his predecessors and by the social and political currents of his time. Darwinian evolution can be summarized as "heritable variation subject to natural selection." Darwin's avowed goal was to counter the theory of special creation. Nevertheless, his theory was not widely embraced. Where did variation come from? How was it inherited? Darwin had no answer to these questions. One class of answers to these questions was provided by the rediscovery of Mendel's work in the early twentieth century and the development of the science of genetics. The merging of Darwin's theory and Mendelian genetics into the Modern Synthesis led naturally to the search for the chemical basis of heredity and the founding of molecular biology. Evolution was reconceptualized as changes in allele frequencies in populations over time. Among other advances, the development of rigorous, sequence-based phylogenetic methods greatly enhanced our understanding of the history of life. Nevertheless, as the modern synthesis emerged in the early twentieth century, the darkest chapter in the history of evolutionary thinking unfolded. Eugenics – controlling breeding to improve the human race – took hold throughout the world. Yet Darwin himself was not a eugenicist. By arguing that controlling

Evidence-Based Evolutionary Medicine, First Edition. John S. Torday, Neil Blackstone, and Virender Kumar Rehan

breeding might be favored at the level of individuals but not at the level of tribes or societies, Darwin both refuted the intellectual basis for eugenics and anticipated the development of a multilevel theory of evolution.

Introduction

As Dobzhansky [1] famously pointed out, "Nothing in biology makes sense except in the light of evolution." Evolution, the closest to a general theory of biology that we have, thus provides common intellectual ground for all biologists. For instance, consider the growing field of genomics. When biologists seek to identify portions of genomes that are functionally important, they compare genomes of different species. Areas that are conserved between species likely reflect such functional importance [2, 3]. The thinking here is entirely evolutionary. Shortly after two species diverge from a common ancestor, their genomes are expected to be highly similar. As time passes, mutation acts to break down this similarity. In species that share a distant ancestor, parts of the genome may show little similarity. However, purifying selection will remove organisms whose genomes contain deleterious mutations in areas that are functionally important. To the extent that deleterious mutations commonly occur, these areas of the genome will thus appear conserved relative to areas that lack functional importance. Genomic studies routinely take advantage of these consequences of evolution.

A cynic, however, might suggest that evolutionary biologists traditionally focus more-or-less exclusively on organisms and genes (and now genomes). Evolutionary theory thus has had little impact on many fields of biology. As Wilkins [4] notes:

> The subject of evolution occupies a special, and paradoxical, place within biology as a whole. While the great majority of biologists would probably agree with Theodosius Dobzhansky's dictum that 'nothing in biology makes sense except in the light of evolution', most can conduct their work quite happily without particular reference to evolutionary ideas. 'Evolution' would appear to be the indispensible unifying idea and, at the same time, a highly superfluous one.

Wilkins [5] later elaborated on these remarks: "…many biologists who investigate proximal causes in various biological processes (in, for instance, biochemistry, physiology, development) often have little or no recourse to evolutionary ideas or explanations." Such statements from the founding editor of the notably interdisciplinary journal *BioEssays* suggest that while evolutionary theory may have the potential to unite all biological disciplines, it has not yet done so. At least some biology continues to be conducted with no particular reference to an evolutionary framework. The role that evolution could play in uniting biological disciplines has thus not yet been fully realized.

Yet this is changing. Minimally, since biology must embrace the history of life, virtually all biologists recognize the need for a historical framework. Further, the tools for providing this framework are increasingly well developed. Modern techniques of phylogenetic systematics analyze increasingly massive nucleotide sequence datasets with more and more sophisticated models of mutational change. As a result, we are progressively better able to apply evolutionary thinking to biological data of all sorts. The promise of over 150 years of evolutionary thinking is beginning to be realized.

Darwin

Remarkably, even in the age of genomics, evolutionary theory can be traced relatively intact back to the work of a nineteenth-century individual, Charles Darwin. The year 2009 was the 200th anniversary of Darwin's birth and the 150th anniversary of publication of one of his most important works, *On the Origin of Species by Means of Natural Selection*. Of course, Darwin built on earlier ideas. In particular, we will mention two.

One of the very powerful ideas that developed in the nineteenth-century Europe was the "uniformitarian" view of the Earth's geology. Developed by James Hutton and others, this view, summarized by "the present is the key to the past," eventually led to the geological time scale, which is central to our understanding of the history of life and is shown in Figure 1.1. Hutton was unfortunately so brilliant that no one could really understand a word he said, and his theory was popularized and

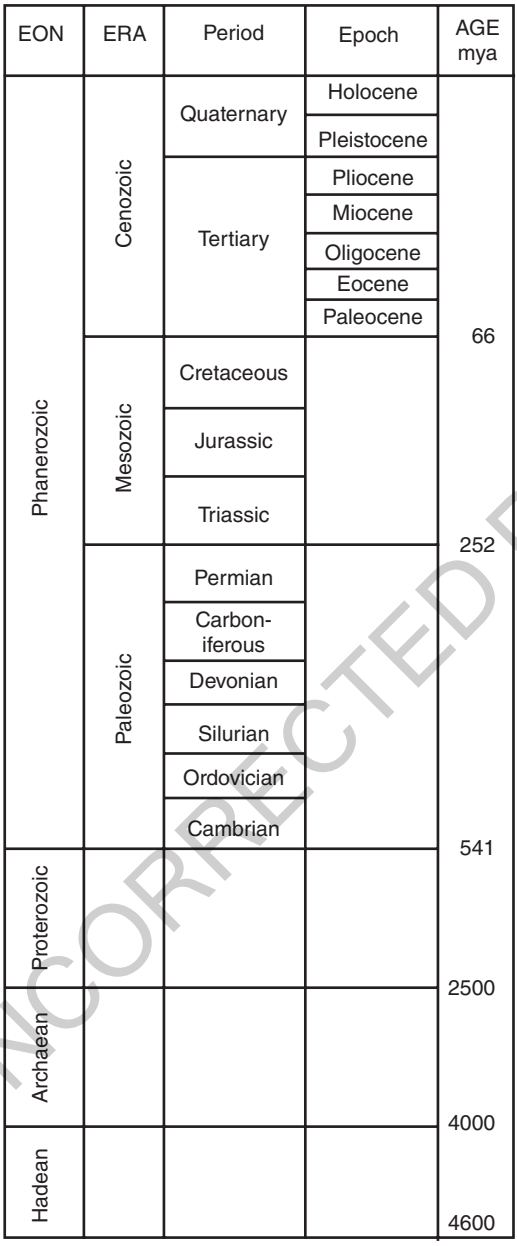

Figure 1.1 *The geological time scale*. Originally based on relative time derived from uniformitarian principles, radiometric dating of geological strata now allows both relative and chronological time measures. Newly ratified periods of the Proterozoic (e.g. the Cryogenian) are based not on stratigraphic events but on measures of chronological time.

made more accessible by Charles Lyell's *Principles of Geology*, which had a lasting influence on Darwin.

Other political and social developments in the nineteenth-century Europe include the Communist Manifesto, published in 1848. Communism closely identifies with the evolutionary theory of Lamarck, a predecessor of Darwin. Lamarck's theory of evolution – usually summarized as "the inheritance of acquired characteristics" – emphasizes that the organism must strive for the acquisition of novel characteristics. For instance, a giraffe with a short neck must struggle to lengthen its neck, stretching it every day, year in, year out. Only then will it acquire and pass on the longer neck. Thus, the parallel to the dialectic of communist ideology is clear.

Darwin's theory, on the other hand, was strongly rooted in capitalistic society. Darwin was from the English middle classes in the nineteenth century. This was Victorian England. Class structure was still very strong in England at this time, although the hereditary English nobility had lost a lot of its power to the English middle classes. This was a very gradual process; there were no revolutions. Numerous vestiges survived from earlier times – the House of Lords in Parliament and Queen Victoria, herself. At the same time, England was carving an empire out of the rest of the world. There was perhaps the need to justify this process in terms of the "natural order." The assumption that because something is natural it is also morally right was widely embraced.

At the same time that English society was changing gradually, and England was conquering much of the world, there was the prevailing view that change was progressive; the world was getting better. The gain of power of the middle classes led to economic advances, industry, science, medicine, and so on; the conquering of other countries was alleged to have a "civilizing" influence, although perhaps those conquered countries would have debated this.

These societal influences were no doubt important, particularly for young Charles Darwin when he set off on his voyage around the world on the HMS *Beagle*, 1831–1836. On this trip Darwin examined various aspects of geological and natural history – coral reefs, finches, tortoises, and so on. Darwin would ask himself: Why were areas of South America that were cli-

matically similar to England nevertheless populated by distinct flora and fauna? And why when he unearthed South American fossils were they more similar to the modern South American creatures, while English fossils were likewise similar to modern English creatures? These were daunting questions to an inquiring mind.

Darwin's Theory

After his return to England, Darwin thought about these and other questions for a number of years and eventually came up with the theory of evolution by natural selection. After resisting publication for some time, a paper by Alfred Russell Wallace forced his hand. Darwin published a short paper in 1858 with Wallace and then in 1859 published *On the Origin of Species*. His goal in the *Origin* was to convince readers that there was no need for "special creation" of each species by God, hence the title "*On the Origin of Species*." It is also noteworthy that he did allow a role for the Creator in the origin of life. While this was implicit in the first edition of the *Origin* [6], it became considerably more explicit by the 6th edition [7]:

> There is grandeur in this view of life, with its several powers, having been originally breathed by the creator into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved.

Darwin most clearly and succinctly describes his theory in the opening paragraphs of perhaps his greatest work, the *Descent of Man* [8], first published in 1871:

> He who wishes to decide whether man is the modified descendent of some pre-existing form, would probably first enquire whether man varies, however slightly, in bodily structure and in mental faculties; and if so, whether variations are transmitted to his offspring in accordance with the laws which prevail with the lower animals…The enquirer would next come to the important point,

> whether man tends to increase at so rapid a rate, as to lead to occasional severe struggles for existence; and consequently to beneficial variants, whether in body or mind, being preserved, and injurious ones eliminated.

Darwin's theory thus consists of three principles:

1) organisms vary
2) this variation is inherited
3) this variation is subject to natural selection

The cause of the variation is completely unspecified; the existence of variation need only be demonstrated empirically. The mechanism of inheritance is also unspecified and could be entirely unknown as long as parent–offspring correlations can be demonstrated empirically. Darwin's theory is thus compatible with genetic or epigenetic mechanisms of inheritance, or even cultural inheritance. The actions of natural selection are often thought of as differential mortality; however, differential reproduction with no mortality is equally effective.

The core of Darwin's theory of evolution is thus: "Heritable variation is subject to natural selection." Yet Darwin's theory was unconvincing to many. Where did variation come from? How is it transmitted? Darwin had no good answers to these questions, and in later editions of the *Origin*, he came up with increasingly fanciful ideas in this regard.

The Modern Synthesis

Meanwhile, in 1865, Gregor Mendel, a German-speaking, Augustinian friar, first presented his experiments that addressed exactly these questions, but the significance of his work was not immediately apparent. Mendel's data were "discovered" in about 1900 and quickly led to the science of genetics. In 1910, Thomas Hunt Morgan began his studies of fruit flies. In the 1930s and 1940s, led by Theodosius Dobzhansky, a student of Morgan's, and a number of other prominent scientists, the modern synthesis of Darwin's theory and Mendel's genetics was conceived. This led naturally to the search for the chemical nature of heredity, the discovery (in 1953) of the structure of DNA, and the founding of the field of molecular biology.

Molecular biology has roots perhaps best described by Francis Crick [9], one of the discoverers of the structure of DNA:

> I myself was forced to call myself a molecular biologist because when inquiring clergymen asked me what I did, I got tired of explaining that I was a mixture of crystallographer, biophysicist, biochemist, and geneticist, an explanation that in any case they found too hard to grasp.

The real focus of molecular biology, however, is on biological information, and great advances have clearly been made in this area. Related to the abundance of biological information now available, there have been correspondingly great advances in phylogenetic methods. Darwin himself recognized that his theory implied that all organisms were connected within a phylogenetic tree. Indeed, the only illustration in the *Origin* was one such tree. Such a tree suggests the history of life as implied by the fossil record (Figure 1.2). At the bottom are the oldest

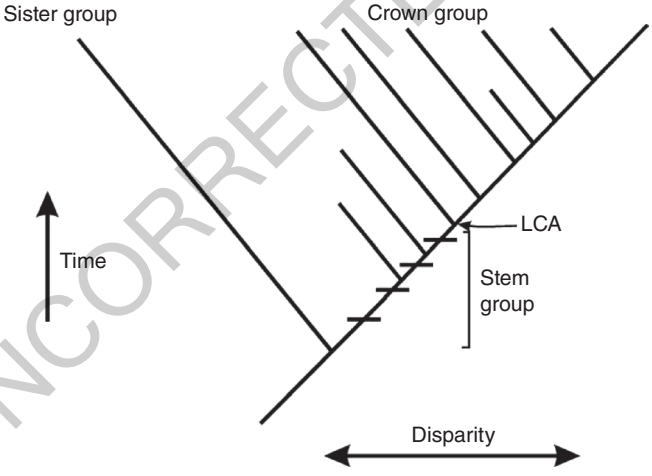

Figure 1.2 *A phylogenetic tree*. Analogous to the fossil record, time proceeds from bottom to top, while diversity and disparity are measured on the *x*-axis. A stem group diverges from its sister group while deriving novel character states (horizontal bars). The stem group taxa are entirely extinct. The last common ancestor (LCA) shares all the derived character states with the crown group, which includes all living and some extinct members of the group or clade.

strata containing the oldest taxa. These taxa diversify along the *x*-axis, while time proceeds on the *y*-axis. Today, such trees are typically built with nucleotide sequence data using statistical and mathematical theory to model mutation rates.

Perhaps the least durable aspects of Darwin's theory relate to gradual, progressive change. Evolution can proceed at various rates, and for some lineages there may be long periods of "stasis," in which little or no change occurs. While complexity does tend to increase during the history of life, secondary simplification also occurs constantly: some eukaryotes have lost their mitochondria, bivalves have lost their heads, snakes have lost their limbs, some birds have lost their wings, and so on.

Evolutionary theory continues to be central to biology in the age of genomics. Finding functional areas of genomes is entirely based on evolutionary thinking. As mentioned above, genomic areas that are conserved in different taxa are typically found to be functionally important. When a mutation occurs in these functional areas of the genome, the mutation is usually detrimental, and the individual containing the mutation is removed by selection or fails to reproduce as rapidly as those without the mutation. Evolutionary thinking has also shown that most large genomes, far from being a "blueprint" of the organism, are actually the evolutionary playground of little bits of DNA called mobile genetic elements, which due to their incessant replication now make up the bulk of the human genome and the genomes of other eukaryotes as well [10]. While Darwin proposed his theory over 100 years before mobile genetic elements were discovered, this theory – heritable variation subject to selection – nevertheless perfectly explains the evolutionary success of mobile genetic elements. Modern evolutionary biology thus encompasses studies ranging from molecular biology to organisms, to human culture and psychology, and everything in between.

The Darkest Chapter

In the glow of the successes in the study and analysis of biological information, it is easy to forget that early twentieth century geneticists and evolutionary biologists embraced the science of

eugenics in the darkest chapter in the history of evolutionary biology. By the 1920s, this movement was at its peak in the United States. At this time, 24 states had passed laws permitting eugenic sterilization [11]. Further, this movement was led by prominent scientists of the day. For instance, in 1921 Henry Fairfield Osborn was both the president of the American Museum of Natural History and the host of the second International Congress of Eugenics, and his signature was prominent on advertisements for the Congress.

In response to the pernicious effects of eugenics, some states passed laws limiting the teaching of evolution. One such state was Tennessee, and this law led to the Scopes trial. Osborn and other prominent scientists may have refused to testify on behalf of the defense at the Scopes trial in part perhaps because of Clarence Darrow's opposition to eugenics. William Jennings Bryan led the opposition to teaching the theory of evolution. Despite this, Bryan was not personally a fundamentalist. Rather, he opposed the political and social aspects of the evolutionary agenda and viewed creationism as a tool to suppress these unsavory offshoots of evolutionary theory.

Yet it was not the opposition of creationists that halted the eugenics movement. Rather, it was the horrible excesses of World War II that made it clear to all that eugenics was no longer politically tenable. Modern evolutionary theory has largely failed to acknowledge this dark chapter. Instead, criticisms of eugenics focus on the problems of classifying the unfit and the difficulty of selecting against deleterious recessive alleles. Because recessive alleles are masked in heterozygotes, selection has little impact when the allele frequency is low. Eugenics seems to be viewed as too difficult to properly implement, rather than as scientifically and morally flawed. Indeed, a number of well-meaning evolutionary biologists continue to sound the alarm concerning mutation unchecked by selection. For instance, in this context Herron and Freeman [12] write: "The implications for the future are ominous, and the obvious solutions unappealing."

Yet Darwin was not a eugenicist. In the *Descent of Man*, Darwin [8] elaborated a subtle and powerful argument against the nascent political movement that later would be called eugenics. In the first step of this argument, he extends his theory of evolution

to multiple levels of selection by pointing out that groups of individuals could be selected under some circumstances:

> It must not be forgotten that although a high standard of morality gives but a slight or no advantage to each individual man and his children over the other men of the same tribe, yet that an increase in the number of well-endowed men and an advancement in the standard of morality will certainly give an immense advantage to one tribe over another. A tribe including many members who, from possessing in a high degree the spirit of patriotism, fidelity, obedience, courage, and sympathy, were always ready to aid one another, and to sacrifice themselves for the common good, would be victorious over most other tribes; and this would be natural selection. At all times throughout the world tribes have supplanted other tribes; and as morality is one important element in their success, the standard of morality and the number of well-endowed men will thus everywhere tend to rise and increase.

In this passage, Darwin focuses on a trait – morality – that is assumed to be inherited at least in part and that "…gives but a slight or no advantage…" at the level of the human individual. In other words, at this level of the biological hierarchy, morality is selectively neutral. When individual-level selection alone operates, moral individuals will on average have no more offspring than immoral ones. Thus, the frequency of moral individuals will neither increase nor decrease. Darwin then points out that at a higher biological level – the tribe – the results of selection are quite different: "A tribe including many members who, from possessing in a high degree the spirit of patriotism, fidelity, obedience, courage, and sympathy… would be victorious over most other tribes…." In other words, when between-tribe conflict occurs, tribes that contain many moral individuals will prevail over tribes with fewer such individuals. Tribes that in aggregate have a high moral standard will increase in frequency relative to tribes that in aggregate have a low moral standard. The effects of tribe-level selection thus differ from the effects of individual-level selection. The latter will not affect the frequency of individuals that vary in moral standard, while the former very clearly

does affect the frequency of tribes that in aggregate vary in moral standard. If between-tribe selection was a potent force in human evolution, the existence of human morality can be explained by this sort of natural selection.

It was this levels-of-selection thinking that caused Darwin to differ profoundly from some of his contemporaries. He continues: "We civilized men… do our utmost to check the process of elimination; we build asylums for the imbecile, the maimed, and the sick; we institute poor-laws; and our medical men exert their utmost skill to save the life of every one to the last moment." He then points out that human sympathy is the basis for both morality and for our caring for the helpless. He concludes: "Nor could we check our sympathy, even at the urging of hard reason, without deterioration in the noblest part of our nature" [8].

In modern terms, Darwin is recognizing a conflict between levels of selection. At the individual level of selection, letting the unfit perish or actively preventing such individuals from reproducing (which is what eugenicists advocate) may well be adaptive. On the other hand, the tribe or group that institutes such policies loses "the noblest part" of its nature – recall "the spirit of patriotism, fidelity, obedience, courage, and sympathy…." All of that is lost. Such a society will fail in competition with other societies that maintain this part of human nature, even against "the urging of hard reason." Thus, eugenics is selected for at the level of the individual, but selected against at the level of the group [13]. Our moral revulsion of eugenics evolved.

While Darwin's wisdom regarding eugenics has been largely forgotten, his view of the evolution of groups in general [14] and of morality in particular is now widely accepted, as we see here [15]:

> Natural selection underpins the evolution of good and evil in human beings. This claim may sound far-fetched, but increasingly archaeological and anthropological evidence and the work of a small coterie of theoreticians indicate that Paleolithic people clustered together using common languages and culture to develop norms that protected equality, liberty, and fraternity, and thus forged cooperative groups that behaved altruistically. Such bonds allowed the group to present a united front to

> less-fortunate neighbors, thereby providing backup for dispatching rivals in combat with little risk to self and probably with considerable benefit in terms of resources grabbed.

As an aside, one can of course see the contradiction of human cooperation: cooperation within a group often facilitates more efficient conquest of other groups.

In any event, there is also no doubt group-level thinking is common in human society, as personified by the motto of the three musketeers: "All for one, and one for all," the immortal words of Winston Churchill describing the Battle of Britain, "Never… was so much owed by so many to so few," and the lyrics to one of the most famous rock-and-roll songs of all time, "When all are one, and one is all" (Led Zeppelin, Stairway to Heaven). And yes, often a common enemy serves as a unifier for a group, as for instance in the unlikely case described by Alexander Solzhenitsyn [16]: "Whatever they'd been talking or thinking about was forgotten. The whole column had one thing and one thing only on its mind. 'Get ahead of ten! Beat them to it!' Things were all mixed up. No more sweet or sour. No more guard or zek. Guards and zeks were friends. The other column was the enemy."

In some cases, we may see even today in modern society the kind of group-level selection that Darwin envisioned. During the Fukushima Daiichi nuclear accident, successful shut down of the doomed power plants depended on the willingness of a number of workers to sacrifice themselves for the "good of the group" as described in some news reports, for instance this one from 5 days after the earthquake titled "50 workers bravely stay at troubled Japan reactors": "Adding to this natural bonding, jobs in Japan confer identity, command loyalty and inspire a particularly fervent kind of dedication. Economic straits have chipped away at the hallowed idea of lifetime employment for many Japanese, but the workplace remains a potent source of community… Japanese are raised to believe that individuals sacrifice for the good of the group" [17]. And without these workers' sacrifice, the release of radioactivity would likely have been much greater and caused greater peril to Japanese society.

Conclusions

In modern biology, some great scientists spend their entire careers without citing articles older than a few years [18]. Yet it is undeniable that the central principles of evolutionary biology can be traced back to Darwin's work over 150 years ago. The modern synthesis of Darwin's theory and Mendelian genetics provided a mechanistic basis for understanding variation and heredity but also ushered in the era of eugenics. Darwin's view of eugenics, however, illustrates the value of a multilevel theory and counters the thinking that led to evolution's darkest chapter.

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2

Outlining the Major Transitions in the History of Life

Summary

In the late twentieth century, a consensus emerged that the history of life shows a repeating pattern. Lower-level biological units repeatedly banded together to form higher-level units. In the process, much of the complexity of life emerged. First, molecules banded together as life emerged, then groups of molecules formed simple cells, simple cells formed complex cells, and complex eukaryotic cells formed multicellular organisms. More minor transitions – and minor only by comparison – include genes banding together to form chromosomes and groups of organisms forming societies. These transitions all share certain evolutionary features. The formation of nascent groups leads to conflicts among the lower-level units. Individuals can cooperate, and risk being exploited by free riders, or individuals can defect and further their selfish replication. In order for a transition to occur, mechanisms have to evolve that mediate these conflicts. Subsequent to the evolution of mechanisms of conflict mediation, higher-level units can emerge. Driven by selection for size increase, much of the complexity of life thus formed. Conceptualizing the history of life in this fashion has allowed evolutionary biology to recognize that group selection has been a potent force in the history of life.

Evidence-Based Evolutionary Medicine, First Edition. John S. Torday, Neil Blackstone, and Virender Kumar Rehan

Introduction

When we think of the vast sweep of geological time and the massive changes that life on Earth and Earth itself have undergone, it seems impossible that anything about the history of life could exhibit a simple, repeating pattern. Yet as remarkable as this may seem, it may also be the case. Indeed, the history of life consists of a series of major transitions in which lower-level biological units cooperatively banded together to form higher-level biological units [1]. First groups of molecules, then molecules within simple cells, then simple cells within complex cells, complex cells within multicellular organisms, and even in some cases, multicellular organisms within societies (Figure 2.1). In the process of these transitions, life became increasingly complex.

While there is simplicity in the repeating pattern, these major transitions themselves were not necessarily simple. In fact, they were perhaps the greatest achievements of organic evolution. In each case, the major obstacle impeding the transition was

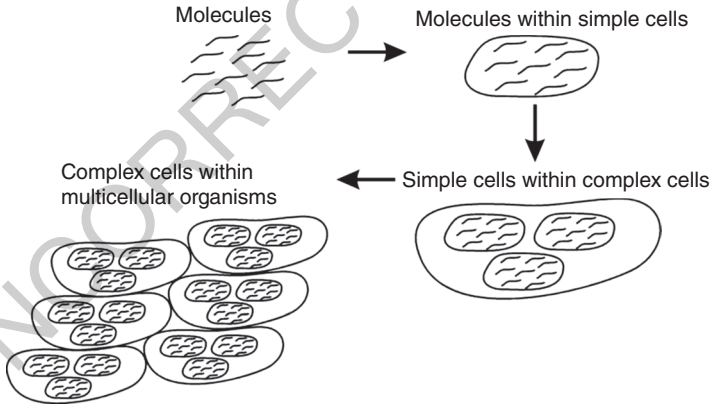

Figure 2.1 Major transitions in the history of life. The origin of life entailed groups of replicating molecules. These groups of molecules formed simple cells. Simple cells banded together in complex cells that became eukaryotes. Eukaryotes repeatedly formed multicellular organisms. The advantages of size increase favored each transition, while conflicts among lower-level units hindered them.

evolutionary conflict. As lower-level units band together, conflicts arise – some units free ride, using group resources without contributing their fair share. These conflicts must be mediated if a higher-level unit is to emerge.

Mechanisms of conflict mediation involve a huge variety of biological features. While these mechanisms were no doubt difficult to evolve, there remains a conceptual simplicity in the nature of conflict mediation. Rick Michod and Aurora Nedelcu [2] point out that mechanisms of conflict mediation in biology typically decrease the variation of the lower-level units (thus decreasing the likelihood that a selfish lower-level unit will evolve) or increase the variation among the higher-level units (thus increasing the likelihood that a cooperative group will be favored by natural selection). Much of the history of life is the story of the derivation of these mechanisms of conflict mediation; in some sense, much of life is like a bad marriage (or maybe a good marriage) – lots of fighting among the lower-level units as to who should do the dishes and take out the trash, until mechanisms to mediate these conflicts evolve, and the higher-level unit emerges.

While evolutionary conflicts impede transitions, other selective forces nevertheless favor banding together. In particular, the resulting groups were larger and tended to be the largest organisms of their time. As described by John Bonner [3], biology has its own version of room at the top: "…the reason for nonstop selection for organisms of increased size is that the top of the size scale is an ever-present open niche, and has been open during the entire course of organic evolution."

Because they were bigger, these higher-level units could successfully outcompete any lower-level units that had not banded together. As compared to the smaller lower-level units, higher-level units could exploit more food resources, they could disperse more efficiently and avoid the constraints of low Reynolds numbers, they could have more offspring, and they could better fend off predators. However, as competition intensified between the higher-level units, some of these would again cooperatively band together to form a new, still larger, higher-level unit. And so the same process was repeated over and over again in the history of life.

The Major Transitions

Well, enough generalities: what are the major transitions? Certainly, there are at least four of them – the origin of life, the origin of simple cells, the origin of complex cells that became eukaryotes, and the origin of multicellular organisms. There are several additional transitions that are perhaps minor by comparison but still very important in the emergence of complex life.

The first major transition, the origin of life, is in many ways a singular event, when "nonliving" became "living." In part this transition hinges on the definition of life, and we lack such a definition. About the best we can do is a good description. Descriptions of life focus on the canonical features of energy conversion and replication (Figure 2.2). All living things need to take up energy from the environment and convert it into a useable form; some of this energy is then used to replicate life's informational content.

There is limited inference about the origin of life that can be drawn from modern life. Comparative methods can be used to understand the features of the Last Universal Common Ancestor (LUCA) of life. However, it is not clear the extent to which we can generalize from modern life to beyond LUCA into the very earliest stages of the history of life. This so-called stem group of life is entirely extinct, and life may have originated in a form very

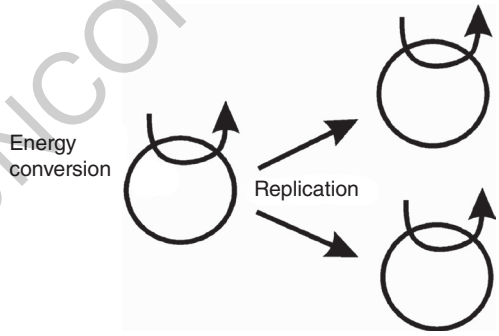

Figure 2.2 A description of life. All living things need to take up energy from the environment and convert it into a useable form; some of this energy is then used to replicate life's informational content.

different even from LUCA. Thus, it is very difficult to infer what happened from the first living things to LUCA.

While there are no fossils of early life, some biochemical clues exist. In particular, life tends to take up more of the most abundant stable isotope of carbon, carbon 12, relative to the slightly larger and much less abundant carbon 13. As compared to nonliving carbon, remnants of living carbon are thus enriched for carbon 12 relative to carbon 13. This carbon "fractionation" provides evidence that life existed on Earth more than 3500 million years ago [4, 5].

Our ability to understand the origin of life is limited by the dramatically different conditions that existed on Earth at that time. Here, we will not delve too deeply into this complex problem but only point out that since life is thermodynamic disequilibrium, life likely arose in an environment of thermodynamic disequilibrium, such as the deep-sea rift vent environments. We may thus think of the origin of life not as an extremely unlikely series of events but as chemical inevitability, "the free lunch you are paid to eat" [6].

In such an environment, the first life consisted of networks of molecules capable of replicating themselves. But the free lunch did not last, and eventually these molecules had to carry out energy conversion all by themselves. Copying errors led to variation then as now. Selection likely favored faster replication and more efficient energy conversion. But some molecules made a discovery that was to resonate throughout the entire history of life. If a molecule relied on its sister molecules to carry out all or some of the tasks of energy conversion, the "selfish" molecule could then replicate at a higher rate and increase its frequency in the population. If the lunch is no longer free, then you can steal someone else's lunch. Indeed, such a molecule could specialize on just one aspect of living things – replication or reproduction – at the expense of the other – energy conversion.

Given the obvious advantages of letting someone else make lunch, how does cooperation evolve? Figure 2.3 examines the selective dynamics of early living molecules with ideas derived from a model of multilevel selection [7, 8]. In a population with two types of molecules – cooperators that convert substantial amounts of energy and defectors that take up the products of energy conversion from the environment – all molecules

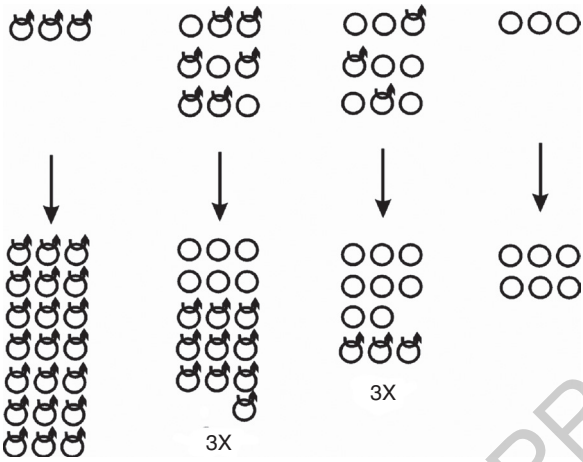

Figure 2.3 A trait-group model of group selection. Two types of molecules exist in a population. Cooperators (circles with arrows) convert energy and release energy-rich products into the environment. Defectors (circles) rely on these products for their own replication. The latter are favored by within-group competition, while the former are favored by between-group competition.

reproduce by copying. Before reproduction, they form groups of three molecules at random. These fleeting groups can be thought of as forming in microhabitats suitable for energy conversion and reproduction. The groups share the products of energy conversion, and the availability of these products determines the extent that reproduction can proceed. A group of all defectors (on the right of the figure) thus has little available energy, so each molecule can only make two copies of itself. A group with one cooperator (shown here next from the right) has more energy, so the cooperator makes three copies of itself, while the defectors make four copies. A group with two cooperators (second from the left) has still more energy, so the cooperators make five copies of themselves, while the selfish molecules make six copies. Finally, in a group with all cooperators (on the left), each cooperator makes seven copies of itself.

Thus, within-group competition favors the selfish molecules, but between-group competition favors the cooperators. Under these specified conditions, between-group competition is stronger, and in the population as a whole the cooperators increase in frequency relative to the defectors.

What if a group of cooperators could surround themselves with a barrier to keep out selfish molecules? This highlights one of the advantages of the next major transition, the Origin of Cells. Generally, cells allow concentrating substances useful to life, while excluding substances that are harmful. Further, because lipid molecules have a hydrophilic end and a hydrophobic one, in water they spontaneously form the lipid bilayer that is characteristic of cell membranes. Likely, the origin of life was followed closely by the origin of simple cells.

Yet the origin of cells did not entirely solve the problem of defector molecules that favor their own replication. Within a cell, mutation leads to variation, and again selection favors molecules that invest more in their own replication and less in energy conversion. Such molecules will inevitably come to predominate in the cellular environment, possibly endangering the entire cell as energy reserves are consumed in favor of short-term replication.

To illustrate these conflicts and how they may be mediated, the "stochastic corrector" model is useful [9]. In protocells, a replicator (circles with arrows) gives rise by mutation to a second replicator (circles without arrows), which replicates more efficiently and faster (Figure 2.4). Recall that selection on the lower-level unit will always favor faster replication; if there were no cells or higher-level units of any kind, type 1 replicators would be eliminated by selection simply because type 2's make more of themselves.

However, the replicators are also catalysts that carry out energy conversion. As we saw previously with the multilevel selection model, cooperation can be favored if the replicators differ in rates of energy conversion. As with the multilevel model, type 2 replicators could be less efficient at converting energy than type 1. In this case, the evolutionary dynamics would parallel the earlier model, with within-cell competition favoring type 2's and between-cell competition favoring type 1's. No doubt such dynamics occurred repeatedly in the early history of cells. But let us take a somewhat different approach here and show how multilevel selection can build complexity: Consider that these replicators might catalyze several steps in energy conversion, and type 1 replicators may be more efficient in some of the steps, while type 2 replicators are more efficient in other steps.

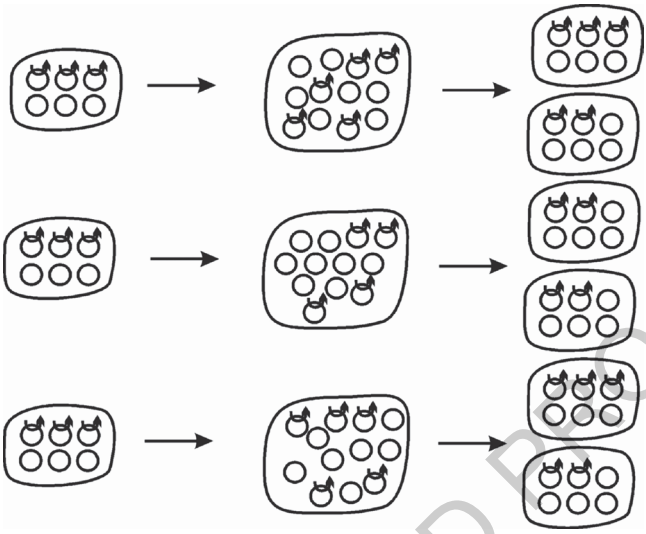

Figure 2.4 The stochastic corrector model. A replicator (circles with arrows) gives rise by mutation to a second replicator (circles without arrows), which replicates more efficiently and faster. Protocells, however, divide when the number of replicators is small, allowing cells with equal numbers of replicators to form purely by chance. If replicators complement each other catalytically, selection will favor the cells with equal numbers.

Now consider the effects of this complementation of energy conversion on the evolutionary dynamics. Type 2 replicators are inexorably favored at the level of the molecule. Cells begin with an equal number of replicators, shown in the left column of the figure. As cells grow, type 2's come to predominate, as shown in the center column of the figure. However, if the cells divide soon enough, there will still be a moderate number of type 1 replicators. And purely by chance, a few of the offspring cells (shown in the right column of the figure) will have an equal number of replicators. Other cells will tend to have more type 2's than type 1's. Now consider the effects of selection on the daughter cells. Since the replicators complement each other in energy conversion reactions, the cells with equal numbers of type 1's and 2's will convert energy faster and thus have a larger steady-state supply of energy than those cells with more type 2's than type 1's. More energy will allow the "optimal compartment" cells to

grow and replicate faster, outcompeting the cells that are biased toward type 2 replicators. In this way, the type 2's are held in check and enzymatic complexity is favored.

Note the interaction of several forces of evolution. First, mutation produces variation at the molecular level in catalytic and replicatory function. Second, chance events, usually termed genetic drift, produce variation in the initial conditions at the cell level. Third, because of functional differences, cells with both replicators are favored by selection at the higher level because their energy conversion as a group is higher. Thus, conflict between the replicators is mediated by the functional complementation and by sampling error or drift. For comparison, consider what would happen if cells did not divide until there were 10 000 replicators inside each cell.

So here conflict is mediated not by constraining the variation of the lower-level units but by increasing the variation among the higher-level units and letting selection act. As long as cell division occurs at a low threshold of lower-level units, the variation among higher-level units will be enhanced, and selection on the higher-level units will lead to cooperative groups being favored. Selection of groups of molecules at the level of the cell can overcome selection at the level of the individual molecule, and both replicators can persist. Selection on the higher level unit results in cooperation, diversity, and complexity.

This sort of selection can continue to build complexity. However, several other transitions had to occur before prokaryotic cells emerged. Independent replicators had to be combined into chromosomes. The "chromosome rule" – if one replicator was copied, all were copied – decreased the competition among replicators. Chromosomes are a nice example of how a higher-level unit – the chromosome in this case – mediates conflict by constraining the variation of a lower-level unit – the replicator or gene in this case. At the same time, the origin of chromosomes would increase the variation between simple cells – some cells would have them and some cells would not. Chromosomes also solve the problem of getting the right number of replicators into each daughter cell. In evolving further from simple cells to prokaryotes, the "RNA world" gave way to the modern world of RNA, DNA, and proteins. The resulting prokaryotes have been the most successful life forms to exist on earth. LUCA was a

prokaryote perhaps 3500 million years ago. All metabolic evolution, including oxygenic photosynthesis which completely altered the biosphere, was accomplished by prokaryotes.

Nevertheless, as pointed out by Nick Lane [10], there may be a fundamental constraint in the design of prokaryotes. In prokaryotic cells, the external membrane system is used in energy conversion to form an electrochemical gradient, which powers the cell. The gradient is formed by the actions of the electron transport chains that extrude protons. Tying this gradient of protons to the external membrane system creates a fundamental surface-to-volume constraint. Increase in size results in relatively less surface area of the cell relative to its volume. As we see in Figure 2.5, in a roughly spherical cell, surface area increases as the square of the radius, while volume increases as the cube. Size increase therefore results in relatively less "proton power" to supply energy while at the same time increasing the cellular "sink" that requires this energy. Energy conversion using the external membrane thus constrains the evolution of prokaryotes – they have to remain small and simple.

One way to circumvent this constraint can be seen in the evolution of complex, eukaryotic cells. Cells with external energy-converting membranes were brought inside a larger complex cell. Thus, with its external membrane system freed from energy conversion, the complex cell could greatly increase in size and become the "elephant" of its time. Indeed, increasing evidence suggests that the key event in the evolution of the eukaryotes was the symbiosis between an archaeon and

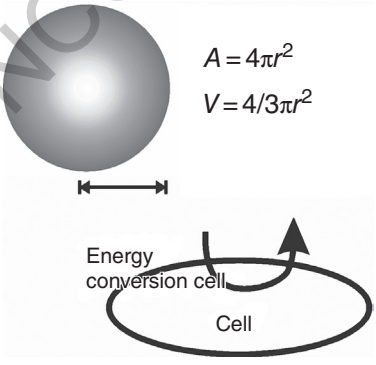

Figure 2.5 Surface-to-volume constraints. In a spherical cell, surface area (*A*) increases as the square of the radius, while volume (*V*) increases as the cube. Prokaryotes with external, energy-converting membranes cannot easily increase in size.

bacterium. The eukaryotic cell is thus a chimera of two types of prokaryotes. Based on well-calibrated molecular phylogenies, the symbiosis occurred perhaps 2000 million years ago.

It remains difficult to understand the process of eukaryogenesis, the evolution of the many eukaryotic features that separate eukaryotes from prokaryotes. Comparative methods suggest that all the features of eukaryotes had evolved by the time of the last eukaryotic common ancestor. All stem eukaryotes are extinct. Given that it took nearly 2000 million years for prokaryotes to evolve eukaryotes, we can infer that this was challenging transition.

Why might this be a challenging transition? What looks like a clever engineering solution to surface-to-volume constraints turns into a levels-of-selection nightmare. What is to prevent a symbiotic bacterium, one of the lower-level units in this transition, from using the products of energy conversion for its own selfish replication? Such a symbiont, shown in Figure 2.6 in red, will replicate faster than the other symbionts. The parallels to selfish replicator molecules are all too obvious.

Eukaryotic sex, which involves whole-cell fusion, could then allow such selfish symbionts to spread from cell to cell. Just as with selfish replicators, mechanisms of conflict mediation had to evolve to hold these selfish lower-level units in check.

In eukaryotic cells, much of the signaling system particularly as it relates to metabolism likely evolved at least in part to

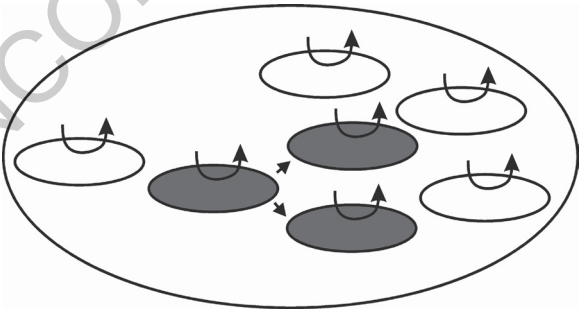

Figure 2.6 The perils of cells within cells. A cell (red) uses the products of energy conversion for its own selfish replication. (*See insert for color representation of the figure.*)

mediate these conflicts. Once they emerged, eukaryotes flourished and were able to outcompete or consume prokaryotes in many circumstances because of their larger size. Meanwhile, competition between eukaryotes favored larger and larger sizes.

And once again, surface-to-volume constraints became relevant. As eukaryotic cells became larger and larger, surface-dependent processes became more and more limiting, again because surface area increases as the square, while volume increases as the cube of the radius. Thus, a chain or a sheet of smaller cells was favored over a single, large cell.

Unlike the other major transitions, this one seems to be easy to accomplish. Eukaryotes have repeatedly evolved multicellularity. In modern groups, perhaps 10–15 different groups independently evolved multicellularity, including the amoebozoan slime molds; land plants and other green algae such as *Volvox*; numerous other algae and protists; and in the opisthokonts, choanoflagellates, fungi, and of course animals. Nevertheless, while multicellularity has evolved a number of times in many distinct eukaryotic groups, conflicts still had to be mediated. We can better understand these conflicts by focusing on perhaps the most successful multicellular eukaryotes, the animals.

Because of the pressures to increase size on one hand, and get material to the cells on the other, it has been said by JBS Haldane [11] that the history of animal life is "largely the story of the struggle to increase surface in proportion to volume." To have a three-dimensional shape, animals require a circulatory system to ventilate the interior. The first circulatory systems, such as those in the sponges, used ciliated cells to move fluids through the interior and collect food from the fluid. The ciliated cells are terminally differentiated and are incapable of cell division or at least they replicate more slowly due to the ciliation constraint (that is, since the microtubule-organizing center cannot make a cilium and the mitotic spindle apparatus at the same time, ciliated cells have to lose their cilia to divide).

In sponges, the interior amoeboid cells carry out cell division and growth, while the ciliated cells provide locomotion and feeding for the entire group. Thus, it is the amoeboid cells that reproduce this simple multicellular organism. Ciliated cells are doing all the work, but they are potentially getting excluded from contributing to the next generation. This is the principal

risk of multicellularity. Ciliated cells and amoeboid cells must remain closely related.

Consider an amoeboid cell that, when it divides, does not produce any ciliated cells, only new amoeboid cells. Since only amoeboid cells divide, the descendants of such a cell will come to predominate in the somatic environment relative to normal amoeboid cells that when they divide may produce, say, half amoeboid cells and half ciliated cells. The arithmetic should be clear: the variant amoeboid type will outcompete normal cells in the somatic environment (in passing, note the parallels to the energetically selfish mitochondrion or the efficient self-replicator in the stochastic corrector model). On the other hand, the benefits to the selfish cell are frequency dependent (as with the previous examples of selfish molecules or organelles): a sponge composed of only such "selfish" variant cells will quickly degenerate to the unicellular state, with the associated costs (again, compare to a complex cell with only selfish mitochondria or a simple cell with only selfish replicators). As in the previous levels of selection transitions, selection at the higher level favors cooperation, while selection at the lower level favors conflicts.

In animals and other multicellular eukaryotes, mechanisms to hold these selfish cells in check include among other features a unicellular stage to the life cycle and programmed cell death. Both of these constrain the variation of the lower-level units. A unicellular stage insures that all cells are genetically identical, at least initially. Programmed cell death removes those cells that have mutated to become genetically different and potentially dangerous.

Finally, in some cases, multicellular animals have formed a still higher-level unit, the society, as a way to achieve still larger sizes. The most successful examples of such societies are the social insects and perhaps also human beings. Many of the same issues of conflict and conflict mediation arise in this transition as well.

Conclusions

The history of life is a history of major transitions. These transitions – the origin of life, the origin of cells, the origin of complex eukaryotic cells, the repeated origins of multicellularity, and

even the origins of societies – led to increased size and complexity. Yet in each transition, conflicts had to be mediated. These mechanisms of conflict mediation are many and various and constitute much of the richness of biology and perhaps even of human society.

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3

One Central Mystery: Why Did Eukaryotes Only Evolve Once?

Summary

With the exception of metabolic complexity, most of the complexity of life on Earth – in structure, function, genes, genomes, life cycles, and life history – is eukaryotic. Yet we remain only dimly aware of the process that gave rise to eukaryotes. For over 100 years, endosymbiosis has been considered central to the origin of eukaryotes. Currently, this endosymbiosis is conceptualized as an archaeon that took up bacterial symbionts 1500–2000 million years ago. Nevertheless, no consensus exists concerning the connection between endosymbiosis and complexity. Was the host cell already complex before the symbiosis? Alternatively, did the endosymbiosis itself trigger much of eukaryotic complexity? Comparative methods can provide some insight. Features of Last Eukaryotic Common Ancestor (LECA) can be reconstructed from modern taxa. Similarly, characteristics of the First Eukaryotic Common Ancestor (FECA) can be inferred from putative archaeal sister taxa of the host cell and putative proteobacterial sister taxa of the symbiont. Extinction nevertheless complicates this approach, since the actual sister taxa of the original partners in the symbiosis are likely long extinct. The process of eukaryogenesis, however, occurred in the eukaryotic stem group, i.e. between FECA and LECA. All representatives of the eukaryotic stem are extinct. Examining modern taxa thus remains largely uninformative in this regard. New conceptual and methodological approaches for understanding the origin of

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eukaryotic complexity continue to be developed. Levels-of-selection theory provides an overarching evolutionary framework. Symbiosis produced conflicts among lower-level units. Conflict mediation allowed the emergence of the higher-level unit. Diverse aspects of eukaryotic complexity can thus be explained as consequences of conflict mediation.

Introduction

Let us very briefly review the history of life, discussed in detail in Chapter 2. This is the history of several major evolutionary transitions – the origin of life, the origin of simple cells, the origin of complex cells that became eukaryotes, and the origin of multicellularity. Much of the complexity of life was built up in this way: first groups of molecules, then molecules within simple cells, then simple cells within complex cells, and finally complex, eukaryotic cells within multicellular organisms. Note that all complex multicellular organisms are derived from eukaryotic not prokaryotic cells.

Each of the major transitions involved lower-level units banding together to form a group. In each case, there was evolutionary conflict among these lower-level units – should they cooperate or should they defect and behave selfishly? For the higher-level unit to emerge, these conflicts had to be mediated. Rick Michod [1] points out that mechanisms of conflict mediation in biology typically decrease the variation of the lower-level units (thus decreasing the likelihood that a selfish lower-level unit will evolve) or increase the variation among the higher-level units (thus increasing the likelihood that a cooperative group will be favored by natural selection).

While evolutionary conflicts impede transitions, other selective forces nevertheless favor banding together. In particular, the resulting groups were larger and tended to be the largest organisms of their time. Because they were bigger, these higher-level units could successfully outcompete any lower-level units that had not banded together. As compared to the smaller lower-level units, higher-level units have a number of ecological advantages such as more food resources, easier dispersal, more offspring, and fewer predators.

So if the evolutionary issues were similar – evolutionary conflict and its mediation on one hand impeding the transition, while selection for size increases on the other hand favoring it – it would seem that each transition would occur at roughly the same pace. Let us very briefly review each transition and see if this expectation is met.

The origin of life remains a mystery in many ways. We do know that remnants of living things tend to be biased toward the lighter, more abundant form of carbon. Such carbon fractionation may suggest evidence for life in some of the oldest sedimentary rocks on Earth – certainly by 3500 million years ago. Geologically speaking, this is nearly immediately after the Earth first became inhabitable. Simple cells likely evolved immediately after the origin of life. Prokaryotes likely evolved rapidly as well. The Last Universal Common Ancestor (abbreviated as LUCA) of all modern life was likely a prokaryote.

Then for about 2000 million years considerable biochemical evolution occurred, most notably the evolution of oxygenic photosynthesis. This process releases molecular oxygen as a by-product of the splitting of water. The ever increasing amounts of molecular oxygen in the atmosphere and the oceans completely changed the nature of the biosphere. Curiously, at least in terms of their structure, prokaryotes remained virtually unchanged during this time and indeed up to the present day. Prokaryotes then and now are small and simple and exhibit little structural complexity. For 2000 million years, there was virtually no increase in the structural complexity of life on Earth!

Perhaps one could argue that the pace of evolution just slowed down as life matured. If we skip ahead for a minute, past the origin of eukaryotes, we see that once eukaryotes evolved, many different forms evolved multicellularity. A phylogeny of the eukaryotes shows that several groups, with the notable exception of the excavates, repeatedly evolved multicellularity (Figure 3.1). Note that the placement of the root of the eukaryotes remains an area of active investigation [2]. Even the simplest such multicellular organisms are considerably more complex than any prokaryotes. And of course, the most elaborate eukaryotes, such as the human species, are indeed fascinating in their complexity.

No doubt, each of the major transitions is an amazing evolutionary accomplishment. Nevertheless, three of the major

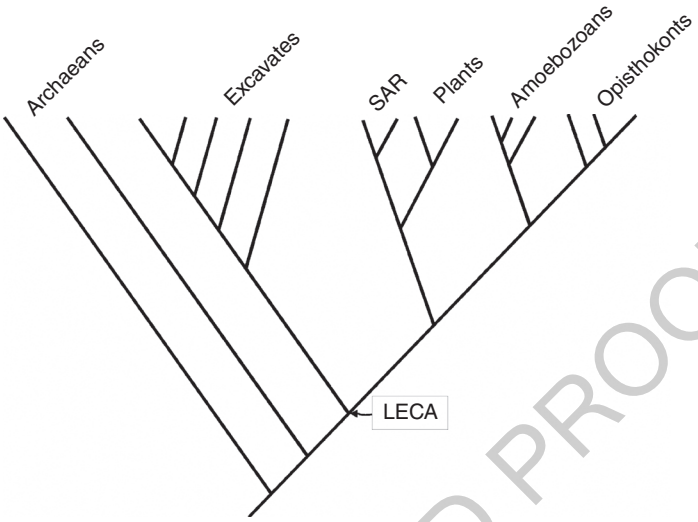

Figure 3.1 A phylogeny of eukaryotes (excavates + SAR + plants + amoebozans + opisthokonts). Multicellular eukaryotes evolved repeatedly in SAR (= stramenopiles, alveolates, and rhizaria), plants, amoebozoans, and opithokonts. Adapted from Butterfield [3]. Reproduced with permission of John Wiley & Sons.

transitions were apparently relatively straightforward or at least proceeded relatively rapidly in geological time as far as we can tell.

On the other hand, one of the major transitions, the origin of eukaryotes, was particularly difficult. Why did life languish as simple cells for 2000 million years? And why, once eukaryotes evolved, did they go on to routinely form complex multicellular organisms and even societies of such organisms? This delayed evolution of eukaryotic complexity is the biggest mystery in the history of life and is central to understanding the origin of eukaryotes. Let us begin to explore this mystery with a review of earlier ideas about the evolution of eukaryotes.

For over a century, discussions of eukaryotic origins have focused on endosymbiosis – in which one cell is taken up by another. Indeed, if you examine the ultrastructure of a eukaryote, it does appear that the organelles are like cells of bacteria. This is particularly the case with the mitochondrion, and most

of the discussion has focused on the endosymbiotic origins of mitochondria. Beyond that, there has been no consensus about what the other partner was, and more-or-less complete denial about the nature of the evolutionary processes that would have occurred during endosymbiosis.

For example, nearly a century ago, Wallin [4] viewed what he called "symbionticism" as an exception to Darwinian evolution. As he stated, "Modern writers have recognized the insufficiency of Darwin's hypothesis to explain the origin of species. The 'unknown factor' in organic evolution has been especially emphasized by Osborne, Bateson, Kellog, and other recent writers. This 'unknown factor' is especially concerned with the origin of species." Of course in Wallin's mind, the unknown factor was what he termed symbionticism.

This view was echoed by Lynn Margulis in her extensive writing about the serial endosymbiosis theory later in the twentieth century, as for instance in the following passage from Margulis and Sagan [5]: "Next, the view of evolution as chronic bloody competition among individuals and species, a popular distortion of Darwin's notion of 'survival of the fittest,' dissolves before a new view of continual cooperation, strong interaction, and mutual dependence among life forms. Life did not take over the globe by combat, but by networking. Life forms multiplied and complexified by co-opting or exapting others, not by killing them."

On the other hand, George Williams [6] pointed out: "The subsequent stability of these eukaryotic cell lineages through geologic time, despite potential disruption from selection among cellular components, presents an evolutionary problem that deserves detailed attention." In other words, selection on the components of the eukaryotic cell could very easily disrupt cooperation. Endosymbiosis is not some alternative to Darwinian evolution, rather it is a clear and necessary part of it that fits well with Darwin's theory as he conceptualized it.

All of the major transitions may involve conflict, but mechanisms must evolve to mediate conflict in order for the higher-level unit to emerge. Generally, levels-of-selection transitions involve stages of nascent cooperation, conflict, conflict mediation, followed by emergence of the higher-level unit (Figure 3.2).

We will return to issues of conflict. But first let us address the related question: if the origin of eukaryotes involved endosymbiosis

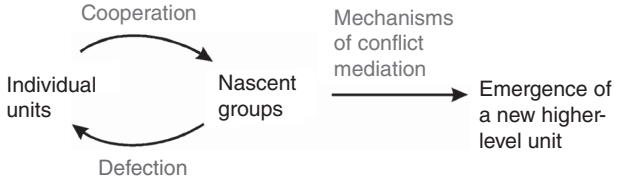

Figure 3.2 Conflict and conflict mediation in the emergence of biological units [7]. Adapted from Butterfield [3]. Reproduced with permission of John Wiley & Sons. (*See insert for color representation of the figure.*)

and none of the other transitions did, maybe eukaryogenesis was difficult because endosymbiosis itself is challenging?

Indeed, many have argued that prokaryotes are unsuitable for undertaking endosymbiosis, for instance, they have a cell wall so they cannot take up the symbiont cell. Thus, by this view the cell that took up the symbiont must have been a eukaryote. Putatively, amitochondriate eukaryotes were once classified as "archezoans," descendents of the first eukaryotes that supposedly never had mitochondria. By this view, one of these archezoans took up the bacteria that became mitochondria around 700 million years ago [8].

This view became less tenable as evidence began to accumulate that the so-called archezoans had genes in their nuclear chromosomes that were undoubtedly of mitochondrial origin [9]. In light of modern evidence, typically archezoans have mitochondria, just in the vestigial form of mitosomes or sometimes hydrogenosomes, although some may have secondarily lost these [10]. A number of prokaryotes have also been found to lack cells walls, and some bacteria even have been found within other bacterial cells as endosymbionts [11]. So not only is the archezoan hypothesis largely discredited, but there also seem to be cases of prokaryotes taking up other prokaryotes as endosymbionts. Thus, it is difficult to see that this is such a major stumbling block that it would have taken thousands of millions of years to accomplish.

Maybe the obstacle to eukaryotic evolution had nothing to do with endosymbiosis. Maybe for 2000 million years the environment simply did not permit large, complex eukaryotic cells? Then when the environment changed, say 1500 million years ago, complex cells suddenly could evolve. One could, for instance,

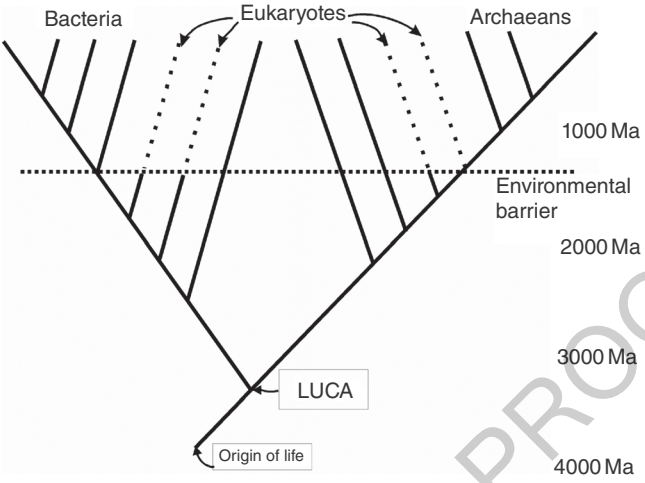

Figure 3.3 Eukaryogenesis following environmental release. The lifting of an environmental barrier allowing the formation of large, complex cells would have led to numerous lineages becoming eukaryotes.

hypothesize that levels of oxygen in the atmosphere acted as this sort of environmental barrier. Notice, however, that once the barrier lifted and suddenly it became easy to become a eukaryote, one would expect that any number of different types of prokaryotes would suddenly be capable of evolving into a eukaryote. As shown in Figure 3.3, the expectation would be for eukaryotes to be polyphyletic, i.e. composed of numerous strains of prokaryotes that independently became eukaryotes. This is decidedly not the case. All evidence suggests that eukaryotes form a monophyletic group and that in nearly four thousand million years of prokaryotic life on this planet, eukaryotes evolved only once.

So in reviewing earlier ideas, we have entertained several hypotheses concerning the observed difficulties in eukaryotic origins – maybe endosymbiosis itself was the obstacle or maybe the environment simply was not suitable for eukaryotes. We have examined evidence relevant to these hypotheses and neither one seems to be consistent with the data. So we discard these hypotheses and move on to others. Parenthetically, this is of course what science is all about – testing and sometimes falsifying hypotheses.

So what else could it be? Recall that one aspect of each major transition is size increase. Maybe this was partly responsible for the delay in the evolution of eukaryotes.

To understand the role of size increase, first consider how prokaryotes convert energy using the process of chemiosmosis. They do this using their external membrane system to form an electrochemical gradient. The gradient is formed by the actions of the electron transport chains that extrude protons. Subsequently, the protons move back inside the membrane through ATP synthase, catalyzing the formation of ATP from ADP and inorganic phosphate. The chemical energy in ATP then serves as the battery of the cell.

Indeed as pointed out by Nick Lane [12], there may be a fundamental constraint in the design of prokaryotes. Tying this gradient of protons to the external membrane system creates a surface-to-volume constraint. Increase in size results in relatively less surface area of the cell relative to its volume. In a roughly spherical cell, surface area increases as the square of the radius, while volume increases as the cube. Size increase therefore results in relatively less "proton power" to supply energy while at the same time increasing the cellular "sink" that requires this energy. Energy conversion using the external membrane thus constrains the evolution of prokaryotes – they have to remain small and simple. There are, by the way, some few exceptions to this rule, but on close examination these exceptions indeed prove the rule.

Now we can see why endosymbiosis is integral to the origin of eukaryotes. One way to circumvent the surface-to-volume constraint is to move cells with external energy-converting membranes inside a larger cell (Figure 3.4). For the larger collective cell, this frees the external membrane system from energy conversion. The complex cell can now greatly increase in size and become the "elephant" of its time.

Indeed, increasing evidence suggests that the key event in the evolution of the eukaryotes was the symbiosis between archaeans and bacteria, based on metabolic complementation [13]. The eukaryotic cell is thus a chimera of two types of prokaryotes. Based on well-calibrated molecular phylogenies, the symbiosis occurred roughly 1.5–2 thousand million years ago [14]. Eukaryotic genes are a mixture of bacterial and archaeal genes [15].

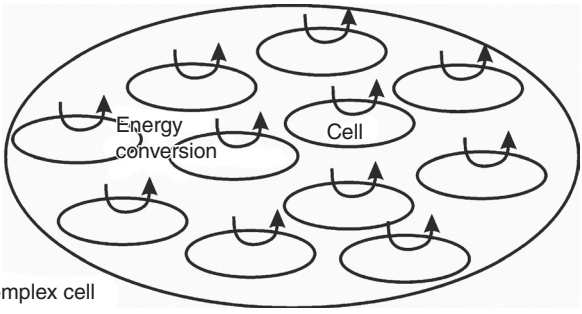

Figure 3.4 Circumventing surface-to-volume constraints in the origin of eukaryotes. By moving small, energy-converting cells inside a larger cell, surface-to-volume constraints could be alleviated, and the complex cell could grow to larger sizes.

Nevertheless, it remains difficult to understand the process of eukaryogenesis, the evolution of the many eukaryotic features that separate eukaryotes from prokaryotes. Comparative methods suggest that all the features of eukaryotes had evolved by the time of LECA, the last eukaryotic common ancestor. All stem eukaryotes are extinct.

How can we make progress in understanding the evolution of eukaryotic features, particularly the timing of eukaryogenesis relative to the mitochondrial symbiosis?

Let us return to the idea of conflict. One form of conflict was likely conflict at the genomic level. Bill Martin and colleagues [16–18] have hypothesized that the proto-mitochondrial genome contained self-splicing introns. When proto-mitochondria died, they released their DNA into the cytoplasm of the larger cell. This DNA became incorporated into the host DNA, and the introns began to multiply. Suddenly, the host genes had introns. These introns were transcribed along with the exons. Self-splicing introns were rather slow, and a lot of them ended up being translated into peptides. These intronic sequences then led to malformed proteins. The nucleus, the defining feature of eukaryotes, evolved as a physical barrier between transcription and translation. Only after all of the introns had spliced themselves out was the messenger RNA exported and subsequently translated. Once DNA was contained in a nucleus, bacterial mechanisms of cell division will no longer work and

new mechanisms (that is, mitosis, meiosis, and the cell cycle) must follow.

A broader view of conflict must take into account bioenergetics. What is to prevent a symbiotic bacterium, one of the lower-level units in this transition, from using the products of energy conversion for its own selfish replication? Eukaryotic sex, which involves whole-cell fusion, could then allow such selfish symbionts to spread from cell to cell (Figure 3.5).

How can these conflicts be mediated? Much of what sets eukaryotes apart from prokaryotes is a much more elaborate system of intracellular communication and signaling. In this context, consider the proto-mitochondrion that finds itself inside a larger cell. For a previously free-living cell, this is a challenging environment in which to maintain homeostasis. The symbiont has no direct interaction with the external environment. Rather, it must interact with the external environment through the internal environment of the larger cell, the proto-eukaryote. Further, it is unrealistic to expect the symbiont to quickly invent new tools for the interaction. It must develop interactions with the tools already at hand, co-opting the existing tool-kit into new functions.

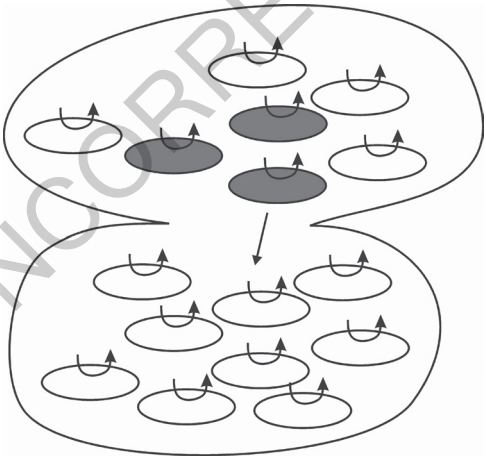

Figure 3.5 Eukaryotic sex allows symbiont migration. Two eukaryotic cells fuse during sex and energetically selfish symbionts migrate from one cell to the other. (*See insert for color representation of the figure.*)

Chemiosmotic mechanisms can provide part of the basis for this interaction. Conceptually, metabolism can be viewed as a series of redox couples linking external electron sources and sinks. Imbalances are thus quickly manifest. Suppose the host cell is a poor environment for the proto-mitochondria. Growth, division, and other activities falter. ATP is no longer broken down to ADP and inorganic phosphate. If substrate is available, the proto-mitochondrion will build the proton gradient to a maximum. The electron carriers will be loaded with electrons and some of these electrons will be picked up by molecular oxygen, forming reactive oxygen species, which are partially reduced forms of oxygen. The term "oxygen free radicals" or "radical oxygen species" is sometimes used in a similar sense, although not all reactive oxygen species are free radicals and vice versa.

In other words, during typical metabolism substrate oxidation and metabolic demand are in balance. When metabolic demand falters, the proton gradient becomes maximal, leading to highly reduced electron carriers and high levels of reactive oxygen formation. The problem is that there is not too little ATP, but too much, resulting in membrane hyper-polarization and reactive oxygen species. Under these conditions, the proto-mitochondria can easily damage themselves.

Consider calcium signaling in this context [19]. Calcium ions are a ubiquitous second messenger in eukaryotes and are also widely used in prokaryotes, including the bacterial relatives of proto-mitochondria. One way for a proto-mitochondrion to create metabolic demand is to depolarize its membrane. This can be done by allowing the influx of positively charged ions such as calcium. Subsequently, electron flow can rebuild the membrane potential and reactive oxygen formation can be alleviated. Further, pumping the calcium ions back out requires the breakdown of ATP, and this creates additional metabolic demand.

However, the interior of the proto-eukaryotic cell, like all cells, likely had very low amounts of calcium ions. High levels of calcium cause precipitation of phosphate, among other difficulties. Three options thus seem likely (Figure 3.6). A healthy proto-mitochondrion distant from the external membrane would incinerate in its own reactive oxygen species, as suggested by (b) in Figure 3.6. Or a proto-mitochondrion could accumulate

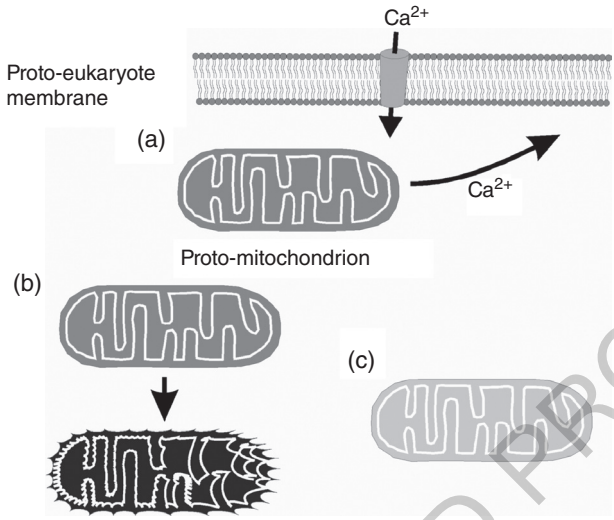

Figure 3.6 Co-option of calcium signaling by proto-mitochondria. To maintain metabolic homeostasis, a proto-mitochondrion near the external membrane (a) takes up calcium ions and pumps them back out. This depolarizes its membrane. A proto-mitochondrion distant from the membrane (b) cannot do this. Membrane hyper-polarization leads to excessive production of reactive oxygen species. Another proto-mitochondrion (c) survives by loose-coupling between substrate oxidation and proton extrusion. Adapted from Blackstone [19]. Reproduced with permission of Elsevier. (*See insert for color representation of the figure.*)

mutations that result in highly inefficient metabolism so that it could persist in the poor internal environment, as in (c). Alternatively, a proto-mitochondrion could station itself near the calcium channels in the proto-eukaryotic membrane, as in (a). An influx of calcium ions from outside the cell would in turn allow uptake by the proto-mitochondrion and a return to metabolic homeostasis as described above.

Continuing with this scenario, healthy proto-mitochondria that are distant from the external membrane would be unlikely to survive, while those near the membrane would survive and would emit an energized calcium pulse, as shown in (a) in Figure 3.6. Such a signal can trigger movement and chemotaxis and cause the proto-eukaryote to relocate to a more suitable environment. Under more favorable conditions, the healthy,

membrane-associated proto-mitochondria would outcompete the slow-metabolizing ones.

This scenario allows us to see not only how eukaryotic signaling pathways evolved, but also how they are based on mechanisms of host–symbiont communication that led to metabolic homeostasis.

Returning to the framework of conflict mediation, we can also see how signaling pathways indeed mediate evolutionary conflict. In the above scenario, within proto-eukaryotes that exhibit calcium signaling, the variation among the lower-level units has decreased. Healthy proto-mitochondria that were not associated with the membrane have perished, while the slow-metabolizing ones have been outcompeted. The population has become relatively uniform with healthy, membrane-associated proto-mitochondria predominating.

At the same time, variation among the higher-level units has increased. There is variation among proto-eukaryotes, with some exhibiting calcium signaling and some that do not. Since the former exhibit metabolic homeostasis, they will be favored by selection relative to the latter.

In this way, eukaryotic signaling pathways evolved from preexisting prokaryotic mechanisms. These mechanisms were co-opted to function in host–symbiont communication and led to metabolic homeostasis. Each pathway added another layer of homeostasis, constraining variation at the lower level and increasing variation at the higher level. As eukaryotes emerged, conflict mediation and metabolic homeostasis complemented each other.

A brief digression concerning the term "co-opt" is warranted here. Co-opt – to use in a role different from the original one – has been widely used in biochemistry and molecular biology for some time. Other biologists, typically focused on morphological structures, sought to describe this process in more detail and used the term "pre-adaptation." Thus a structure or mechanism that was co-opted was in some sense "pre-adapted" for its later role. Noting the awkwardness of this term, Gould and Vrba [20] proposed the term "exaptation" instead, essentially duplicating the use of co-option. Nevertheless, co-option, exaptation, and even pre-adaption remain in use today.

This analysis of conflict can inform our understanding of the process of eukaryogenesis relative to the timing of the symbiosis.

Since a number of eukaryotic signaling pathways plausibly evolved in the context of conflict mediation, these features of eukaryotes had to follow the symbiosis. By this view, mitochondrial symbionts were likely acquired very early in the process of eukaryogenesis.

Innovative traits of eukaryotes subsequently evolved and built on these early co-opted processes. Notable in this regard was a family of proteins that evolved to allow transport in and out of mitochondria. These include membrane uncouplers and inorganic phosphate carriers, but the most notable is called adenine nucleotide translocase, or alternatively ADP/ATP translocase or ADP/ATP carrier. With this carrier, the proto-mitochondria can bring in ADP from the cytosol and get rid of excess ATP. This reinforces metabolic homeostasis and relegates earlier mechanisms to lesser roles of fine-tuning homeostasis. Further, now the proto-eukaryote could utilize ATP from proto-mitochondria, and the promise of moving energy-converting smaller cells inside a larger cell could begin to be realized.

Another major step was the evolution of the protein import apparatus. Very early in the transition, mitochondrial genes and genomes released by dying symbionts would have combined with the host genome to form the chimeric proto-nuclear genome. Recombination and association of mitochondrial genes with host promoters would allow expression of mitochondrial proteins in the cytosol. However, these could not be imported back into mitochondria until the protein import apparatus evolved much later in the transition. Subsequent to the mediation of conflicts between the higher and lower levels, this innovation allowed mitochondria to lose genes and to import the necessary proteins from the nuclear genome. Mitochondrial genome reduction has occurred to a greater or lesser extent in various eukaryotic lineages. This is a very effective mechanism to mediate conflicts because it clearly constrains the heritable variation of the lower-level units.

Nevertheless, even modern mitochondria retain a few genes. Again, this can be understood in terms of metabolic homeostasis. As pointed out by John Allen [21, 22], metabolic homeostasis depends on rapid signaling between chemiosmotic mechanisms and the nearby genome. These signaling mechanisms evolved under these circumstances in prokaryotes, and they have not really changed in eukaryotes. A small organelle genome is always

necessary to receive these signals from chemiosmotic mechanisms and then respond with the appropriate gene activity. Mitochondria that lost their genome entirely could not do this and hence were selected against.

Much of the evolution of eukaryotes thus involved grafting prokaryotic metabolisms together. Achieving both conflict mediation and metabolic homeostasis under these conditions was challenging and required a number of evolutionary steps. First, preexisting prokaryotic signaling mechanisms were co-opted into new functions. Then eukaryotic innovations, particularly the ADP/ATP carriers, better accomplished these functions, relegating earlier signaling mechanisms to a secondary role. Finally, the loss of heritable variation up to the point at which it threatened homeostasis completed the transition.

Why was the evolution of eukaryotes so challenging? Likely, there was no single reason. Rather, it was a complicated transition, involving many steps. Endosymbiosis led to evolutionary conflict. These conflicts had to be mediated and in the process a number of evolutionary innovations had to arise. No single step was the deciding factor, but probabilistically it was challenging to accomplish all of the steps. Likely, many evolutionary experiments failed before one succeeded.

Conclusions

The evolution of eukaryotes was the most challenging of the major transitions. Endosymbiosis alleviated surface-to-volume constraints but led to severe evolutionary conflicts. Conflict mediation required co-opting existing mechanisms as well as evolving new ones to achieve metabolic homeostasis. In this way, all of the major features of eukaryotes were derived subsequent to the endosymbiosis.

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4

A Levels-of-Selection View of Evolutionary Physiology

Summary

From an evolutionary perspective, what we call animal physiology can be seen as inextricably bound up in the unicellular to multicellular transition. Cellular requirements must be met by the multicellular organism via systems for respiration, digestion, circulation, and so on. At the same time, the requirements of these physiological systems when imposed on cells result in cell–cell conflicts, which in turn must be mediated. Physiological systems thus lead to conflict mediation, and both lead to greater complexity. Nevertheless, complex physiological systems have enhanced the evolutionary success of animals contributing to their dominance of marine, freshwater, and terrestrial ecosystems.

> I could have been describing the inside of one of your own cells, or equally a plant cell, or fungus, or a single-celled protozoon swimming around in your local pond. There is a marvelous unity to the world of the cell, which gives a deep sense of connection and fellowship with the world around. From the point of view of the cell, you are just another variation in body plan, just another way of building something wonderful with similar bricks.
>
> *Nick Lane, Life Ascending*

A number of lessons for physiology and medicine are apparent from the foregoing discussion of evolutionary biology. Clearly, biology must be examined from a historical perspective. Life is

Evidence-Based Evolutionary Medicine, First Edition. John S. Torday, Neil Blackstone, and Virender Kumar Rehan

fundamentally a historical process with all the quirks and vestiges that entail. Life is ancient, with its origins dating back more than 3500 million years [1]. On the other hand, complex eukaryotic life is considerably less ancient, perhaps only half as old. In any considerations of physiology and medicine, the origin of eukaryotes looms large. Not only did this transition lead to all complex life, but it was also a wrenching transition, an improbable melding of prokaryotic cells that had diverged since the time of the Last Universal Common Ancestor. Evolutionary conflict during this transition was all but overwhelming. Numerous features of prokaryotes were relentlessly co-opted into conflict mediation. As these homeostatic mechanisms of conflict mediation produced a working cell, co-option of these mechanisms likely occurred again and again as complexity was further built up into multicellular organisms. Indeed, with the exception of metabolism, most complexity – in genes, genomes, structure, function, life cycles – is eukaryotic. Yet remarkably, all multicellular eukaryotic organisms have a unicellular stage in their life cycles. Eukaryotic life, however complex, is never far from the unicellular condition.

The familiar subjects of physiology – respiration, digestion, metabolism, circulation, and so on – are driven by the needs of cells. In the case of eukaryotic cells, these needs are often, but not always, complicated by the grouping of cells into multicellular organisms. Trophic levels occupied by these organisms (e.g. producers, consumers, and decomposers) also strongly impact their physiology. Nevertheless, as Lane [2] points out, underlying all of this complexity, remarkable commonalities emerge. These commonalities trace back to the inherited similarities of all cells, or at least of all eukaryotic cells.

Many of the issues in physiology can be understood in terms of the historical transition from single cells to multicellular organisms that has already been mentioned in Chapter 1. Since subsequent chapters focus primarily on animals, they will be emphasized here as well. Recall that by liberating their external membranes from functions related to energy conversion, eukaryotes thereby diminished surface-to-volume constraints. The result was much larger cell sizes. Increasing cell size eventually led to other limiting factors. Indeed, aerobic eukaryotes (which are the ones that evolved complexity) are faced with other

surface-to-volume constraints, particularly those related to getting oxygen into the cell. Why should size matter in this regard? Oxygen diffuses into the cell and such diffusion is dependent on the surface area of the cell. As with energy conversion, this introduces problems of scale because roughly speaking the volume of a geometric object increases with the cube of a linear dimension, while the surface area increases as the square of a linear dimension. Thus, as size increases, surface oxygen diffusion becomes less and less effective, because there is a smaller surface area supplying a larger volume of tissue. Bigger objects have a relatively smaller surface (resulting in for instance, American football players being very sensitive to heat stress). So a colony of cells in a chain, or a sheet, or a hollow sphere can escape surface-to-volume constraints, while a single large cell cannot (Figure 4.1).

To the extent that there are a number of circumstances in which larger size is desirable, so too will multicellularity be selected for. Besides football, these circumstances include a moderately long list, e.g. efficient dispersal, exploitation of more or different food sources, producing more offspring, escaping predators, and avoidance of the constraints of low Reynolds numbers (*Re*). The last refers to the ratio of forces of momentum to viscosity; fluids behave differently depending on *Re*, e.g. little, slow-moving things can "stop still," unlike the RMS *Titanic* or freight trains. Throughout the entire history of life, the top of the size scale has been an open niche [3]. Perhaps the only consistent disadvantage of large size other than surface-to-volume constraints is a slower replication rate; at times selection for the various advantages may have overcome these two disadvantages.

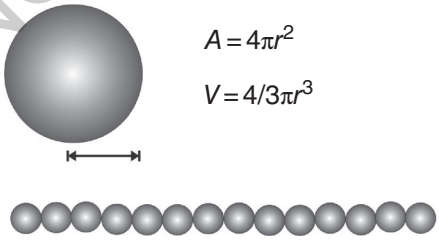

Figure 4.1 Surface-to-volume constraints again. A chain or sheet of cells has relatively more surface relative to volume than a single large cell.

Because of the pressures to increase size on one hand, and supply metabolites and remove waste on the other, it can be said, to paraphrase Haldane [4], that much of physiology is the struggle to increase surface in proportion to volume. The concentration of oxygen in seawater (where animals evolved) is also much less than in air: today, depending on temperature, pressure, and salinity, there is roughly 0–10 ml/l oxygen in marine environments versus nearly 210 ml/l in dry air at sea level. Surface area to volume constraints likely dictate the upper limits of cell size. Further, these constraints influence how animals can become multicellular – in lieu of some special mechanisms for carrying oxygen, better be a chain, or flat, or hollow. Indeed, the first animals likely were flat and sheet-like. The first fossils of the Metazoa and probably several other kinds of multicellular life are found in the late Proterozoic about 565–541 million years ago, perhaps in shallow water reef environments [5].

While the first Great Oxidation Event was more than 2200 million years ago, the second "great oxidation event" was occurring at this time and oxygen levels in the atmosphere may have been approaching those of modern environments. The evolution of multicellular life may have been tied to oxygen levels [6]. A number of factors may have influenced oxygen levels, including extrinsic ones such as an increase of dissolved oxygen in the ocean. This in turn may have resulted from increased atmospheric O_2 or a decrease in ocean temperature, allowing greater oxygen solubility. With regard to the former, O_2 may have increased as a consequence of greater burial of organic carbon. Given the stoichiometry of oxygenic photosynthesis and respiration, molecular oxygen can only be liberated into the atmosphere to the extent that reduced carbon is sequestered in living things or buried. This may have occurred because of greater erosion on land (perhaps in turn caused by the emergence of fungi), or because of the origin of macroscopic animals with guts in the ocean (i.e. macroscopic feces are less likely to be consumed by bacteria before being buried). Thus, macroscopic life may have exerted positive feedback on O_2 levels by increasing carbon burial.

On the other hand, perhaps these extrinsic factors were not particularly influential for the origin of animals [7]. Intrinsic factors such as the evolution of oxygen-carrying proteins may have

also been involved. Such oxygen-carrying proteins would effectively increase the concentration of oxygen inside an animal regardless of the environment. Key innovations in animal development and cell–cell signaling may also have had to occur. From an evolutionary perspective, the appropriate down-regulation of cell division, and hence mediating of cell–cell conflicts, may have been crucial. [8, 9] Thus, features of the environment had to be permissive, but intrinsic features of stem-group animals may have also affected animal origins.

In any event, the late Proterozoic was a very different world from that at present. Because the Sun was not yet in its mature phase, solar incidence was perhaps only 95% that of today. There may have been ice formation at sea level in the tropics (i.e. periodic "snowball earth"). The oceans were likely turbid because no suspension-feeding animals existed, and oceanic waters may have been considerably less basic [10]. The strange and wonderful Ediacarian assemblages [11] that appeared at this time likely included some of the first animals. Many of these organisms were flat, only a few millimeters thick. Oxygen diffusion may have dictated this geometry; these first multicellular organisms may have acted to increase their surface area by flattening out. Some modern animals remain small and flat such as placozoans or flatworms. Such a design clearly facilitates exchange between component cells and the environment.

Nevertheless, some simple animals such as sponges exhibit complex branching and folding of the body. Sponges have the essential design of a primitive circulatory system, that is, they do not rely on simple diffusion of, say, oxygen; they use convection, that is, they ventilate their interiors with a continual supply of seawater (at least in marine forms). Much more elaborate transport systems have evolved in other animals, but this essential feature of moving fluid is central to all. This design can be taken further by adding oxygen-carrying proteins, allowing less fluid to be moved for relatively more oxygen and less energy expended.

Much of our understanding of the evolutionary physiology of animals remains obscured by an ongoing dispute over the relationships of modern animals (Figure 4.2). Sponges have long been considered the sister group to all other animals [12, 13]. Such a sister-group relationship instills a clear polarity to the evolution of major animal features, e.g. sponges lack tissues,

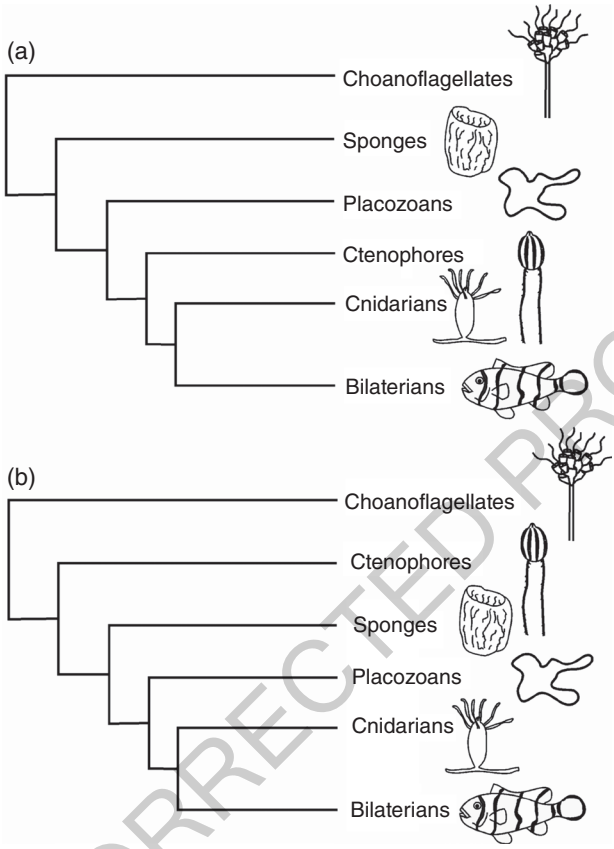

Figure 4.2 Contrasting views of the relationships of the five early-diverging groups of animals. The "sponge sister" view (a) and the "ctenophore sister" view (b) agree that animals are monophyletic and sister to choanoflagellates but differ in the placement of the group sister to all other animals.

nerves, muscles, and have a simple system for ventilating their interiors. On the other hand, some suggest that ctenophores, rather than sponges, are sister to all other animals [14]. Ctenophores are considerably more complex than sponges with tissues, nerves, muscles, and a moderately advanced internal transport system. Such a phylogeny suggests that complex physiological and anatomical features have evolved in parallel in several animal groups.

The debate about the origin of muscles should be mentioned in this context. [15] Based primarily on experimental, developmental, and molecular studies of hydromedusae, a number of workers have suggested that striated muscle in hydroids is homologous to that of vertebrates [16]. Given the phylogenetic position of hydroids, such arguments rest on tenuous evolutionary logic [9, 17]. In a more complete conceptualization of this hypothesis, however, Seipel and Schmid [18] summarize data from all cnidarian classes and explicitly suggest that cnidarians and bilaterians descended from a triploblastic ancestor with muscle-based locomotion. On the other hand, fulfilling earlier predictions [16], "there is no simple relationship between genetic and morphological complexity," Steinmetz and colleagues [19] show that no reliable molecular markers exist for muscle development, whether for smooth- or striated-cell types. A core set of contractile proteins, including a type II myosin heavy chain (MyHC) protein characteristic of striated muscles in vertebrates, was found to be conserved not only among metazoans but even in some unicellular organisms (i.e. prior to the origin of multicellularity and, obviously, muscles). While sponges lack muscles entirely, representatives of both MyHC orthology groups are differentially expressed in various cell types. Although some cnidarians have striated muscles that express the corresponding MyHC ortholog, they completely lack other crucial components of bilaterian striated muscles such as the troponin complex. The results of Steinmetz et al. [19] thus suggest that there is little molecular support for homology between bilaterian and cnidarian muscles.

Focusing on ctenophores, Dayraud and colleagues [20] carry these results a step further. They found a ctenophore-specific duplication of the striated-muscle *MyHC* gene and the association of only one of the resultant paralogs with muscle cells. The association between *MyHC* genes and muscles was thus likely derived after the divergence of ctenophores from both bilaterians and cnidarians. While other scenarios are possible, the simplest interpretation is that ctenophores independently derived muscles subsequent to divergence from the common ancestor with cnidarians and bilaterians.

Muscles are a quintessential feature of animal physiology, allowing their distinctive, "high-powered" life styles and no

doubt contributing considerably to their evolutionary success. Nevertheless, the data suggest that the groups of animals that have muscles – ctenophores, cnidarians, and bilaterians – independently derived these muscles. Nonhomologous muscles, however, seem to use homologous molecular "tools" at least up to a point. While this sort of parallelism is compatible with the "ctenophore sister" view, it does not necessarily support this interpretation. After all, bilaterians and cnidarians are considered as closely related by both the "ctenophore sister" and the "sponge sister" views (Figure 4.2), but these groups also apparently independently derived their muscles.

While there remains no resolution to the debate over the relationships of early-diverging animal groups, it does appear that muscles were independently derived in several of these groups. The stem-group animals likely did not have muscles, similar to choanoflagellates, sponges, and placozoans. Locomotion in these organisms is achieved by cilia, and as mentioned above, for sponges internal transport is also accomplished by cilia, thus allowing complex, three-dimensional shapes. To provide such internal circulation, many somatic cells must become specialized, nondividing, and dedicated to functions other than the reproduction of the organism. Since animal cells primitively exhibit one cilium per cell, and since the microtubule organizing center can form either a cilium or the apparatus for cell division, but not both simultaneously, ciliated cells in animals generally do not divide.

Because of surface-to-volume constraints, larger size therefore entailed multicellularity and ultimately circulation, whether driven by cilia or muscles, or both. This in turn required that some cells of the animal be dedicated to specialized, nonreproductive functions. In animals, there is thus usually a distinction between somatic cells (specialized, nondividing cells that carry out circulation and other critical physiological functions) and germinal cells (cells that are capable of unlimited division and producing a new multicellular individual).

As briefly discussed in Chapter 1, herein lies the major peril of multicellularity [21, 22]. When an organism evolves from unicellular protists to a multicellular grade, the benefits of multicellularity may be clear, but what are the potential costs to the cells involved? Consider a choanoflagellate (Figure 4.3), which

forms a simple multicellular morphology similar to sponges in which amoeboid cells in a gelatinous matrix are surrounded by ciliated cells. The ciliated cells are incapable of cell division, or at least have to shed their cilia before dividing and hence replicate more slowly. Hence, the interior amoeboid cells carry out cell division and growth, while the ciliated cells provide locomotion, or moving fluid, for the entire group. Thus, it is the amoeboid cells that reproduce this simple sponge-like colony. Ciliated cells are thus fulfilling functional needs, but they are potentially getting excluded from contributing to the next generation. This is the principal risk of multicellularity. The ciliated cells must somehow be "sure," speaking informally and teleologically, that they are genetically identical to the amoeboid cells. There must be some sort of evolutionary "agreement" or conflict mediation between the cells of a multicellular organism.

In more formal terms, Darwinian selection at the level of the cell may favor "somatic cell parasites," that is, genetically variant cells that do not contribute to somatic duties but rather

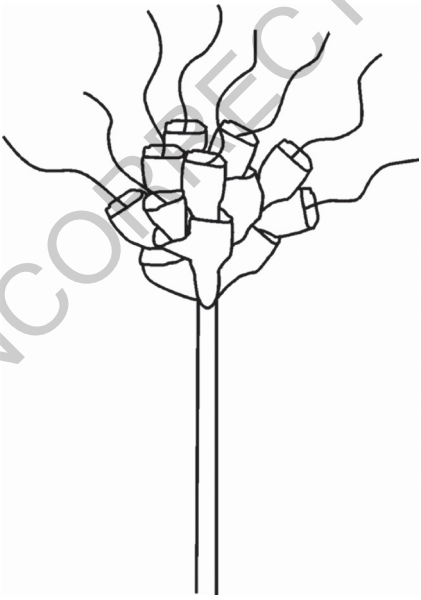

Figure 4.3 A simple colony of choanoflagellates.

monopolize reproduction functions (i.e. selection at the lower level produces competition). The variant amoeboid cell will be favored because when it divides, it does not produce any ciliated cells, only new amoeboid cells. On the other hand, a choanoflagellate composed of only such "selfish" variants will quickly degenerate to the unicellular state, with the associated costs. Selection at the level of the cell will favor somatic cell parasites; selection at the level of the multicellular organism will oppose these variants (i.e. selection at the higher level produces cooperation).

In the context of physiology, the broader point is that common physiological functions (respiration, digestion, metabolism, circulation, etc.) themselves require specialized somatic cells. These cells typically replicate more slowly than stem cells, or not at all, because of the demands of their somatic functions. Physiology thus leads to cell–cell conflicts that in turn require mechanisms of conflict mediation. These mechanisms in turn entail other multicellular features. For instance, the unicellular condition helps to mediate conflicts – the organism is genetically homogenous, at least initially – but complex mechanisms of development are required to obtain the multicellular organism. Programmed cell death can eliminate actually or potentially dangerous cells, but complex mechanisms (caspase cascades, apoptosomes, etc.) and their regulation are required. Complexity, conflict, and conflict mediation evolve in a continuous cycle (Figure 4.4).

Nevertheless, there is no doubt that these physiological systems provided great advantages for multicellular organisms. In this context, consider the mouth and the digestive system of animals. The simplest animals – sponges, placozoans – lack a mouth and digestion occurs at the cellular level. These animals likely have little or no advantage vis-à-vis other multicellular, heterotrophic eukaryotes. The addition of a mouth and gut in cnidarians, ctenophores, and bilaterians, however, may have been a critical innovation. Sequestering and monopolizing a large quantity of substrate likely provided representatives of these animal groups with decisive advantages in competition with other heterotrophs [23]. Refinements of the bidirectional gut in cnidarians, possibly in ctenophores and certainly in bilaterians, enhanced this adaptation, allowing regionalization of digestion. While physiological systems required cellular specialization and

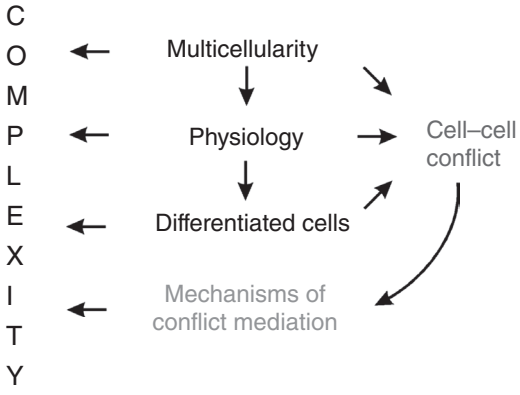

Figure 4.4 Complexity and conflict. Multicellular organisms meet the physiological requirements of individual cells by differentiating some cells into physiological systems. Complexity and cell–cell conflicts both increase. Mediation of cell–cell conflicts also increases complexity. (*See insert for color representation of the figure.*)

enhance competition, animals successfully mediated this cell-level competition and emerged as a dominant life form.

Conclusions

Physiological systems maintain cells within multicellular organisms. Nevertheless, these systems require large numbers of differentiated cells, and this enhances cell–cell competition. Numerous mechanisms mediate this competition, further increasing complexity. In spite of high levels of cell–cell competition generated by their physiological complexity, animals are extraordinarily successful as judged by both their diversity and abundance.

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5

The Cell as the Smallest Functional Unit of Biology/Physiology

Summary

The whole of biology is not equal to the sum of its parts. As a continuum from unicellular to multicellular organisms, fundamental insights into ontogeny and phylogeny can be seen as a functionally integral whole, linking the external physical environment with the milieu interieur of physiology. Primitive cells provided a protected space for electrochemistry that decreased and stabilized the internal energy state within the cell, from which life emerged. The existence of a protected compartment within such primitive "cells" allowed for the formation of the endomembrane system, giving rise to chemiosmosis, or the generation of bioenergy through the partitioning of ions within the cell, like a storage battery. The internalization of the external environment by this mechanism reciprocally conveyed functional biologic information about the external surroundings, and promoted intracellular communication, or the milieu interieur.

Specific duplications of the PTHrP receptor, β adrenergic receptor, and glucocorticoid receptor due to environmental stress facilitated vertebrate adaptation to land. By looking at the process of evolution from its unicellular origins, the causal relationships between genotype and phenotype are revealed, as are many other aspects of biology and medicine that have remained dogmatic, anecdotal, and counterintuitive.

Evidence-Based Evolutionary Medicine, First Edition. John S. Torday, Neil Blackstone, and Virender Kumar Rehan

In the Beginning

Physiology is conventionally thought of as an assemblage of loosely linked biologic events that give rise to, maintain, and repair an entire organism. Yet we know that the whole of biology is not equal to the sum of its parts. Alternatively, a mechanistic approach can be asserted founded on the First Principles of Physiology – negentropy, chemiosmosis, and homeostasis – beginning with the unicellular stage of life. By viewing physiology at the cellular-molecular level as a continuum from unicellular to multicellular organisms, fundamental insights into ontogeny and phylogeny can be seen as a functionally integral whole, linking the external physical environment with the milieu interieur of physiology. And even extending beyond that, to the metaphysical realm by bearing in mind that the calcium waves that mediate consciousness in paramecia form a continuous arc with the axons of our brains as one and the same fundamental mechanism.

Life probably formed like the sea foam you can observe on any shoreline, since such lipids naturally produce primitive soap bubble-like "cells" when vigorously agitated in water. Such primitive cells provided a protected space for electrochemistry that decreased and stabilized the internal energy state within the cell, from which life emerged. Formation of that cellular compartment permitted circumvention of the Second Law of Thermodynamics. That deception of physical law is the essential property of life as self-referential, self-organizing, and self-perpetuating, always in flux, keeping apace with, and yet continually separable from a stressful, ever-changing external environment. That is the bargain life forms have struck with Nature.

Even from the inception of life, rising calcium levels in the oceans have driven a perpetual balancing selection for calcium homeostasis, epistatically counterbalanced by lipid metabolism. Metaphorically, the Greeks called it Ouroboros (Figure 5.1), an ancient symbol depicting a serpent eating its own tail. Ouroboros embodies self-reflexivity or cyclicity, especially in the sense of something constantly recreating itself. Just like the mythological Phoenix, it operates in cycles that begin anew as soon as they end. Critically, the basic cell permits the internalization of factors in the environment that would otherwise have destroyed

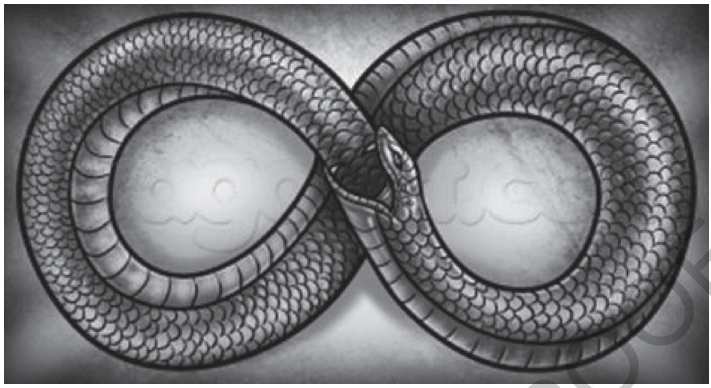

Figure 5.1 Ouroboros. After the Greek legend of a snake catching its tail.

it – oxygen, minerals, heavy metals, micro-gravitational effects, and even bacteria – all facilitated by an internal endomembrane system that compartmentalized those factors within the cell, making them biologically useful. These membrane interfaces are the biologic imperative that separates life from nonlife – "Good walls make good neighbors."

The Advent of Multicellularity

Unicellular organisms dominated the Earth for the first and only 4.5 billion years. These organisms were constantly adapting. From them, the simplest cyanobacteria evolved first, producing the oxygen and carbon dioxide that modified the nitrogen-filled atmosphere. The rising levels of atmospheric carbon dioxide, largely generated by volcanoes and metamorphic degassing acidified the oceans by forming carbonic acid, progressively leaching more and more calcium from rock into the ocean waters. A period of rising levels of atmospheric carbon dioxide caused a "greenhouse effect," partially drying up the oceans, eventually forcing migration of life from sea to land.

The existence of a protected compartment within such primitive "cells" allowed for the formation of the endomembrane system, giving rise to chemiosmosis, or the generation of bioenergy through the partitioning of ions within the cell, like a storage

battery. Early in this progression, the otherwise toxic ambient calcium concentrations within primitive cells had to be lowered by forming calcium channels, composed of lipids embedded within the cell membrane, and the complementary formation of the Endoplasmic Reticulum, an internal membrane system for the compartmentalization of intracellular calcium (Figure 5.2). Ultimately, the advent of cholesterol synthesis facilitated its incorporation into the cell membrane of eukaryotes, differentiating them (our ancestors) from prokaryotes (bacteria), which are devoid of cholesterol. This process was contingent on an enriched oxygen atmosphere, since it takes 11 oxygen atoms to synthesize one cholesterol molecule. The cholesterol-containing cell membrane thins out, critically increasing oxygen transport, enhancing motility through increased cytoplasmic streaming, and is also conducive to endocytosis, or cell eating, exocytosis, or cell secretion. All three of these processes are the cardinal characteristics of vertebrate evolution.

At some point in this progression of cellular complexity, impelled by oxygen promoting metabolic demand, the evolving physiologic load on the system resulted in Endoplasmic Reticulum Stress, periodically causing the release of toxic levels of calcium into the cytoplasm of the cell. The counterbalancing or epistatic mechanism was marked by the advent of the Peroxisome, an organelle that utilizes lipids to buffer such excess intracellular calcium. That mechanism ultimately became homeostatically fixed, further promoting the movement of ions into and out of the cell. Importantly, the internalization of the external environment by this mechanism reciprocally conveyed functional biologic information about the external surroundings, and promoted intracellular communication – what Claude Bernard referred to as the *Internal Milieu* [1]. Walter B. Cannon [2] later formulated the concept that biological systems are designed to "trigger physiological responses to maintain the constancy of the internal environment in face of disturbances of external surroundings," which he termed homeostasis. He emphasized the need for reassembling the data being amassed for the components of biological systems into the context of whole organism function. Hence, in 1991, Weibel, Taylor, and Hoppeler [3] tested their theory of "Symmorphosis," the idea that physiology has evolved to optimize the economy of biologic function. Interestingly, they found that the one exception to this

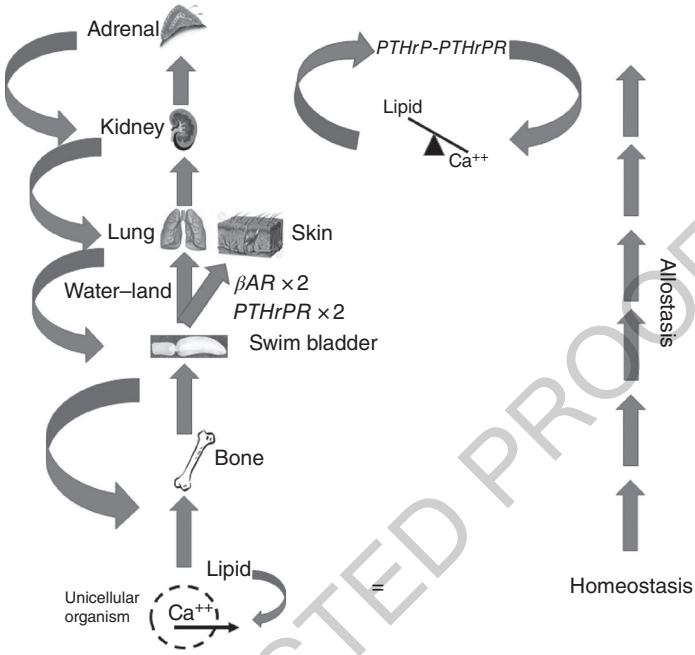

Figure 5.2 From lipid–calcium homeostasis to complex physiology. The ontogenetic and phylogenetic integration (∫) of calcium–lipid homeostasis, from unicellular organism incorporation of lipid into the plasma lemma to multicellular organism calcium/lipid epistatic homeostasis fostered the evolution of metazoans. This figure focuses on the specific stress of the water–land transition on the evolution of a wide variety of organs – bone, lung, skin, kidney, adrenal – resulting from the duplication of the *PTHrP* receptor gene in fish, followed by the β adrenergic receptor (*βAR*) gene, culminating in integrated physiology, or allostasis (on far right). Internal selection was mediated through selection pressure on homeostatic mechanisms mediated by paracrine cell–cell interactions; as vertebrates adapted to land, the *PTHrP* signaling mechanism iteratively allowed for physiologic adaptations to air breathing (skin, lung), prevention of dessication (skin, kidney), and "fight or flight" (adrenal). The blue arrows on the far left signify how evolved traits refer back to their antecedents, or are *exapted*. (*See insert for color representation of the figure.*)

otherwise ubiquitous theory was the lung, which they described as "over-engineered," but more about that later.

Claude Bernard [1] is a proponent of the concept that the energy that flows through a system also organizes that system. West et al. have likewise derived a general model for allometry

(the study of the relationship of body size to shape, anatomy, physiology, and behavior). They have proposed a mathematical model demonstrating that metabolism complies with the 3/4 power law for metabolic rates (i.e. the rate of energy use in mammals increases with mass based on a 3/4 exponent). Back in 1945, Norman Horowitz [4] speculated that all of biochemistry could be reduced to hierarchical networks, or "shells." Based on decades of study, investigators acknowledge that there are fundamental rules of physiology, but they do not address either how or why these rules have evolved.

As eukaryotes thrived, they experienced increasing pressure for metabolic efficiency in competition with their prokaryotic cousins. They are hypothesized to have ingested bacteria via endocytosis, which were subsequently assimilated as mitochondria, providing more bioenergy to the cell for homeostasis. Eventually, eukaryotic metabolic cooperativity between cells gave rise to multicellular organisms, which were effectively able to compete with prokaryotes. As Simon Conway Morris has archly noted, "Once there were bacteria, now there is New York." Bacteria can function as pseudo-multicellular organisms through such behavioral traits as quorum sensing and biofilm formation. The subsequent counterbalancing selection by cellular growth factors and their signal-mediating receptors in our ancestors facilitated cell–cell signaling, forming the basis for eukaryotic metazoan evolution. It is this same process that is recapitulated each time the organism undergoes embryogenesis.

This cellular focus on the process of evolution serves a number of purposes. First, it regards the mechanism of evolution from its unicellular origins as the epitome of the integrated genotype and phenotype. This provides a means of thinking about how and why multicellular organisms evolved, starting with the unicellular cell membrane as the common source for all evolved complex traits. Further, it offers a discrete direction for experimentally determining the constituents of evolution based on the ontogeny and phylogeny of cellular processes. For example, it is commonplace for evolutionists to emphasize the fact that any given evolved trait had its antecedents in an earlier phylogenetic species as a pre-adapted or exapted trait. These ancestral traits can then subsequently be cobbled together to form a novel structure and/or function. If followed

to its logical extension, all metazoan traits must have evolved from their unicellular origins.

Evolution: Cellular Style

Moving forward in biologic space and time, how might such complex biologic traits have come about? Physiologic stress must have been the primary force behind such a generative process, transduced by changes in the homeostatic control mechanisms of cellular communication. Mechanistically, when physiologic stress occurs in any complex organism, it increases blood pressure, causing vascular wall shear stress, particularly in the microvascular beds of visceral organs. Shear stress generates Reactive or Radical Oxygen Species (ROS, which can be used interchangeably but are not one and the same), specifically at sites of greatest vascular wall strain. ROS are known to damage DNA, RNA, and protein, and to particularly do so at those sites most affected by the prevailing external stress. This can result in context-specific gene mutations, and even gene duplications, all of which can profoundly affect the processes of evolution by favoring selection for such adaptations. So it should be borne in mind that such genetic changes are occurring within the integrated structural–functional context of the specific tissues and organs being affected. However, understanding the biochemical processes facilitating the genetics provides profound and testable mechanisms for understanding the aggregate of genetic changes as both modifications of prior genetic lineages, and yet "fit enough to survive" in their new form.

Over evolutionary time, such varying modifications of structure and function would iteratively have altered various internal organs. These divergences would either successfully accommodate the conditions at hand or, failing that, would cause yet another round of damage-repair. So either an existential solution was found or the organism became extinct; either way, such physiologic changes would have translated into both phylogenetic and ontogenetic evolution.

Such an evolutionary process need not be unidirectional. In the forward direction, developmental mechanisms recapitulate phylogenetic structures and functions, culminating in homeostatically controlled processes. And in the reverse direction, the

best illustration lies with the genetic changes that occur under conditions of chronic disease, usually characterized by simplification of structure and function. For example, scarring mechanisms are typified by fibroblastic reversion to their primordial signaling pathways. This sustains the integrity of the tissue or organ through the formation of scar tissue, albeit less efficiently than homeostasis, but still allowing the organism to reproduce before being overwhelmed by the ongoing injury-repair.

Jean Guex has provided empiric evidence for reverse evolution in ammonites. Severe environmental changes have brought about commensurate changes in these organisms. Most of the evolutionary trends described in the following pages concern more or less gradual geometrical and ornamental transformations occurring over long periods of ecologically stable periods. By contrast, major evolutionary jumps in several invertebrate groups occur during massive extinction periods, which are characterized by the appearance of primitive forms resembling remote ancestors of their immediate progenitors. These forms are defined as atavistic. Homeomorphic species generated during sublethal environmental stress can be separated from the ancestral group by several millions of years.

Guex presents a new theoretical model of retrograde evolutionary changes during sublethal environmental stress and analyzes the evolutionary patterns for some planktonic foraminifera, radiolarians, nautiloids, conodonts, corals, and ammonoids during major extinction periods. In ecologically stable periods, the transformations of the skeletons are characterized by an increase of shell curvature, corresponding to an increase in the apparent geometrical complexity. During periods of sublethal environmental stress, rapid retrograde evolution occurs in many invertebrates. The evolution of silicoflagellids is discussed as an example of application of artificial stress to modern organisms.

The Water–Land Transition and Vertebrate Evolution

Nowhere are such mechanisms of molecular evolution more evident than during the water–land transition (Figure 5.2). Net rises in oxygen and carbon dioxide in the Phanerozoic

atmosphere over the course of the last 500 million years partially dried up the oceans, lakes, and rivers, forcing organisms to adapt to land by remodeling tissues and organs, or alternatively becoming extinct. There were two known gene duplications that occurred during this period of terrestrial adaptation – the parathyroid hormone-related protein (*PTHrP*) receptor, and the β adrenergic receptor (*βAR*). The cause of these gene duplications can be reconstructed based on their effects on vertebrate physiology. *PTHrP* is necessary for a variety of traits relevant to land adaptation – ossification of bone, skin barrier development, and the formation of alveoli in the lung. Bone had to ossify to maintain the integrity of skeletal elements under the stress of higher gravitational forces on land compared to buoyancy in water. *PTHrP* signaling is necessary for calcium incorporation into bone. It is known from the fossil record that there were at least five independent attempts to breach land by fish ancestors based on fossilized skeletal remains. Those events must have been accompanied by the evolution of visceral organs, based on both a priori reasoning and the fact that the genes involved in skeletal development are also integral to the morphogenesis of critical internal organs, particularly *PTHrP*. In the aggregate, the net effect of shear stress on *PTHrP*-expressing organs like bone, lung, skin, and kidney would have precipitated the duplication of the *PTHrP* receptor, facilitating the evolution of those progeny best suited for adaptation to land. These were the founders of the subsequent terrestrial species.

As a result of such positive selection pressure for *PTHrP* signaling, its genetic expression ultimately evolved in both the anterior pituitary and adrenal cortex of land vertebrates, further stimulating adrenocorticotrophic hormone and corticoids, respectively, in response to the stresses of land adaptation. This evolved pituitary–adrenal cascade would have amplified the production of adrenaline, since corticoids produced in the adrenal cortex pass through the microvascular arcades of the adrenal medulla on their passage to the systemic bloodstream. This flow of corticoids through the medullary microvascular labyrinth enzymatically stimulates the rate-limiting step in adrenaline synthesis, phenyethanolamine-*N*-methyltransferase, or PNMT. Positive selection pressure for this functional trait may have resulted from cyclic bouts of hypoxic stress, as follows. Episodes

of intermittently large increases and decreases in atmospheric oxygen over geologic time, known as the Berner Hypothesis, may have precipitated lapses in the capacity of the lung to oxygenate efficiently, forcing alternating antagonistic adaptations to hyperoxia and hypoxia as a result. The periodic increases in oxygen sufficiency gave rise to well-known increases in body size, whereas the subsequent bouts of hypoxia are the most potent vertebrate physiologic stressors known. Such intermittent periods of pulmonary insufficiency would have been alleviated by increased adrenaline production, stimulating lung alveolar surfactant secretion, transiently increasing gas exchange by facilitating the distension of the existing alveoli. The increased distention of the existing alveoli, in turn, would have fostered the generation of more alveoli by stimulating stretch-regulated PTHrP secretion, which is both mitogenic for alveolarization, and angiogenic for the alveolar capillary bed, aided and abetted by its potent vasodilatory activity. In the aggregate, this process would have allowed for the iterative evolution of the alveolar bed through positive selection pressure for those members of the species most capable of increasing their PTHrP secretion, though the only "fossil evidence" resides in the cellular-molecular ontogeny and phylogeny of land vertebrates.

And it is worthwhile highlighting the fact that the increased amounts of PTHrP flowing out of the adrenal cortex into the medulla may have caused the evolution of the capillary arcade system of the latter in mammals and birds. Such pleiotropic effects typify the positive selection that has occurred during the evolutionary process, yet they are never seen as both evolutionarily and physiologically functionally integrated based on the top-down descriptive perspective.

This scenario is also consistent with the duplication of the *βARs*. The increase in their density within the alveolar capillary bed was existential for relieving a major constraint during the evolution of the lung in adaptation to land – the *βARs* are a ubiquitous mechanism for blood pressure control in both the lung alveoli and the systemic blood pressure. The pulmonary system had limited capacity to withstand the swings in blood pressure to which the other visceral organs were being subjected, having evolved for optimal surface area-to-blood volume ratio. PTHrP produced by the alveolar epithelium is a potent vasodilator,

so it served to compensate for this constraint on elevated blood pressure in the interim. But eventually the *βARs* had to evolve to coordinately accommodate both the systemic and local pulmonary blood pressure control within the alveolar space.

The glucocorticoid (GC) receptor evolved from the mineralocorticoid (MC) receptor during this same period through a third gene mutation. Since blood pressure would have tended to increase during the vertebrate adaptation to land in response to gravitational demands, there would have been positive selection pressure for reducing the vascular stress caused by the blood pressure stimulation by the MC aldosterone during this phase of land vertebrate evolution. The evolution of the GC receptor would have placed positive selection on GC regulation by reducing the hypertensive effect of the MCs by diverting steroidogenesis toward cortisol production. In turn, the positive selection for cortisol production would have stimulated *βAR* expression, potentially explaining how and why the *βARs* superseded the blood pressure reducing function of *PTHrP*. It was these *ad hoc* existential interactions that promoted land adaptation through independent local blood pressure regulation within the alveolus. This integration of blood pressure control in the lung and periphery by catecholamines represents allostatic evolution.

The net result of *PTHrP*-mediated pituitary–adrenal corticoid production would have fostered a more potent "fight or flight" response in mammalian ancestors. They were small, shrew-like organisms that would have been advantaged by such a mechanism, making them "friskier" and more nimble, able to more likely survive the onslaught of much larger predators during that turbulent era.

Moreover, increased episodes of adrenaline production in response to stress may have fostered the evolution of the central nervous system. Peripheral adrenaline mediates and limits blood flow through the blood–brain barrier, which would have caused increased adrenaline and noradrenaline production within the evolving brain. Both adrenaline and noradrenaline promote neuronal development. It might even be speculated that this cascade led to human creativity and problem solving as an evolved expression of that same axis as an alternative to "fight or flight," since it is well-known that learning requires a modicum of catecholamine-mediated stress.

The bottom-line is that all of the molecular pathways that evolved in service to the water–land transition – the PTHrP receptor, the βAR, and GCs – were aided and abetted by the evolution of the vertebrate lung, the rate-limiting step in land adaptation. Perhaps that is why Weibel, Taylor, and Hoppeler observed that the lung had more physiologic capacity than was necessary for its normal range of function (see above), since only those organisms that were pre-adapted to amplify their *PTHrP* expression survived the stress of the water–land transition. The synergistic interactions between the hypoxic lung and pituitary–adrenal axis producing adrenaline relieved the constraint on the lung through increased PTHrP production, fostering more alveoli; perhaps this is the reason why the lung has such excess capacity – organisms thus overexpressing *PTHrP* signaling having had higher fitness.

The Cellular Approach to Evolution Is Predictive

This reduction of the process of lung evolution to cell biology has an important scientific feature – it is predictive, in contrast to conventional physiology, which is descriptive and postdictive. For example, it may answer the currently untenable question as to why organisms return to their unicellular origins during their life cycles. Perhaps, as Samuel Butler surmised, "a hen is just an egg's way of making another egg." It is worth considering the hypothesis that since all complex organisms originated from the unicellular state, a return to the unicellular state is a necessary fail-safe mechanism for ensuring the fidelity of any given mutation with all of the subsequently evolved homeostatic mechanisms, from its origins during phylogeny, through all the elaborating mutational permutations and combinations of that trait during the process of evolution. One way of thinking about this concept is to consider that perhaps Haeckel's Biogenetic Law is correct after all – that ontogeny actually does recapitulate phylogeny. His theory was dismissed for lack of scientific evidence for the intermediary steps in phylogeny occurring during embryonic development, like gill slits and tails. However, that transpired during an era when the cellular–molecular mechanisms of development were unknown.

A testament to the existence of such molecular lapses is the term "ghost lineage," which fills such gaps in the fossil record metaphorically. We now know that there are such cellular–molecular physiologic changes over evolutionary time that are expressed in bone, and are equally as important, if not more so in other organ systems. In all likelihood, ontogeny must recapitulate phylogeny in order to vouchsafe the integrity of all the homeostatic mechanisms that each and every gene supports in facilitating evolutionary development. Without such a "fail-safe" mechanism for the foundational principles of life, there would inevitably have been drift away from such First Principles, putting the core process of evolution in response to environmental change itself at risk of extinction. The only organism that comes to mind that may have been founded on another set of principles is viruses. Stephen J. Gould famously wondered whether an evolutionary "tape" replayed would recapitulate? In this construct, the answer would resoundingly be "no," since the fluctuations in carbon dioxide and oxygen do not occur now as they did when the atmosphere was in flux.

One implication of this perspective on evolution, starting from the unicellular state phylogenetically, being recapitulated ontogenetically, is the possibility that it is the unicellular state that is actually the primary level of selection. The multicellular state that which Gould and Lewontin called "Spandrels" is merely a biologic "agent" for monitoring the environment between unicellular stages in order to register and facilitate adaptive changes. This consideration is based on both a priori and empiric data. Regarding the former, emerging evidence for epigenetic inheritance demonstrates that the environment can cause heritable changes in the genome, but they would only take effect phenotypically in successive generations. This would suggest that it is actually the germ cells of the offspring that are being selected for.

The starvation model of metabolic syndrome may illustrate this experimentally. Maternal diet can cause obesity, hypertension, and diabetes in the offspring. But they also mature sexually at an earlier stage due to the excess amount of body fat. Though seemingly incongruous, this may represent the primary strategy to accelerate the genetic transfer of information to the next generation (positive selection), effectively overarching the expected

paucity of food. The concomitant obesity, hypertension, and type II diabetes are unfortunate side-effects of this otherwise adaptive process in the adults. Under these circumstances, it can be surmised that it is the germ cells that are being selected for; in other words, the adults are disposable, as Kirkwood has suggested.

Hologenomic evolution theory provides yet another mechanism for selection emerging from the unicellular state. According to that theory, all complex organisms actually represent a vast collaborative of linked, codependent, cooperative, and competitively localized environments and ecologies functioning as a unitary organism toward the external environment. These co-linked ecologies are comprised of both the innate cells of that organism and all of the microbial life that cohabits with it. The singular function of these ecologies is to maintain the homeostatic preferences of their constituent cells. In this theory, evolutionary development is the further expression of cooperation, competition, and connections between the cellular constituents in each of those linked ecologies in successive iterations as they successfully sustain themselves against a hostile external environment. Ontogeny would then recapitulate phylogeny, since the integrity of the linked environments that constitute a fully developed organism can only be maintained by reiterating those environmental ecologies in succession toward their full expression in the organism as a whole.

Another way to think about the notion of the unicellular state as the one being primarily selected for is to focus on calcium signaling as the initiating event for all of biology. There is experimental evidence that the increases in carbon dioxide that occurred during the Phanerozoic Era caused acidification of the oceans, causing leaching of calcium from the ocean floor. The rise in calcium levels can causally be linked to the evolution of the biota and is intimately involved with nearly all biologic processes. For example, fertilization of the ovum by sperm causes a wave of calcium that triggers embryogenesis. These same sorts of processes continue throughout the life cycle, until the organism ultimately dies. There seems to be a disproportionate investment in the zygote from a purely biologic perspective. However, given the prevalence of calcium signaling at every stage of life, on the one hand, and the participation of the gonadocytes

in epigenetic inheritance on the other, the reality of the vectorial trajectory of the life cycle becomes apparent. It cannot remain static, it must move either toward or away from change.

By using the cellular–molecular ontogenetic and phylogenetic approach described above for the water–land transition as a major impetus for evolution, a similar approach can be used moving both forward and backward from that critically important phase of vertebrate evolution. In so doing, the gaps between unicellular and multicellular genotypes and phenotypes can realistically be filled in systematically. But it should be borne in mind that until experimentation is done, these linkages remain hypothetical. Importantly though, there are now model organisms and molecular tools to test these hypotheses, finally looking at evolution in the direction in which it actually occurred, from the earliest iteration forward. This approach will yield a priori knowledge about the First Principles of Physiology, and how they have evolved to generate form and function from their unicellular origins. AQ2

We Are Not Just *in* This Environment, We Are *of* It

The realization that there are First Principles in Physiology, as predicted by the cellular–molecular approach to evolution, is important because of its impact on how we think of ourselves as individual humans, as a species, and our relationships to other species. Once it is recognized and understood that we, as our own unique species, have evolved from unicellular organisms, and that this is the case for all of the other organisms on Earth, including plant life, the intense and intimate interrelationships between all of us must be embraced. This kind of thinking has previously been considered in the form of genes that are common to plants and animals alike, but not as part of a larger and even more comprehensive, elemental process of evolution from the physical firmament. This perspective is on par with the reorientation of hominins to their surroundings once we acknowledged that the Sun, not the Earth was the center of the Solar System. That shift in thought gave rise to the Age of Enlightenment! Perhaps in our present age, such a paradigm

shift will provide insight into such big picture problems as Black Matter, String Theory, and Multiverses.

In retrospect, it should come as no surprise that we have misapprehended our own physiology. Many discoveries in biomedicine are serendipitous, medicine is post-dictive, and the Human Genome Project has not yet yielded any of its predicted medicinal breakthroughs. However, moving forward, knowing what we now do, we should countenance our own existence as part of the wider environment … that we are not merely in this world, but literally of this world… with an intimacy that we had never previously imagined.

This unicellular-centric vantage point is heretical, but like the shift from Geocentrism to Heliocentrism, our species would be vastly improved by recognizing this persistent, systematic error in self-perception. We are not the pinnacle of biologic existence, and we would be better stewards of our planet if we realized it. We have learned that we must share resources with all of our biological relatives. Perhaps through a fundamental, scientifically testable, and demonstrable understanding of what we are and how we came to be so, more of us would behave more consistently with Nature's needs instead of subordinating them to our own narcissistic whims. As we become deeply aware of our true place in the biologic realm, such as we are already witnessing through our increasing recognition of an immense microbial array of fellow travelers as our microbiome, we may find a more ecumenical approach to life than we have been practicing for the last 5 000 years.

Bioethics Based on Evolutionary Ontology and Epistemology, Not Descriptive Phenotypes, and Genes

By definition, a fundamental change in the way we perceive ourselves as a species would demand a commensurate change in our ethical behavior. Such thoughts are reminiscent of a comment in a recent biography of the British philosopher Derek Parfit in *The New Yorker* magazine, entitled "How to be Good," in which he puzzles over the inherent paradox between empathy

and Darwinian Survival of the Fittest. These two concepts would seem to be irreconcilable, yet that is only because the latter is based on a false premise. Darwin's great success was in making the subject of evolution user-friendly by providing a narrative that was simple and direct. Pleasing as it may be, it is at best, entirely incomplete. Think of it like the transition from Newtonian Mechanics to Einsteinian Relativity Theory. As much is learned about the unicellular world with its surprising mechanisms and capacities, new pathways must be imagined. It is clear that we as humans are hologenomes, and all the other complex creatures are too. In fact, there are no exceptions. The reasons for this can only be understood properly through a journey from the "Big Bang" of the cell forward, with all its faculties and strictures. By concentrating on cellular dynamics, an entirely coherent path is empowered. Tennyson's line about "Nature, red in tooth and claw" is only the tip of what the iceberg of evolution really constitutes. As pointed out earlier, we evolved from unicellular organisms through cooperation, codependence, collaboration, and competition. These are all archetypical cellular capacities. Would we not then ourselves, as an example of cellular reiteration, have just those self-same and self-similar behaviors?

The Theory of Everything (Toe)

All multicellular life expresses from an initiating unicell state. There are no exceptions.

Therefore, it is proper to consider the unicell state as the object of evolution, even as it seems not to our human observation. The development of life as compartmentalized in a capable unicell was an acquisitive act between the cellular environment and the larger external one as both intracellular engineering and an extended epigenetic process. In that sense, all of evolutionary development must be reconsidered in a continuum as an interactive epigenetic process unfurling at multiple levels, though first, within the cell by multiple means (e.g. gene transfer, micro RNA, etc.). Multicellularity is an effective mechanism of further maintaining the integrity of the unicellular state by extending its ability to encounter and cope with environmental stimuli

and stresses. Multicellularity is subject to the same epigenetic influences that governed stages from the origin of life to the unicell.

Embryogenesis and organic development must be regarded then as an act of cellular choreography, elaborating developmental stages as the means of enabling the acquisition of pertinent epigenetic experiences. The sum total of these will be recapitulated again in the unicell (zygote state). This explains the primacy of meiosis, which is mathematically the best means of averaging the communal epigenetic experiences of populations of like organisms (which now is the same as saying "like unicells").

The return of multicellular organisms to the unicell state is a requisite period of reassortment and recentering of the genome and entire transcriptome in support of the unicellular state as it reexpresses itself in the macro organism. The unicellular state (zygote) assesses the epigenetic incursions of the macro organism to determine those that are permitted, those that need to be expunged, or adjusting to necessarily accept others that cannot be repelled.

Importantly, this must be examined from within the framework of that unicellular assessment.

This is both a deterministic form of internal selection and cellular engineering to best cope with the environment and its random and nonrandom stresses. In this sense, all complex organisms in macro form are "scouting parties" of the environment, assuring the perpetuation of the unicell in its preferred state that can only be accomplished through constantly interpreting, responding to, and complying with its environment.

It is through this means that unicellar homeostasis is actually maintained, as a continuously balanced reciprocality between unicell, macro organism, and the larger ecology. Hologenomes as a further elaboration of the eukaryotic multicellular state are a more elaborate means of assessing the environment; hence, all complex multicellular creatures are hologenomes.

This further explains why the hologenome is collaborative, cooperative, and competitively linked cellular ecologies that serve to continually experience the variety of stimuli in the larger external ecology, to then be recapitulated in the unicell, maintaining its preferred homeostasis. Speciation is the permanent

shift of the unicell state from one set of homeostatic boundary conditions to the next.

Evolutionary development can best be considered as a cyclical epigenetic reiterative environmental assessment phenomenon, originating from the unicellular state to sustain and perpetuate homeostasis.

Coda

In summary, by looking at the process of evolution from its unicellular origins, the causal relationships between genotype and phenotype are revealed, as are many other aspects of biology and medicine that have remained dogmatic, anecdotal, and counterintuitive. That is because the prevailing descriptive, top-down portrayal of physiology under Darwinism as tautologic. In contrast to that, the cellular–molecular, middle-out approach is conducive to prediction, which is the most powerful test of any scientific concept. Though there is not a great deal of experimental evidence for the intermediate steps between unicellular and multicellular organisms compared to what is known of ontogeny and phylogeny of metazoans, it is hoped that the perspectives expressed in this essay will encourage more such fundamental physiologic experimentation in the future.

In closing, rather than a refutation of Darwinian Evolution Theory, the position taken in this chapter is intended as a further extension of the Modern Synthesis, and as a way for evolution to contribute to evidence-based medicine.

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6

Development of Tissues and Organs

Summary

In the last fifty years great advances have been made in understanding the principles of embryologic pattern formation. The discovery of growth factor receptors and their second messengers for cell–cell signaling has offered the opportunity to determine the cellular–molecular mechanisms of embryogenesis. Wedding growth factor signaling to ontogeny and phylogeny has provided a way to actualize Haeckel's Biogenetic Law, particularly as it applies to lung evolution. By tracing the cellular–molecular developmental and phylogenetic changes in structure and function, the evolution of all of the other physiologic traits can also be elucidated.

Introduction

The science of developmental biology can be traced back to the ancient Greeks five hundred years before the common era, theorizing about the origins of life. Some fourteen hundred years later, at the end of the nineteenth century Haeckel had formulated his Biogenetic Law [1], merging development and phylogeny into one continuous process of "ontogeny recapitulating phylogeny." And Spemann [2] provided the mechanism for embryologic development, claiming that there was an "organizing principle" that was produced within the developing

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tissue that determined morphogenesis. Unfortunately, neither Haeckel nor Spemann was able to offer scientific evidence for their ideas, so they were by-passed in favor of the emerging discipline of genetics as the explanation for all things evolutionary. Yet genetics could not explain pattern formation during embryogenesis, leaving a void in our knowledge of this process for many decades.

It is only in the last fifty years that great advances have been made in understanding the principles of embryologic pattern formation, starting with the work of Clifford Grobstein [3], who showed experimentally that there were soluble factors that passed between the endoderm and mesoderm to mediate the spatial and temporal changes during embryonic development. That observation lay fallow until the discovery that cells in culture produce specific soluble growth factors (which were probably Spemann's "organizer"). And equally important were the discoveries of growth factor receptors and their second messengers, finally offering the opportunity to determine the cellular–molecular mechanisms of embryogenesis. Ironically, wedding growth factor signaling to ontogeny and phylogeny has provided a way of actualizing Haeckel's Biogenetic Law that ontogeny recapitulates phylogeny. The best example of such a process of embryogenesis is that of the lung, whose complete formation has been revealed through decades of study in many laboratories.

In the course of studying lung development, particular attention has been paid to the role of surfactant in lung alveolar development and homeostasis. The recognition of the fundamental relationship between lung surfactant and gas exchange was the "Rosetta Stone" that allowed for the link between current physiology and the origins of life. Lipids were instrumental in the formation of the first protocell, and the optimization of gas exchange from the inception of life. This is particularly true in the case of cholesterol, which facilitated the evolution of eukaryotes, beginning several billion years ago because of its dual biologic properties of increasing oxygenation and protecting against oxidant injury. Such epistatic balancing selection fostered the metabolic drive responsible for the evolution of complex physiologic traits. It is because of this insight into the organ of gas exchange that a far deeper recognition of how and

why the lung evolved has been gained. For these reasons, we will describe the process of lung alveolar development as a template for physiologic evolution.

Lung Alveolar Morphogenesis

The breakthrough understanding of the spatiotemporal interrelationship between the endodermal and mesodermal components of the lung (Figure 6.1) began with the serendipitous finding that the hormone cortisol could accelerate the production of surfactant by the alveolar type II cell (ATII). That finding literally launched hundreds of studies of the mechanism of glucocorticoid induction of lung development, culminating in a comprehensive model of cell–cell interactions mediated by soluble growth factors

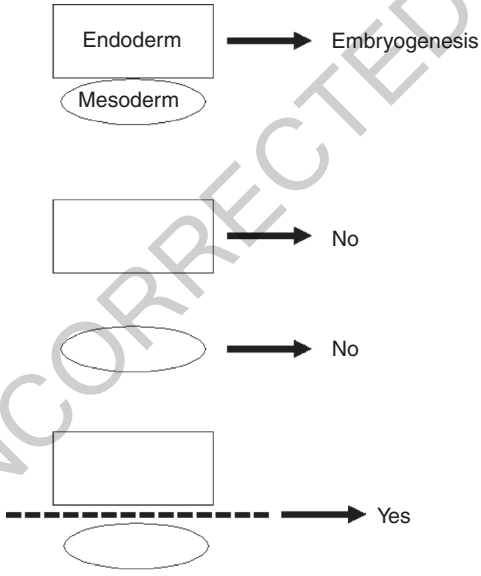

Figure 6.1 Cell–Cell Communication and Lung Embryogenesis. Depicted here (from the top down) is the development of the intact lung mediated by endodermal–mesodermal interactions. In contrast, the isolated endoderm and mesoderm, respectively, do not develop in isolation, whereas the recombination of these tissue layers will develop comparably with the intact tissue.

and their cognate receptors. The key experimental observation for this understanding of vertebrate adaptation for gas exchange was the discovery that the effect of cortisol was not directly on the ATII cell, the site of lung surfactant production, as expected, but on the neighboring fibroblast, which produces a soluble factor originally termed fibroblast pneumonocyte factor. This elusive molecule has more recently been identified as leptin, a soluble product of the adipocyte-like lipofibroblast (LIF) of the alveolar acinus. LIFs played a critical role in the evolution of the mammalian lung, initially protecting the alveolus against oxygen injury by actively accumulating neutral lipid from the microcirculation and storing it as substrate for ATII surfactant phospholipid synthesis, a process termed neutral lipid trafficking. Over the ensuing course of land vertebrate evolution, those stored lipids provided the substrate for the well-recognized "on-demand" production of lung surfactant, the individual steps in the regulated uptake and recruitment of the neutral lipid being stretch-regulated in response to the distention of the alveolar wall upon inspiration of air into the lung. The determination of the molecular mechanisms involved in the "trafficking" of neutral lipid from the LIF to the ATII cell was the "Rosetta Stone" for deconvoluting the evolution of the lung.

Parathyroid Hormone-Related Protein

The principle behind lung morphogenesis is a series of cell–cell interactions mediated by soluble growth factors and their cognate receptors on neighboring interstitial cells, alternating between the endoderm and mesoderm (Figure 6.2). Having established the presence of the glucocorticoid receptor (GR) in the LIF, the question arose as to what factor was signaling from the endoderm to induce it. The seminal observation that deletion of the parathyroid hormone-related protein (*PTHrP*) gene in the embryonic mouse prevented the formation of lung alveoli was the key to unraveling the cellular–molecular cascade of alveolar development. *PTHrP* is produced by the ATII cell, binding to its receptor on the LIF, where it triggers G protein-coupled receptor cyclic adenosine monophosphate production, ultimately stimulating the expression of LIF differentiation, including the

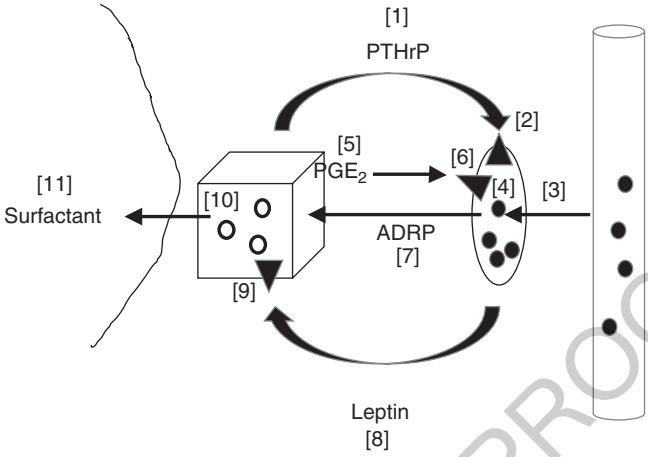

Figure 6.2 Mesodermal–Endodermal Growth Factor-Receptor Interactions Mediate Lung Alveolar Development. Triggered by the distension of the alveolar wall, [1] parathyroid hormone-related protein (PTHrP) produced by the endoderm [2] binds to its receptor on the mesoderm [3], stimulating uptake and storage of neutral lipid [4]. Prostaglandin E_2 [5] produced by the endoderm binds to its receptor on the mesoderm [6], stimulating the release of lipid-bound adipocyte differentiation related protein (ADRP) [7]. Leptin [8] secreted by the mesoderm binds to its receptor on the endoderm [9], stimulating neutral lipid incorporation into surfactant phospholipid [10], which is subsequently secreted into the alveolar space [11].

GR. PTHrP also stimulates the expression of leptin, which acts in a retrograde manner to stimulate surfactant synthesis by the ATII cell. Additionally, *PTHrP* stimulates LIF expression of adipocyte differentiation related protein (ADRP), which mediates the uptake, storage, and release of neutral lipid for surfactant phospholipid synthesis by the ATII cell.

The discovery of this cascade was made with the aid of isolated monolayer cell culture of the endodermal and mesodermal cells of the developing lung. By harvesting the secretions of these cells separately from one another, the independent effects of the epithelial and fibroblastic cells on surfactant biosynthesis could be discerned. For example, it was found that the isolated fibroblasts could readily take up neutral lipid from the cell-culture medium and store it within them, whereas the isolated ATII cells could not; importantly, the fibroblasts could not

release the stored neutral lipid unless they were exposed to the secretions of the ATII cells. In contrast to these observations, when the two cell-types were recombined in culture, the neutral lipid rapidly transited from the fibroblasts to the ATII cells, where they were actively incorporated specifically into surfactant phospholipid, inferring a dedicated mechanism of surfactant phospholipid synthesis via neutral lipid supplied by the interstitial lung fibroblast. Follow-up experiments revealed that the ATII cell produces prostaglandin E_2, which causes release of the neutral lipid from the lung fibroblast via a receptor-mediated pathway. Importantly, this process of neutral lipid trafficking was stimulated by cortisol, providing an integrated mechanism for the effect of cortisol on lung development and homeostasis for the first time.

Stretch-Induced Cell–Cell Interactions

The recognition that the phenotypic expression of the ATII and LIF were mediated by specific soluble growth factors was a breakthrough in understanding this mechanism of complex physiology. It predicted that those interactions were coordinated by the distension of the alveoli with lung liquid since it was known that the fluid expansion and contraction of the lung increased or decreased surfactant production. It was subsequently shown that all of the mediators of lung surfactant production and their receptors were stimulated by stretching the isolated ATIIs and LIFs, respectively, both in vitro and in vivo. The realization of these developmental physiologic principles was the key to recognizing the arc of lung evolution from unicellular to multicellular vertebrates since lipids are used throughout this process to generate, maintain, and establish homeostasis. As such, the lung, as the organ of oxygenation, provides a "Rosetta Stone" of properties for understanding the complementary evolution of other tissues and organs based on developmental and phylogenetic cellular–molecular homologies.

For example, the lung alveolus and kidney glomerulus are homologous, both acting physiologically as pressure transducers. In the case of the lung, the distension of the alveolus

causes increased surfactant production due to the epithelial–mesenchymal interactions mediated by *PTHrP*, as described above, maintaining alveolar homeostasis; in the case of the kidney, signaling from the podocytes that line the glomerular space to the mesangial fibroblasts on the surface of the kidney tubule by *PTHrP* regulates glomerular mediation of systemic fluid and electrolyte homeostasis. *PTHrP* also determines homeostatic balance in the bone, uterus, skin, and brain. Indicative of the relevance of these mechanisms to health, breakdowns in these signaling pathways funnel through the fibrotic process as a means of stabilizing this wide array of tissues and organs, mediated by the Wnt/beta-catenin pathway.

By tracing the developmental and phylogenetic changes in structure and function based on cell–cell interactions mediated by soluble growth factors and their cognate receptors, the evolution of many other physiologic traits can also be elucidated.

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7

When Homeostasis Fails

Summary

Life is self-organizing and self-referential, and the determinant of these mechanisms is homeostasis, which is the self-regulating means for supporting these principles. Implicit in this perspective is that under duress homeostasis can and will fail, but the organism is able to cope with such existential conditions by implementing its evolutionary properties, referring backward in its developmental and phylogenetic history to homologous conditions. If we begin with the precept that cell–cell communication is what has fostered complex physiology, then loss of such communication can be seen as a stepwise, reverse-evolutionary maintenance of homeostatic capacity over time.

Introduction

The premise of this book is that life is self-organizing and self-referential, and that the determinant of these mechanisms is homeostasis, which is the self-regulating means for supporting these principles.

Implicit in this perspective is that under duress homeostasis can and will fail, but that the organism copes with such existential conditions by implementing its evolutionary properties, referring backward in its developmental and phylogenetic history to homologous conditions. If we begin with the precept that cell–cell communication is what has fostered complex physiology,

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then loss of such communication can be seen as a stepwise, reverse-evolutionary maintenance of homeostatic capacity over time. Nowhere is this interrelationship seen more clearly than in the lung, where failure to fully develop at the time of birth results in lung-surfactant deficiency, placing excess strain on the alveolar wall due to increased surface tension. That failure to adapt to the environment causes dissociation of growth factor communication between the mesodermal and endodermal compartments of the alveolus due to cellular molecular damage by physical stress and oxidant damage. In response to these challenges to the homeostatic integrity of the alveolar acinus, the mesodermal cells default (regressively) to their developmental, phylogenetic, and evolutionary origins in the myofibroblast phenotype as a physical stop-gap measure, or "band-aid" for structurally maintaining the alveolus in lieu of the highly evolved coordinated mechanisms for lowering surface tension – parathyroid hormone-related protein (*PTHrP*) signaling for leptin stimulation of alveolar type II (ATII) cell surfactant production, aided and abetted by prostaglandin E_2 (PGE_2) signaling for the release of surfactant phospholipid substrate from the adipocyte-like lipofibroblast (LIF), mediated by adipocyte differentiation related protein (ADRP). Based on this continuum of cell–cell signaling from physiologic homeostasis to pathologic fibrosis, the alveolar acinus can heal itself if the injury has not become irreversible. The excess production of matrix proteins by myofibroblast matrix metalloproteases (MMPs) would obviate the possibility of any reversal of the fibrotic mechanism, though the balancing production of MMPs that breakdown matrix proteins would allow for reversal of the fibrotic process. For example, our laboratory has shown experimentally that the mesodermal cellular environment within the alveolus is critical for homeostatic balance, lipofibroblasts (LIFs) acting to support the growth and differentiation of ATII cells that produce surfactant as the determinant of alveolar homeostasis; conversely, myofibroblasts do not promote either the proliferation or differentiation of the ATIIs, reverting to fibrotic scarring as the means of maintaining the gas exchange surface seen in amphibian and reptilian lungs. Using the LIF agonist peroxisome proliferator activator receptor gamma (PPARγ), the balance of LIFs and myofibroblasts (MYFs) was shown to determine the homeostatic and dyshomeostatic conditions of the gas exchange surface.

Peroxisome Proliferator Activated Receptor Gamma as a Connection to the Evolution of the LIF

The utility of a PPARγ agonist to reestablish alveolar homeostasis, as indicated earlier, is more than just what is referred to as "replacement therapy," i.e. rejuvenating loss of PPARγ activity. This functional relationship refers all the way back to prototypical eukaryotic unicellular organisms like yeast that experience endoplasmic reticulum (ER) stress due to oxidant injury. As a result, calcium leaks from the ER, potentially poisoning the cell due to its deleterious effects on lipids, proteins, and nucleotides by denaturing them. Christian de Duve, who discovered the peroxisome, hypothesized that it evolved due to positive selection pressure for the use of lipids in neutralizing the intracellular calcium. It is because of this atavistic trait that PPARγ ubiquitously prevents fibrosis in a wide variety of tissues, ranging from the lung to the liver, kidney, and brain. Indeed, PPARγ agonists have been found to extend the life span of mice in the laboratory, likely due to the fact that nongenetic determinants of longevity are damaged by oxidants.

PPARγ, Statins, and TOR as Mechanisms for Homeostasis

Homeostatic Control of What?

We make a systematic error when we think of physiology only in contemporary terms, as a synchronic "snapshot." In reality, what we observe in the present is the net result of the evolution of the unicellular organism, annealed by the ever-changing environment as a diachronic, across space-time process of history, both short-term ontogeny and long-term phylogeny. In fact, evolution only occurs because the environment is constantly changing, largely due to the tilt of the Earth's axis, causing seasonality. But even earlier, the separation of the moon from the Earth only 100 million years after its formation has had a profound effect due to its gravitational attraction causing the ebb and flow of the oceans.

Although these influences are ancient and difficult to ferret out, there are still "fingerprints" of these processes that give insight to how they affected the evolution of physiology. For example, the effect of fluid distension on lung development in utero is a vestige of the effect of gravity. Evidence for this has come from both observation and experimentation. When astronauts enter deep space, they must wear pressurized suits in order to maintain the alveolar gradient of the lung to breathe; they also lose calcium from their bones because of the lack of gravitational pull on their skeleton. In both cases, *PTHrP* signaling is decreased, resulting in the physiologic effects on both the lung and bone. But the most telling role of gravity in eukaryotic physiology is seen when yeast is put into microgravity. These simple unicellular eukaryotes lose both the ability to polarize and to bud. The former results failure of calcium flux, and the latter prevents reproduction. Both of these are fundamental properties of life. The mechanism underlying the effect of gravity on the yeast phenotype is the target of rapamycin (*TOR*) gene, which is functionally linked to the cytoskeleton, which determines its activity.

Another "portal" through which to understand the basis for physiology derives from a model of metabolic syndrome, i.e. obesity, hypertension, and diabetes. If a mother rat is deprived of half of her daily food intake in the second half of pregnancy, her offspring will develop metabolic syndrome. However, these chronic diseases may only be an epiphenomenon because another consequence of the food restriction model is that the offspring are growth-retarded. As a result, they enter puberty precociously because of the process of adrenarche, which is the ability of the adrenal gland to produce male hormone. This may be the underlying strategy compensating for low food abundance in the womb since there is acceleration of the reproductive mechanism that would hasten entry of the offspring into a potentially food-abundant environment.

Evidence for such a fundamental mechanism interlinking the organism and its food environment is dictyostelium, or the slime mold. Under abundant food conditions, the slime mold is in a free-swimming amoeboid form. When food is in short supply, the slime mold will revert to its sessile colonial form. The ability of the organism to explore its environment and acquire epigenetic

marks is dramatically different in the amoeboid and colonial forms, offering a mechanistic explanation for the two phenotypes. The *TOR* gene is the sensor for these phenotypic differences as well. The cytoskeleton is more than just the infrastructural support mechanism of the cell. Its conformation dictates its phenotypic functional status, whether it is meiotic, mitotic, or homeostatic.

Pleiotropy: The Deus ex Machina (Ghost in the Machine)

Based on the conventional "snapshot" of an organism's physiology, pleiotropy is descriptively viewed as the same gene randomly utilized for various differing and flexible purposes. As a classic example of pleiotropy's pervasive effects, the preeminent evolutionist George Williams utilized this phenomenon to explain, for example, that senescence occurs as the price for Darwinian reproductive advantage. He described this phenomenon as Antagonistic Pleiotropy – when one gene controls more than one trait, acting beneficially to the organism prior to and during its reproductive phase, but detrimentally post-reproductively, contributing to senescence and aging.

However, pleiotropy may actually occur deterministically rather than by chance, based on the origins of life, thereby revealing the true nature of evolution (Figure 7.1). Pleiotropy has fostered evolution through iterative interactions between the First Principles of Physiology and the ever-changing environment. Pleiotropic novelties emerge through recombinations and permutations of cell–cell interactions for phenotypic adaptation based on both past and present conditions, in support of the present and future needs of the organism for its continued survival. Thus, in contrast to Antagonistic Pleiotropy based on descriptive biology, the cellular–molecular mechanistic approach to aging can be seen as the loss of cellular communication due to the decline in bioenergetics, resulting from selection pressure for the "cost shift" in favor of reproductive success earlier in the life cycle of the organism, i.e. aging is the "price" we pay for reproductive success.

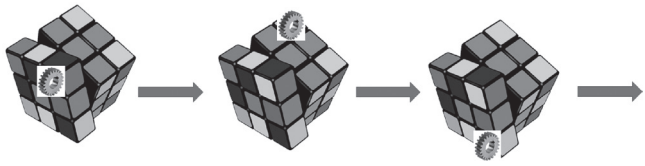


Figure 7.1 The Evolutionary Mechanism of Pleiotropy. The pleiotropic distribution of a gene (symbolized by a "gear" on the surface of a Rubik's Cube) within the organism is determined by its utility during evolution. The gene may have subsequently been co-opted for a different biologic trait necessitated by the prevailing environmental conditions. Under different environmental conditions, that same gene may have been delegated for a different purpose (depicted by the "twisting of the Rubik's Cube," changing the distribution of the gene on the surface of the cube), bearing in mind that the process must always comply with the First Principles of Physiology. That centralized control is the key to understanding the integrated homeostatic control of physiology. (*See insert for color representation of the figure.*)

Rubik's Cube as a Metaphor for Pleiotropic Evolution

Erno Rubik invented his "cube" to teach his students about spatial relationships and Group Theory, the twisting and turning cube generating 4×10^{19} permutations and combinations of colors, like an embryo gyrating during development. By doing so, it generates hundreds myriad cell-types through cell–cell signaling to form the human body. Wolpert has said that "It is not birth, marriage, or death, but gastrulation, which is truly the most important time in your life" because at this stage in embryogenesis the bilayered cell membrane of the embryo becomes three-layered. The spatiotemporal interactions between the endoderm, mesoderm, and ectoderm generate the 200+ cell-types of the organism. Those cell-types determine the tissue-specific homeostatic and allostatic interactions that accommodate organismal structure and function.

Pleiotropy is the phenomenon by which a single gene generates two or more distinct phenotypic traits. If this process is mapped out phylogenetically and ontogenetically, it provides the mechanism of evolution from the protocell to unicellular and multicellular organisms under the iterative, interactive influences

of both internal and external environmental selection pressures. The reappropriation of genes and their phenotypic manifestations are not due to random selection, they are determined by homeostatic constraints within each newly established cellular niche. Those constraints evolved from the First Principles of Physiology, imbuing the transition from one life cycle to the next, remaining consistent with those homeostatic constraints at every scale, phylogenetically, developmentally, and physiologically. If fail to do so, they are either compensated for by other "helper genes," they can be "silenced," or they can be embryonically lethal. It is this process that explains how and why physiologic traits are pleiotropically distributed throughout biologic systems. More importantly, it provides a mechanism for evolutionary novelty, since pleiotropy "repurposes" genes ad hoc for emerging environmental conditions.

In the book *Evolutionary Biology, Cell-Cell Communication, and Complex Disease*, the pleiotropic property of biology was utilized to explain the evolutionary mechanisms for both physiology and pathophysiology. As a prime example, the alveolus and the glomerulus are homologous at the cell signaling level (Figure 7.2), even though they superficially appear to be structurally and functionally unrelated when seen descriptively as organs of gas exchange and fluid and electrolyte balance. Yet from a purely mechanistic vantage point, both of these organs sense and transduce pressure signals, thereby regulating homeostasis through stretch-regulation of *PTHrP* production by the epithelium and its receptor-mediated signaling to specialized neighboring fibroblasts.

Ironically, their homologous physiologic origins are recognizable through such life-threatening losses of homestasis/allostasis as congestive heart failure and Goodpasture's Syndrome. In the case of the former, heart failure commonly disrupts homeostatic control in both the lung and kidney due to *PTHrP* dyshomeostasis in both the organs. In the case of the latter, the evolutionary adaptation to land mediated by the Goodpasture's Syndrome Type IV collagen alpha3(IV)NC1 isomer, which is hydrophobic, protecting against water loss, can cause death due to the generation of autoantibodies that can cause tandem heart and kidney failure.

Regarding the physiologic commonalities between the lung and kidney, in the case of the lung, the stretch-regulated *PTHrP*

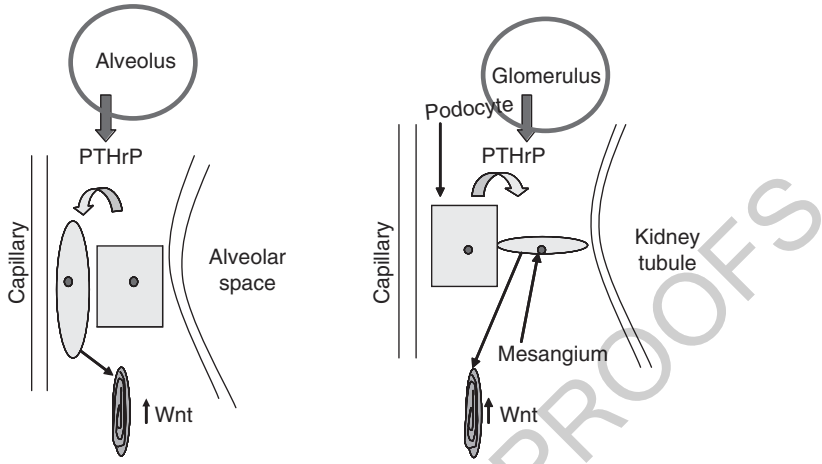


Figure 7.2 Homology between Alveolus and Glomerulus. Both structures are "stretch" transducers whose function is mediated by *PTHrP* signaling. Loss of *PTHrP* signaling causes "default" of the specialized fibroblast to a myofibroblast (depicted as a brown fibrous cell dominated by the Wingless/Int (Wnt) pathway, characteristic of fibrosis. (*See insert for color representation of the figure.*)

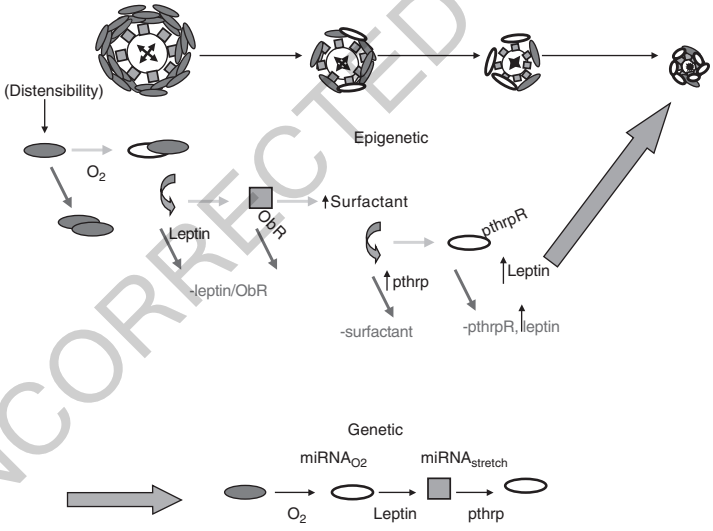
produced by the epithelial type II cell feeds back to its receptor on the lipofibroblast to regulate lung surfactant production (see above), reducing surface tension to maintain alveolar homeostasis; in the case of the kidney, *PTHrP* produced by the epithelial podocytes that surround the fluid-filled space within the glomerulus regulate the mesangium, the thin mesodermal membrane supporting the glomerular capillary loops, homeostatically monitoring and regulating fluid and electrolyte balance in the systemic circulation.

These *PTHrP*-regulated physiologic traits evolved during the water–land transition in response to the "greenhouse" effect caused by accumulating levels of carbon dioxide in the atmosphere. Since skeletal, gas exchange, barrier function, and kidney function are all under *PTHrP* signaling control, the physiologic stress on the microcirculations of all of these structures would have generated shear stress and radical oxygen species, culminating in the duplication of the *PTHrP* receptor [1]. As a consequence, the amplification of *PTHrP* signaling "amplified"

the downstream effects of *PTHrP* on all of these structures, culminating in bone, lung, skin, and kidney, as a attested to experimentally by the loss of the evolved status of all of these physiologic traits when the *PTHrP* gene is deleted. AQ1

The Lung as the Prototypical Pleiotropic Mechanism

The evolution of the lung was existential for the survival of land-dwelling vertebrates, since the rise in atmospheric temperature due to the greenhouse effect of rising levels of carbon dioxide caused the drying up of bodies of water, forcing our forebears to adapt to land (Figure 7.3). The physicochemically integrated



AQ2 **Figure 7.3** Alveolar Evolution. The top of the schematic describes the cellular development of the alveolus. Below it are the mesenchymal–epithelial interactions that caused the developmental and phylogenetic adaptations over the course of vertebrate evolution. The progressive decrease in alveolar diameter increased the surface area to blood volume ratio, facilitating gas exchange in support of metabolic demand over the course of vertebrate evolution from water to land. (*See insert for color representation of the figure.*)

developmental and phylogenetic cell–cell interactions regulating lung surfactant offer the means of understanding the ontogenetic and phylogenetic structural–functional interrelationships at the cellular–molecular level between the decrease in alveolar diameter and the increased lung surface area for gas exchange. The counterbalancing of the otherwise pathological increase in alveolar surface tension due to the decrease in alveolar diameter would have resulted in its collapse, or atelectasis; conversely, the evolution of epithelial–mesenchymal interactions for the concomitant thinning of the alveolar wall and the progressive efficiency of the surfactant system facilitated alveolar accommodation of gas exchange. This is the only biologic means for increasing oxygenation.

It is helpful to realize how lipids have fostered vertebrate evolution. At the inception of life on Earth, polycyclic hydrocarbons contained in the asteroids that delivered water to the surface spontaneously formed micelles or liposomes, semipermeable membranes forming spheres. Sometime between the primordial atmosphere and the rise in oxygen due to plant life, cholesterol synthesis began since it requires 11 atoms of oxygen for each molecule of cholesterol [2]. The cholesterol inserted itself into the primitive bilayered cell membrane, fostering metabolism, gas exchange, and locomotion, the three basics of vertebrate evolution. These same characteristics acted synergistically to facilitate the evolution of the vertebrate lung, which was also contingent on surfactant lipids fostering gas exchange, beginning with cholesterol.

Beginning with the fish swim bladder as a biologic mechanism for adapting to water buoyancy – inflating to float, deflating to sink – fish have successfully exploited gas to optimize their adaptation for buoyancy in water. All of the key molecular features of the mammalian lung as a reciprocating gas exchanger were already present in the fish swim bladder (Figure 7.4) – surfactant lipid and protein to prevent the walls of the bladder from sticking together, *PTHrP* gene expression during swim bladder development, and the β adrenergic receptor regulating the filling and emptying of the swim bladder with gas absorbed from or secreted into the circulation. These components of the evolutionary process were capable of re-permutation and recombination within the physiologic constraints of the existing

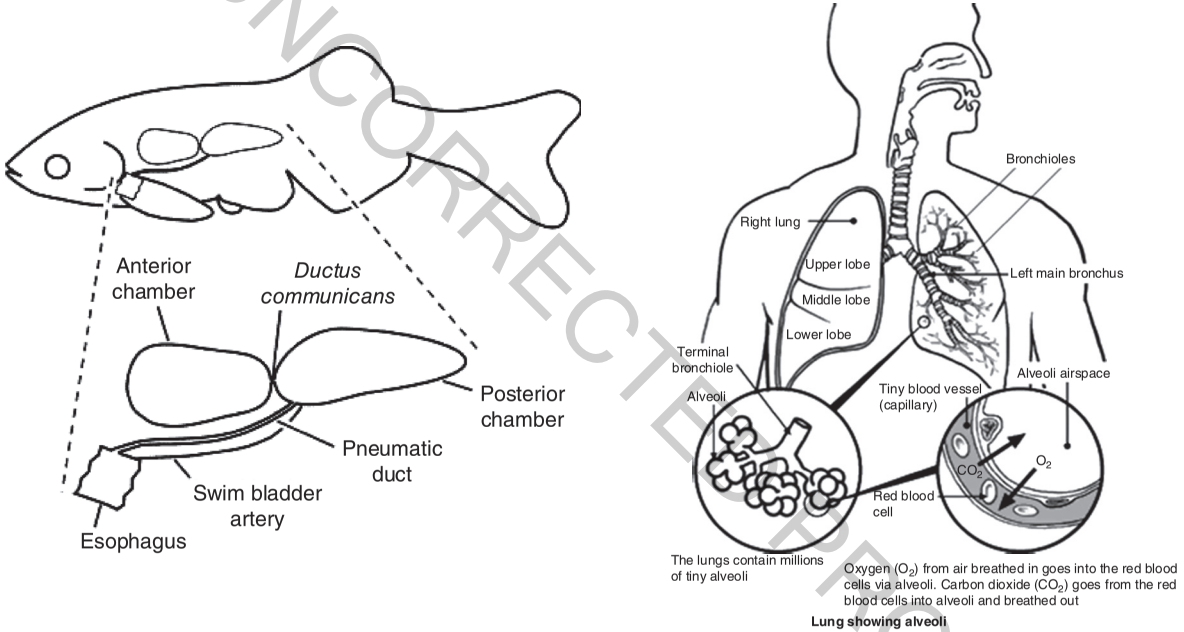


Figure 7.4 Homology between the Swim Bladder and Lung. The swim bladder of physostomous fish (left) develops as an out-pouching of the esophagus to form an inflatable structure that facilitates gas exchange for buoyancy, maximizing metabolic activity in water. The surface of the swim bladder is coated with surfactant to prevent the walls of the bladder from adhering to one another. The lung (right) is also an out-pouching of the esophagus, the alveoli mediating gas exchange for optimally metabolic function.

structure and function to form the lung. The only additional critical physiologic adaptation to be acquired was neutral lipid trafficking (NLT), mediated by ADRP, a member of the ubiquitous PAT (Perilipin, ADRP, TIP47) family of lipid transport and storage proteins. NLT likely evolved from the adaptive advantage of lipofibroblast neutral lipid storage, initially for protecting the lung gas exchange surface against oxidant injury, followed by its regulatory role as a means of more efficiently producing surfactant in response to the ever-increasing distension of the alveolar wall in response to metabolic demand. This is the epitome of the mechanism of pleiotropy, repurposing adipocyte metabolism for both the respiratory system and for the emergence of homeothermy, synergistically facilitating vertebrate adaptation to land through a common functional homolog.

The Lung as an Interactive Barrier: Homolog of the Plasma Membrane, Skin, and Brain

Developmentally, the lung emerges from the foregut as the expansion of the surface of the alimentary tract. As a homolog of the gut, the lung also acts as an interface between the internal and external environments of the body. However, the homology goes much deeper molecularly since the stratum corneum of the skin forms a lipid barrier on its surface much like the alveolar surfactant, forming tubular myelin as a membrane barrier (Figure 7.5) – in either case, the epithelium secretes lamellar bodies composed of lipid–protein complexed with antimicrobial peptides. And the skin and brain are structurally and functionally homologous, both phylogenetically and pathophysiologically – the nervous system of the skin in worms gave rise to the central nervous system of the skin and brain share common lipodystrophies in such neurodegenerative diseases as Niemann–Pick, Tay Sachs, and Gaucher's Disease. It has been speculated by some that this is a reflection of "too much of a 'good thing' going bad." In this case, the excessive myelination of axons in the brain causes tandem skin lipid lesions in association with brain neuronal pathology.

AQ3

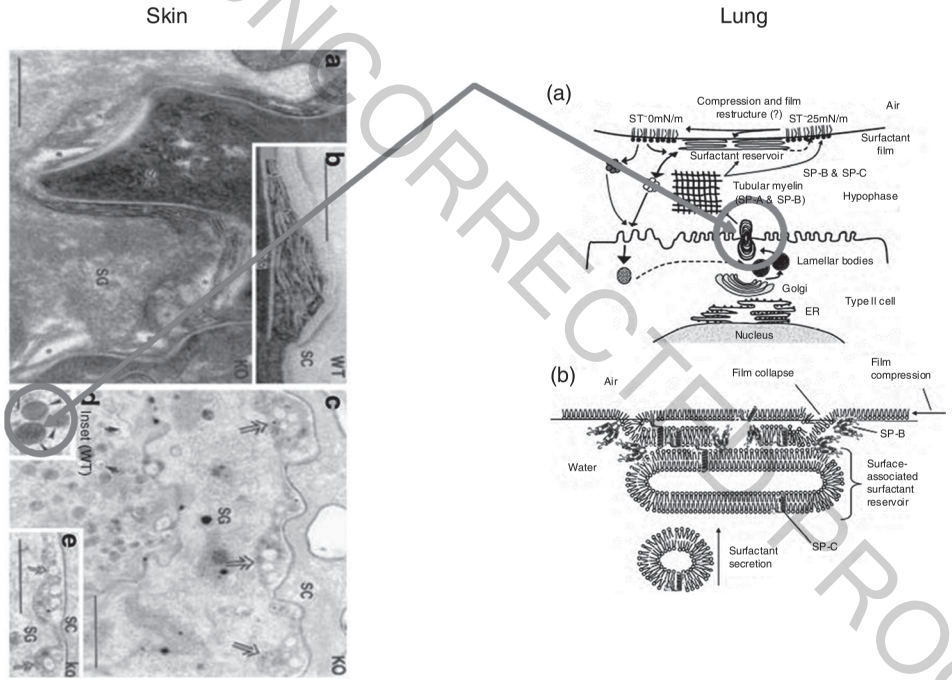


Figure 7.5 Homology between Lung and Skin. The skin and lung are barriers between the external and internal environments. The stratum corneum of the skin forms a lipid barrier containing antimicrobial peptides, and the alveolus generates surfactant, which has the same property. In both cases, the epithelium synthesizes and secretes lamellar bodies (red arrows) composed of lipids complexed with defensins to prevent microbial invasion. (*See insert for color representation of the figure.*)

For example, the functional homology between the lung alveolus and kidney glomerulus are enacted by shared mechanotransducers for the physiologic stretching of their respective walls – in the case of the lung, alveolar *PTHrP* signals to stimulate surfactant production, preventing its collapse due to increased surface tension. In the case of the kidney, the epithelial podocytes lining the glomerulus also secrete *PTHrP*, which then signals the mesangium to regulate water and electrolyte economy as a function of glomerular fluid distension. In both instances, the calcium-regulatory activity of *PTHrP*, which is ubiquitously expressed in all epithelial cells, has been embellished due to its myriad functionally evolved properties. For example, due to its angiogenic property, *PTHrP* promotes microcirculatory capillary formation for gas exchange in the alveolar bed, and fluid and electrolytes in the glomeruli. Phylogenetically, within the fish kidney, the growth of the primitive filtering capillaries of the glomus would presumably have been enhanced by the local production of *PTHrP*, ultimately culminating in the expansion of the capillary network to form glomeruli, increasing the efficiency of water and electrolyte homeostasis in service to land adaptation.

NKX2.1, Thyroid, Pituitary, and Lung Pleiotropy

The foregut is a plastic structure from which the thyroid, lung, and pituitary arise through the Nkx2.1/TTF-1 genetic pathway. Evolutionarily, this is consistent with the concept of terminal addition, since the deuterostome gut develops from the anus to the mouth. Developmentally, when Nkx2.1/TTF-1 is deleted in embryonic mice, the thyroid, lung, and pituitary do not form during embryogenesis. This provides direct experimental evidence for a genetic common denominator for all three organs (Figure 7.6). Their phylogenetic relationship has been traced back to amphioxus, and to cyclostomes, since the larval endostyle (a longitudinal ciliated groove on the ventral wall of the pharynx for gathering food particles) is the structural homolog of the adult thyroid gland.

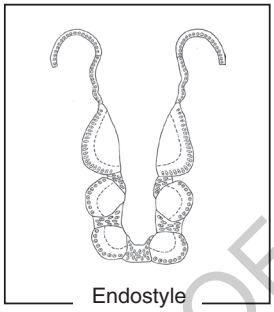


Figure 7.6 Phylogenetic Homology between Thyroid, Lung, and Pituitary Based on Nkx 2.1/TTF-1. The thyroid, lung, and pituitary all develop from the foregut under the genetic control of Nkx 2.1/TTF-1.

The Phylogeny of the Thyroid

The endostyle is retained in post-metamorphic urochordates, and in adult amphioxus, but the post-metamorphic lamprey has a follicular thyroid gland, which is an evolved endostyle. The presence of an endostyle in larval lampreys does not suggest direct descent of lampreys from protochordates, but rather that the evolutionary history of the lamprey is deep and ancient in origin, and that it shares the common feature of having a filter-feeding mechanism during its larval stage of development. However, it is noteworthy that the other extant agnathan, the hagfish, possesses thyroid follicles before hatching. Since hagfish evolution is considered to be conservative, going back 550 million years, this suggests that thyroid follicles could also be considered to have an ancient history.

An Evolutionary Vertical Integration of the Phylogeny and Ontogeny of the Thyroid

Mechanistically, the increased bacterial load consequent to the facilitation of feeding by the endostyle may have stimulated the cyclic AMP-dependent protein kinase A (PKA) pathway, since bacteria produce endotoxin, a potent PKA agonist. This cascade may have evolved into regulation of the thyroid by thyroid-stimulating hormone (TSH), since TSH acts on the thyroid via the cAMP-dependent PKA signaling pathway. This mechanism

potentially generated novel structures such as the thyroid, lung, and pituitary, all of which are developmentally induced by the PKA-sensitive Nkx2.1/TTF-1 pathway. The brain–lung–thyroid syndrome, in which infants with Nkx2.1/TTF-1 mutations develop hypotonia, hypothyroidism, and respiratory distress syndrome, or surfactant deficiency disease, provides further evidence for the coevolution of the lung, thyroid, and pituitary.

Developmentally, the thyroid evaginates from the foregut in the embryonic mouse beginning on day 8.5, about 1 day before the lung and pituitary emerge, suggesting that the thyroid may have been a molecular prototype for the lung during evolution, providing a testable and refutable hypothesis. Adaptationally, the thyroid rendered molecular iodine in the environment bioavailable by binding it to threonine to synthesize thyroid hormone, whereas the lung made molecular oxygen tolerable, first by inducing fat cell-like lipofibroblasts as cytoprotectants, which then stimulated surfactant production by producing leptin, relieving the physiologic oxygenation constraint on the blood–gas barrier by making the alveoli more distensible. This, in turn, would have further facilitated the use of rising oxygen in the atmosphere metabolically, placing further selection pressure on the alveoli, giving rise to the stretch-regulated surfactant system mediated by *PTHrP* and leptin. Subsequent selection pressure on the cardiopulmonary system may have facilitated liver evolution, since the phylogenetically increasing size of the heart, accommodating the water–land transition, would have induced precocious liver development – developmental induction of the liver is caused by the physical interaction between the heart and liver – fostering increased glucose regulation, e.g. gluconeogenesis and glycogen storage/release. In turn, this may have fostered brain evolution since the brain is a metabolic glucose "sink." Further evolution of the brain, specifically the pituitary, would have served to foster the evolution of complex physiologic systems, culminating in endothermy/homeothermy in mammals and birds.

Both the thyroid and lung have played similar adaptive roles by accommodating otherwise toxic substances in the environment during vertebrate evolution. The thyroid has facilitated the utility of iodine ingested from the environment, whereas the lung has accommodated the rising oxygen levels during the

Phanerozoic era. Importantly, both the thyroid and lung have interacted synergistically in facilitating vertebrate evolution – for example, thyroid hormone stimulates embryonic lung morphogenesis during development, while also accommodating the increased lipid metabolism needed for surfactant production by driving fatty acids into muscle to increase motility, as opposed to maladaptively oxidizing circulating lipids to form toxic lipoperoxides. The selection pressure for metabolism was clearly facilitated by the synergy between these foregut derivatives.

A Retrospective Understanding of Evolution

Looking at the definitive structure and function of the mammalian alveolus (Figure 7.3), one can see the signature for phylogenetic traits that facilitated the evolution of land vertebrates from fish in a stepwise fashion. Referring to the schematic, at the far left is the molecular transition from prokaryotes to eukaryotes, which may have been caused by rising oxygen tension in the atmosphere on sterol production since hypoxia-inducible factor-1 (Hif-1) mediates the molecular effect of oxygen on sterols in both prokaryotes and eukaryotes. This scenario would resolve the age-old debate as to whether evolution was gradual or salutatory – it was both. This is a key insight to understanding mechanistic evolution. Historically, Darwin thought that evolution was a gradual process. He did not think that this process was smooth, but rather, that it should be presumed to be stepwise, with species evolving and accumulating through small variations over long periods of time. Darwin further speculated that if evolution were gradual, that there would be fossil evidence for small incremental change within species. Yet Darwin and his supporters have been unable to find most of these hypothesized "missing links." Darwin surmised that the lack of fossil evidence was due to the low likelihood that such critical transitions would have been preserved. Then, in 1972 evolutionary biologists Stephen Jay Gould and Niles Eldredge suggested that the "gaps" in the fossil record were real, representing periods of stasis in morphology, calling this mode of evolution "punctuated equilibrium." This infers that species are generally morpholgically stable, changing little for millions of years.

This slow pace is "punctuated" by rapid bursts of change, resulting in new species. According to this theory, changes leading to new species do not result from slow, incremental changes in the mainstream population. Instead, changes occur in populations living on the periphery, or in isolated populations where their gene pools vary more widely due to slightly different environmental conditions. When the environment changes, such peripheral or isolated species possess variations in morphology that might allow them an adaptive advantage.

A bridging concept can account for both gradualism and punctuated equilibrium. The kinds of mechanisms that have been invoked for pleiotropy would account for both scenarios. As Darwin had surmised, evolution could have occurred on a continuous molecular basis microscopically in response to physiologic stress, occasionally leaving fossilized evidence once form and function reached a macroscale, only making it seem as though evolution had occurred in bursts (yet the molecular evidence can be seen in the continuum from ontogeny and phylogeny to pathophysiology!).

A scenario for differing rates of evolutionary change is all the more cogent when one superimposes the episodic increases and decreases in atmospheric oxygen that have been documented over the last 500 million years, referred to as the Berner Hypothesis. Within this theory, the increases in atmospheric oxygen caused the well-documented increases in the size of land animals. However, the consequences of the decreases have never been considered before, yet would predictably have had profound effects on vertebrae evolution, given that hypoxia is the most potent physiologic effector of complex bilogic systems. Elsewhere, a novel mechanism for the evolution of endothermy/homeothermy based on the interactions between the pulmonary and neuroendocrine/endocrine systems has been invoked that allows for the arc of the Cambrian Burst, culminating in the crown species of mammals and birds. This perspective is validated by pleiotropic effects of the specific gene duplications for the *PTHrP* receptor and the β adrenergic receptor, as well as the mutation of the mineralocorticoid gene to produce glucocorticoids, and the evolution of the Goodpasture's Syndrome Type IV collagen isomer, all of which occurred during the water–land transition. These events corroborate the repurposing of preexisting genes for novel phenotypic adaptations.

Even earlier in vertebrate evolution, sterols may have liquified the bacterial cell wall, possibly due to rising levels of oxygen in the atmosphere, stimulating sterol production. That event would have marked the phenotypic transition from prokaryotes to eukaryotes, the former having hard exterior walls, the latter having compliant cell membranes. That transition may have been further catalyzed by the nascent synthesis of cholesterol, under positive control by Hif-1 [90], catalyzing the evolution of eukaryotes. AQ4

Denouement

The seemingly serendipitous occurrences of pleiotropy based on the conventionally descriptive understanding of biology are overarched by the synchronically mechanistic basis for pleiotropy, emanating from the cell–cell signaling principles elucidated earlier. Thus, the deep, otherwise-unobvious pleiotropic homologies transcend the superficialities of comparative anatomy, only being revealed by knowledge of molecular developmental and phylogenetic physiologic motifs. The deepest of these are related to the physiologic effects of stretching, or mechanotransduction, on surfactant metabolism, which refers all the way back to biologic adaptation to gravitational force, the most ancient, omnipresent, and constant of all environmental effectors of evolution.

For example, the ATII cells produce PGE_2, particularly when they are distended, causing secretion of lipid substrate from lipofibroblasts for lung surfactant phospholipid production by the ATII cells; without PGE_2, the lipids would remain bound within the lipofibroblasts. This effect of PGE_2 on the secretion of free fatty acids (FFAs) from lipofibroblasts is homologous with the release of FFAs from peripheral fat cells, a trait that hypothetically evolved as a consequence of the evolution of endothermy. To alleviate the periodic hypoxic constraints on the evolving alveolar bed, stress-induced adrenalin stimulated surfactant secretion to increase gas exchange transiently until the endogenous *PTHrP* mechanism could generate more alveoli. Thus, the pleiotropic coevolution of the PGE_2 mechanism facilitating FFA utilization in both the lung and fat pad was not a

chance event; it was synergistic when viewed within the context of the evolving lung's effect on endothermy. In further support of this hypothesis, the role of the lung in the evolution of endothermy is further evidence for the causal evolutionary interrelationship between the pulmonary and neuroendocrine systems, both mediated by *PTHrP* signaling. Yet again, this is not a chance event; periods of hypoxia due to the continuous evolution of the lung would have caused physiologic stress, stimulating adrenaline production by the adrenal medulla. Adrenaline production would have had the dual adaptive benefit of increasing alveolar oxygenation, and releasing FFAs from the peripheral fat pads. The release of excess FFAs from the fat pad would otherwise have been toxic, but instead adaptively increased body temperature, complementing the concommitant evolution of dipalmitoylphosphatidylcholine, the surface-active phospholipid in mammalian alveoli, which is 300% more surface-active at 37 °C than at 25 °C.

A similar physiologic evolutionary interrelationship emerges from the etiology of Goodpasture's Syndrome. The disease is caused by an autoimmune reaction to an evolved isoform of Type IV collagen. Alpha 3(IV)NC1 Type IV collagen is absent from worms and flies, but it appears in fish. However, it does not generate the pathogenic Goodpasture's Syndrome antibody. It is ubiquitous in amphibians, reptiles, birds, and mammals. It has the evolutionarily relevant physicochemical characteristic of being more hydrophobic than other Type IV collagens, offering a functional role in preventing water loss across the lung and kidney epithelia in adaptation to land. The fact that this specific Type IV collagen isoform evolved during the process of land adaptation is unlikely to have occurred merely by chance, given its ability to prevent water loss on land.

Thus, not unlike chemistry and physics, biology is also founded on First Principles that can be understood ontologically and epistemologically rather than through dogmatic teleologic mechanisms and tautologic concepts. George Williams' Antagonistic Pleiotropy hypothesis for senescence was alluded to above – in large part, this perspective is reflective of the systematic error authored by Ernst Mayr that there are proximate and ultimate mechanisms of evolution that must be dissociated from one another based on Darwinian principles of mutation and selection. However, that dictum was formulated more than 60 years ago.

Theorists that offered differing perspectives, such as Haeckel, Spemann, and Lamarck have generally been dismissed. However, in the interim a great deal more about biology has been learned that reenergizes some previously disregarded principles toward understanding evolutionary development. This is particularly true within cell biology, where pathways can be identified that inform us that there is a continuum between the proximate and ultimate mechanisms of evolution – Mayr exemplified this principle using bird migration, which was then too complex to be understood as one continuous process, yet we now know how ambient light affects the neuroendocrine system to foster migratory behavior.

As an extension of the insights gained by seeing pleiotropy through the lens of mechanistic pleiotropy, repurposing of the same genetic signaling cascade to form novel phenotypes, heterochrony can be seen in the same way – the mechanism of heterochrony has never been provided before, it has only been described. Haeckel described the concept of heterochrony as a way of expressing how development could facilitate evolutionary change. To this day, no one has expressed heterochrony as a mechanism for reallocating cell–cell signaling to accommodate adaptive change, yet it is the premise we have used throughout this book.

Conclusions

It was Thomas Kuhn, the author of *The Structure of Scientific Revolutions* [3], who said that an indicator of a paradigm shift was a change in the language – going from a descriptive to a mechanistic way of thinking about pleiotropy and heterochrony would reflect such a paradigm shift.

Indeed, Haeckel, Spemann, and Lamarck had many correct surmises about the mechanistic biologic principles that they each addressed – recapitulation theory, the embryologic "organizer," and acquired characteristics. In their time, they lacked the technical ability to support their hypotheses. However, the novel perspective on pleiotropy expressed herein honors both old concepts and new. Our own evolving understanding of evolutionary mechanisms generates a compelling narrative for evolution as a continuum of physiologic adaptations toward rewarding homeostatic mechanisms that permit cells to thrive in diverse environments.

Cells solve problems – they use the tools that they have or can generate. Many generations of scientists have attempted to discern the puzzle of evolutionary development, yet they have lacked the tools that can be productively employed today. What we have now learned is in many ways unexpected. Contrary to our expectation, what was old can again become new. In that sense, this paper is dedicated to those who have labored before us. Their efforts can now be married to compelling research. Through this combination, a new paradigm for evolutionary development unfurls that is congruent with the dominant truth that can be asserted about our physiologic path from First Principles. It is clearly evident that all complex organisms unavoidably must return to their unicellular roots. The physiological pathways and the cellular communication mechanisms that underscore it explain the imperative for this immutable recapitulation.

The resolution of the evolutionary significance of pleiotropy is tantamount to Niels Bohr's eloquent explanation for how light could be both wave and particle based on principles of quantum mechanics. In his complementarity lecture at Lake Como, Switzerland in 1927, he resolved this paradoxical duality by explaining that it was an artifact of the way in which the light was measured (Bohr Como Lecture). Similarly, the cell is both genetic and phenotypic, depending upon the metric, yet in reality it is integral whole whose fate is determined by the ever-transcendent mechanisms that perpetuate it. In his groundbreaking book entitled *Wholeness and the Implicate Order*, the physicist David Bohm explains how our subjective senses cloud our perception of reality. As in physics, recognizing this dichotomy is key to future progress in biology and medicine.

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8

Wnt Signaling During Development

Summary

Growth factor signaling is central to homeostasis and dyshomeostasis alike, rendering it a common "language" for both biology and medicine. The consequences of lost paracrine signaling can readily be observed when the target cell-type is propagated in the absence of the signaling that induced its differentiation developmentally. Both the alveolar lipofibroblast and glomerular mesangial cell are specialized fibroblasts. The gene that determines the phenotypic expression of both of these phenotypes is peroxisome proliferator activated gamma (*PPARγ*). *PPARγ* agonists such as thiazolidinediones and prostaglandin J_2 (PGJ_2) stimulate *PPARγ*, enabling both lipofibroblasts and mesangial cells to establish or reestablish their normal phenotypic expression, molecularly determining alveolar and glomerular homeostasis. By tracing the signaling pathway for the dedifferentiation of the lipofibroblast, the common "language" of physiologic homeostasis and pathologic dyshomeostasis has been realized.

Introduction

Growth factor signaling is central to homeostasis and dyshomeostasis alike, rendering it a common "language" for both biology and medicine. By determining the patterning of the lung by growth factors and the establishment of homeostasis, we gain an understanding of how and why dyshomeostasis occurs when

Evidence-Based Evolutionary Medicine, First Edition. John S. Torday, Neil Blackstone, and Virender Kumar Rehan

such signaling is disrupted, offering the opportunity for early detection, targeted treatment, and effective prevention of chronic disease.

Role of Growth Factors in Alveolar Homeostasis

Much of what we have learned about the dynamics of growth factors in normal alveolar homeostasis has come from a deep understanding of how fluid distension affects the cell–cell interactions responsible for development, homeostasis, and repair. Jost and Policard [1] had established that the lung produces lung liquid during intrauterine development, distending the alveolar bed and contributing to the production of amniotic fluid. Three decades later it was discovered that the distension of the alveoli plays a critical role in the physiologic timing of normal lung development. Experimentally, draining the amniotic fluid surrounding the fetal sheep caused delay of lung development and lung surfactant production, whereas ligation of the trachea accelerated it. More careful study of this phenomenon revealed the structural effect of fluid distension on the alveolar acinus. Several years later it was shown that distension of alveolar type II cells in cell culture stimulated the expression of parathyroid hormone-related protein (PTHrP), which is necessary for alveolarization – if the *PTHrP* gene is deleted in the embryonic mouse the lung does not form alveoli. It had long been known that the alveolar fibroblasts neighboring the alveolar type II cells became glucocorticoid sensitive during the course of fetal development, expressing the glucocorticoid receptor at roughly 75% of term gestation, but how that occurred remained unknown. It did not occur spontaneously since cultured fetal lung fibroblasts in culture do not develop the capacity to express the glucocorticoid receptor over time. AQ1 On the other hand, *PTHrP* stimulates fetal lung fibroblast expression of the glucocorticoid receptor, inducing the structure and function of the lipofibroblast phenotype, which protects the alveolar acinus against oxidant injury by accumulating neutral lipid. The mechanism for this property is due to lipofibroblast expression of adipocyte differentiation related protein (ADRP), a protein that actively "trafficks" neutral lipid from the

alveolar capillaries to the lipofibroblast, and from the lipofibroblast to the alveolar type II cell for surfactant phospholipid synthesis. During the course of studying this mechanism of neutral lipid trafficking between cultured alveolar type II cells and lipofibroblasts, both in isolation and in coculture, it came to light that there were other functionally related properties of these cells that further explained how neutral lipids were trafficked from the microcirculation to the alveolar type II cell. For example, the lipofibroblasts would avidly absorb neutral lipids in the culture medium but would not secrete them unless they were in the presence of the alveolar type II cells themselves, or their secretions (so-called "conditioned medium"). Using a combination of bioassays and biochemical analysis of the content of the conditioned medium allowed for the identification of the bioactive constituent produced by the alveolar type II cells that causes the secretion of neutral lipid from the lipofibroblast to be prostaglandin E_2. Moreover, the prostaglandin E_2 receptor resides on the surface of the lipofibroblast, where it specifically binds prostaglandin E_2, which actively stimulates neutral lipid secretion by the lipofibroblast. Furthermore, the lipofibroblasts produce leptin, which stimulates the synthesis of both surfactant phospholipid and surfactant protein by alveolar type II cells, providing a mechanistic basis for the coordinate paracrine stimulation of surfactant production mediated by *PTHrP*.

As for the functional significance of these cell–cell interactions mediated by cell-specific mediators of both epithelial and mesenchymal origins, it has come to light that all of them act in a concerted fashion to stimulate surfactant production in response to the distension of the alveolar wall (see Figure 7.2). It had long been known that both in utero during morphogenesis and postnatally for homeostatic control of surfactant production for air breathing that alveolar homeostasis was stretch-dependent, the latter referred to physiologically as ventilation-perfusion matching – the capacity of the alveolus to maintain oxygenation homeostatically by coordinating gas exchange with the flow of blood through the alveolar capillaries. The principle behind this property of the alveolus is the stretch-regulated production of *PTHrP* by the alveolar type II cell, because it determined both the rate of surfactant production and is also a potent vasodilator – the

coordinate stretch regulation of both surfactant production and alveolar capillary perfusion constitute the cellular–molecular elements of ventilation–perfusion matching.

Clinically, the significance of this mechano-transductive physiologic mechanism was determined in both animal and human studies. In ventilated newborn lambs, it was determined that over-distension of the lung inhibited *PTHrP* production, providing experimental evidence for the mechanism of barotrauma, which causes alveolar inflammation and scarring, resulting in the chronic lung disease bronchopulmonary dysplasia (BPD). And clinically, in preterm newborns it was determined that their risk of developing BPD, the chronic fibrotic disease of preterm newborns, *PTHrP* in the lung effluent was significantly lower than in age-matched control infants who did not develop BPD.

The Kidney Glomerulus as a Homolog of the Lung Alveolus

The kidney glomerulus is the site of fluid and electrolyte homeostatic control. The glomerulus is lined by epithelial podocytes that synthesize and secrete *PTHrP* (Figure 5.2). The secreted *PTHrP* binds to its receptor on the specialized fibroblasts that underpin the kidney tubules, called mesangial cells. Fluid distension of the glomerulus stimulates *PTHrP* production, stimulating mesangial secretion of fluid and electrolytes into the kidney tubule. This mechanism is homologous with the effect of air distension of the alveolus, stimulating surfactant production by the alveolar type II cell, maintaining alveolar homeostasis by reducing alveolar surface tension. As an aside, in the womb both the alveolus and the glomerulus produce amniotic fluid. Postnatally, the alveolus and glomerulus act synergistically to maintain allostasis.

It is important to point out that the barotrauma of hypertension can inhibit the *PTHrP* signaling between the podocytes and the mesangial cells, causing glomerular scarring, just as it does alveolar fibrosis due to over-distension of the lung.

Pathologic Consequences of Failed Paracrine Signaling

The consequences of lost paracrine signaling can readily be observed when the target cell-type is propagated in the absence of the signaling that induced its differentiation developmentally. For example, when lipofibroblasts are cultured in the absence of *PTHrP* they revert back developmentally and phylogenetically to their myofibroblastic phenotypic origins. This not only represents "reverse evolution" but also acts as a fail-safe mechanism for the preservation of lung structure and function. Myofibroblasts form scar tissue that physically acts to maintain the integrity of the lung parenchyma in lieu of the physiologic mechanisms that evolved to normally lower alveolar surface tension through the production of lung surfactant.

The same loss of *PTHrP* signaling occurs in the kidney, causing glomerular sclerosis. Damage to the podocytes lining the glomeruli inhibits the production of *PTHrP*. In the absence of *PTHrP* production, the mesangial fibroblasts revert back to their developmental and evolutionary origins as myofibroblasts, maintaining the structure of the kidney tubule using scar tissue instead of the evolved efficiency of the mesangium. The scarring mechanism is less than optimal physiologically, but it allows the organism to survive and ultimately reproduce, whereas in the absence of such a fail-safe mechanism the animal would probably die.

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9

Integrated Regulation of Homeostasis – Vascular, Nervous, Endocrine, Neuroendocrine, Autonomic

Summary

The process of vertebrate evolution chronicles the history of oxidative metabolism. Seen in their present forms, it would appear vertebrates evolved in direct response to metabolic drive. However, seen through the lens of cell–cell interactions, this process is highly robust, the effects of oxygen on ontogeny and phylogeny providing a Rosetta Stone for deciphering evolutionary change. When seen longitudinally as a functionally linked continuum of emergent and contingent processes resulting from the recombination and permutation of genetic traits first expressed in unicellular organisms, a very different picture emerges. It is like doing a crossword puzzle, the answer spontaneously emerging from the algorithm.

Introduction

Aristotle coined the term "entelechy" in the fifth century BCE, suggesting the unity of Nature. This notion was revisited in the twentieth century, LL Whyte proposing a Unitary Biology, but it had no basis in mechanism, so it was untestable. Bohm and Benson have also offered insights into such a unity, acknowledging the underlying problem of our own anthropocentric self-perception. The present chapter is predicated on complex physiology evolving from the cell membrane of protocellular organisms, offering a scientific basis for biology as unicellular,

Evidence-Based Evolutionary Medicine, First Edition. John S. Torday, Neil Blackstone, and Virender Kumar Rehan

multicellularity being an epiphenomenon. This conceptualization is scale-free and predictive.

The following is a systems approach to the problem of biologic unity at several different levels – the gene, the transcript, the protein, the cell, the organ, the organ system, or the population. Evolution impacted biology at all of these levels. There are many such analyses in the literature, but they do not provide a vertically integrated, functional genomic, cell–molecular evolutionary process that renders novel insights into the underlying mechanisms, let alone to further experimentation, and ultimately to predictions. Selection pressure must be applied where it has the appropriate effect for optimal survival, i.e. the level where the genetic expression is functionally integrated with the phenotype. The cohesive "middle-out" evolutionary approach described herein offers the advantage of minimizing a posteriori assumptions by focusing on gene regulatory networks (GRNs). GRNs govern the levels of mRNAs and protein that generate form and function, particularly those that have evolved using the same conserved ontogenetic/phylogenetic, homeostatic, and regenerative cell–molecular motifs.

The process of vertebrate evolution chronicles the history of oxidative metabolism. Seen in their present forms, it would appear vertebrates evolved in direct response to metabolic drive. However, seen through the lens of cell–cell interactions, this process is highly interactive, the effects of oxygen on ontogeny and phylogeny providing a Rosetta Stone for deciphering evolutionary change. When seen longitudinally as a functionally linked continuum of emergent and contingent processes resulting from the recombination and permutation of genetic traits first expressed in unicellular organisms, a very different picture emerges, like doing a crossword puzzle, the answer spontaneously emerging from the matrix.

Evolutionary biology is teleologic and tautologic, subverting its ability to explain the processes involved. Conversely, identifying mechanisms that were exapted from seemingly unrelated ancestral traits is of particular value in avoiding such pitfalls. In this regard, the events surrounding the water–land transition that gave rise to vertebrate adaptation to land are instructive and are highly relevant to human physiology. Moreover, because they provide insight into the emergent and contingent mechanisms underlying endothermy/homeothermy in mammals and birds, they can be reverse-engineered to determine the intermediate physiologic steps in land vertebrate evolution.

Water–Land Transition as the Catalyst for Vertebrate Evolution

Based on the Romer Hypothesis [1], the "greenhouse effect" caused by rising carbon dioxide in the atmosphere drying up bodies of water forced land vertebrates to emerge from water some 400 million years ago. Based on the fossil record, vertebrates breached land on at least five occasions, indicating the magnitude and direction of the selection pressure to "gain ground." Yet despite such knowledge, virtually no attention has been paid to the obligatory evolution of the internal organs necessary for this key transitional period. The glaring gap between such phenotypic observations and the underlying mechanisms of evolution is due to the heavy emphasis on random mutation and selection imposed by Darwinian evolutionists. By contrast, Torday and Rehan have shown the value of determining the cellular–molecular adaptation to oxygenation in forming the mammalian lung through specific cell–cell interactions that determine its embryogenesis mediated by soluble growth factors and their receptors. Such interactions have evolved under alternating external and internal selection pressures, generating form and function. Such cellular–molecular interrelationships refer all the way back to the unicellular state by following the pathways formed by lipids in accommodating calcium homeostasis, and their consequent effects on oxygen uptake by cells, tissues, and organs. Through this a priori understanding of the fundamentals of evolution, the traditional pitfalls of teleology and tautology can be avoided, and instead a predictive model of evolutionary biology can be formulated as follows.

Parathyroid Hormone-Related Protein Signaling Is Key to Understanding the Evolution of the Lung

In the absence of the parathyroid hormone-related protein (*PTHrP*) gene, the lung will not form alveoli. *PTHrP* is synthesized and secreted by the alveolar epithelial type II cell, binding to the surface of neighboring lung fibroblasts via the G protein-coupled PTHrP receptor (PTHrPR). This triggers the intracellular protein kinase A pathway, inducing the lipofibroblast phenotype. AQ1

These cells protect the lung against oxidant injury by actively accumulating and storing neutral lipids. Lipofibroblasts subsequently evolved the capacity to actively provide neutral lipid substrate for lung surfactant phospholipid synthesis. Paracrine signaling from the lipofibroblast to the alveolar type II cell is mediated by the locally acting paracrine hormone leptin, which stimulates lung surfactant synthesis by the alveolar type II cell. These complementary interactive cell–cell interactions facilitate the molecular cross-communication between *PTHrP* and leptin for the mechanically regulated production of surfactant, since *PTHrP*, leptin, and their respective cell-surface receptors are all coordinately stretch-regulated genes. The neutral lipid trafficking process is orchestrated by adipocyte differentiation related protein, which mediates the uptake, storage, and transit of neutral lipid from the lipofibroblast to the alveolar type II cell.

It became evident that this cellular configuration must have resulted from evolutionary selection pressure for specific physiologic functions once these cellular–molecular aspects of the functionally integrated mechanism for homeostatic regulation of lung surfactant was reconstructed, i.e. since the lipofibroblast and alveolar type II cell each took a minimum of 3×10^9 years to evolve the mammalian lung, the probability of this occurring by chance alone would have taken the multiplicative product of the two, which is longer than the existence of the Earth, or the Universe itself for that matter ($>9 \times 10^{18}$ years). Thus, deconvoluting the functional interrelationships between the individual molecular mechanisms involved in their phenotypes lay in how the lung surfactant subserves the alveoli ontogenetically, phylogenetically, and pathophysiologically. To recap, the overarching process of lung evolution is characterized by a progressive decrease in alveolar diameter, which facilitates gas exchange by increasing the surface area-to-blood volume ratio between the alveolus and the alveolar capillaries that transfer oxygen to the peripheral tissues and organs.

The Physics of Lung Evolution

Based on the Law of Laplace, the surface tension of a sphere is inversely proportional to its diameter, as in the case of the alveolus. The composition of the surfactant, and therefore its surface

tension-reducing capacity, has changed progressively to compensate for the increasing surface tension caused by the evolutionary decrease in alveolar diameter based on extensive comparative phylogenetic studies by Daniels and Orgeig [2], begging the question as to what cellular–molecular mechanisms facilitated such accommodations. Developmentally, epithelial–mesenchymal interactions form the basis for alveolar morphogenesis, resulting in surfactant-mediated alveolar homeostasis; so the logical hypothesis was that the epithelial and mesenchymal cells generating the alveoli evolved under positive selection to modify the composition and production of the surfactant, causing both the phylogenetic and ontogenetic decreases in alveolar diameter.

The mammalian lung evolved from the fish swim bladder, which uses gases to regulate buoyancy for feeding efficiency. The swim bladder of physostomous fish is an outpouching of the esophagus, connected to the alimentary tract by the pneumatic duct, which is homologous with the mammalian trachea. For example, at the cellular–molecular level both the pneumatic duct and trachea are formed from smooth muscle controlled by the interaction between Hedgehog and FGF10. Furthermore, the swim bladder is lined by gas gland epithelial cells that synthesize and secrete cholesterol, the most primitive form of lung surfactant. Moreover, *PTHrP* is among the top 50 genes expressed in the developing zebra fish swim bladder. Therefore, the functional homology between the swim bladder and lung can be discerned as the utilization of lipid to facilitate gas exchange. Utilizing cholesterol, the most primitive surfactant to lubricate the inner surface of the swim bladder facilitates buoyancy for feeding on algae, which are 90% lipid. This gas exchange mechanism is functionally homologous with the mammalian lung, utilizing surfactant phospholipids to facilitate gas exchange for efficient metabolism. This is essentially how Francois Jacob famously described evolution as "tinkering" [3]. However, up until now this process has been seen as the chance result of Darwinian mutation and selection, whereas in the present model structure and function have evolved from preexisting cellular–molecular traits, determined by homeostatic changes in growth factor–mediated cell–cell communication. The mechanism of selection remains traditional fitness.

Functional Homology between Membrane Lipids and Oxygenation

These cellular–molecular homologies raise the question as to what atavistic unicellular trait or traits might have formed the basis for the functional interrelationships between membrane lipids and oxygenation. Early in the evolution of unicellular organisms, oxidant stress caused endoplasmic reticulum stress, resulting in the release of potentially toxic levels of stored calcium into the cytoplasm. de Duve, who discovered the peroxisome, hypothesized that it evolved to protect against excess intracellular calcium. This ancient functional relationship between the peroxisome and endoplasmic reticulum stress may form the basis for the ubiquitous effects of peroxisome proliferator activated receptor gamma (PPARγ) in preventing and treating a wide variety of inflammatory diseases. And this same receptor is crucial to longevity for laboratory mice, life span being determined by the same cell–cell communications that evolved to maintain calcium–lipid homeostasis. PPARγ is the nuclear transcription factor that determines the adipocyte phenotype, which protects against oxidant injury. When PPARγ is inhibited, the adipocyte defaults to its atavistic muscle phenotype, characterized by alpha smooth muscle actin (αSMA). The contrast between the adipocyte and muscle phenotypes reprises the seminal role of cholesterol in facilitating the evolution of eukaryotes. As for the evolutionary basis for this relationship, Barbara Wold's research [4] has shown that cultured muscle cells will spontaneously differentiate into adipocytes in 21% oxygen (room air), but not if cultured in 6% oxygen, bespeaking the role of atmospheric oxygen in the origins of the adipocyte phenotype.

Another functional indication for the role of lipid–calcium epistasis in evolution is the homology between the lung and skin. Both organs synthesize and secrete lipid-containing lamellar bodies in combination with host defense peptides to form watertight, antimicrobial "barriers." In the case of the skin, the stratum corneum secretes such an extracellular lipid–antimicrobial barrier. In the case of the alveolus, the alveolar type II cell secretes the surfactant film, termed tubular myelin, a lipid–protein complex composed of phospholipids and surfactant protein A

(an antimicrobial peptide), similar in composition and structure to the lipid barrier formed by the stratum corneum.

So there is a fundamental homology between lipids, antimicrobial peptides, and barrier function exhibited by both the lung and skin. These structural–functional homologies refer as far back in phylogeny as the unicellular state, at which point the cell membranes of eukaryotes were populated by cholesterol. In turn, the advent of cell membrane cholesterol promoted gas exchange, motility, and metabolism, the major evolutionary characteristics of all vertebrates. And since we now have experimental evidence that the unicellular form expresses the complete "toolkit" for multicellular organisms, it is feasible that the lipid-oxygen-barrier homology between the lung and skin evolved from the plasma membranes of unicellular organisms. Experimentally, manipulation of cell membrane cholesterol has shown that increasing the cholesterol content is cytoprotective, whereas loss of membrane cholesterol can cause cell death.

Atmospheric Oxygen, Physiologic Stress, Gene Duplication, and Lung Evolution

The hypothesis to be tested is that visceral organ changes during the water–land transition were caused by physiologic stress. Based on the adaptive changes cited earlier, consider the consequences of episodic fluctuations in environmental oxygen, initially protected against by sterol hopanoids found in prokaryotic bacteria. Mechanistically, oxygen stimulates the SREBP/Scap family of enzymes that regulate sterol biosynthesis in both prokaryotes and eukaryotes, reflecting the ubiquity of this evolved trait. Konrad Bloch [5] had hypothesized that the synthesis of cholesterol was due to the increased availability of atmospheric oxygen, since it takes 11 atoms of oxygen to synthesize one molecule of cholesterol; however, bacteria are devoid of cholesterol, so the oxygen–sterol connection must have some other origin.

Deamer has written extensively on the role of polycyclic hydrocarbons, omnipresent throughout the Universe, in the origins of life. Polycyclic hydrocarbons delivered to the nascent Earth during the heavy bombardment phase in the early history of our solar system were likely to be among the most abundant

and stable organic compounds. The Aromatic World Hypothesis suggests that aromatic molecules might function as container elements, energy transduction elements, and templating genetic components for early life forms. These molecules can stabilize fatty acid vesicles much like cholesterol does in contemporary cell membranes and can foster the biosynthesis of nucleotides.

During the Phanerozoic period, much larger fluctuations in atmospheric oxygen, ranging between 15 and 35% are widely recognized to have caused dramatic increases in animal body size; however, the episodic decreases in oxygen that followed the increases, documented by Berner et al., have been ignored. The resulting effect of hypoxia, the most potent physiologic stressor known in vertebrates, is mediated by the hypothalamic–pituitary–adrenal axis. Pituitary ACTH stimulating corticoid production by the adrenal cortex subsequently stimulates catecholamine production by the adrenal medulla, which is downstream of the cortex in amphibians, reptiles, mammals, and birds. This physiologic mechanism is of evolutionary significance because catecholamines cause surfactant secretion from the lung alveoli. That effect would have acutely relieved the hypoxic stress by further reducing surface tension, consequently increasing the distention of the alveolar wall, increasing oxygenation. That, in turn, would have stimulated alveolar type II cell *PTHrP* production, coordinately increasing both alveolarization and alveolar vascular perfusion. *PTHrP* is both a potent vasodilator, and an angiogenic factor, thus comprehensively promoting the physiologic increase in gas exchange surface area over the course of evolutionary time.

Most importantly, the PTHrP receptor duplicated during the water–land transition, amplifying the PTHrP signaling pathway, thus validating this hypothetical evolutionary mechanism based on empiric evidence. One might wonder why the *PTHrP* receptor gene duplicated at this critical juncture in vertebrate evolution. As mentioned earlier, the visceral adaptive changes occurred in concert with at least five independent skeletal remodelings in the effort to breach land. The success of this mechanism may specifically relate to the *PTHrP* signaling pathway, which directly affects bone mineralization and remodeling. Bone will re-conform structurally in response to physical force, referred to as Wolff's Law. The only known mechanism for

this effect is mediated by *PTHrP*, a gravisensor that regulates calcium uptake and accumulation by bone locally.

Duplication of the β Adrenergic Receptor and the Glucocorticoid Receptor Genes

The other two gene duplications known to have occurred during the water–land transition were the β adrenergic receptor (βAR) and the glucocorticoid receptor (GR), both of which facilitated vertebrate land adaptation. The increase in βARs alleviated the constraint on pulmonary blood pressure independent of systemic blood pressure. The GR evolved from the mineralocorticoid receptor (MR), likely due to the constraint of the orthostatic increase in blood pressure due to the increased force of gravity on land-adapting vertebrates; this was exacerbated by the effect of stress on mineralocorticoid stimulation of blood pressure, now offset by diverting some MR expression to the GR due to the acquisition of three amino acid residues. This combined with the synergistic effect of adrenocortical glucocorticoid production on adrenomedullary βAR production synergized integrated physiology.

Increased *PTHrP* signaling in soft tissues such as the lung during the water–land transition would initially have promoted positive selection for those members of the species adapting to land having relatively higher levels of *PTHrP* to facilitate bone adaptation. Moreover, physiologic stress is known to cause microvascular capillary shear stress, which causes genetic mutations and duplications. Such an effect, particularly on the nascent pulmonary microvasculature, was critical for land adaptation, increased breathing causing stress on the lung microvasulature in particular.

Evolution of Endothermy/Homeothermy as Evidence for the Effect of Stress on Vertebrate Physiologic Evolution

Since there is no "hard" fossil evidence for this sequence of events, it is difficult to argue for this mechanism of evolution, though the functional relationships are consistent with their

contemporary roles in ontogenetically forming and phylogenetically maintaining homeostasis a posteriori. There is also an a priori scenario for the subsequent evolution of these integrated physiologic traits that is internally consistent with both their ontogeny and phylogeny through the advent of endothermy/homeothermy. Since a non-teleologic explanation for the evolution of endothermy/homeothermy has not previously been formulated, by exploiting the above-mentioned gene duplications, a mechanism that entails such preexisting physiologic traits that may conditionally have given rise to endothermy/homeothermy is proposed. In the scenario cited earlier for the selection advantage of catecholamines alleviating the constraint on air breathing, catecholamines would secondarily have caused lipolysis, stimulating the secretion of fatty acids from peripheral fat cells. As a consequence, metabolism would have increased, transiently raising body temperature.

In tandem with the effect of intermittent hypoxia on catecholamine release of fatty acids from fat cells, adrenaline has also been shown to stimulate leptin secretion by adipocytes. Leptin, in turn, has been shown to increase the basal metabolic rate of ectothermic Fence Lizards, consistent with the putative role of adrenaline in the evolution of endothermy.

The increase in body temperature would have synergized with the evolved mammalian lung surfactant, composed of dipalmitoylphosphatidylcholine, which is 300% more active in reducing surface tension at 37 °C than at 25 °C. This effect is due to the elevated phase transition temperature of saturated phosphatidylcholine, the temperature at which the lung surfactant film collapses, no longer acting to reduce surface tension. The selection pressure for the coevolution of dipalmitoylphosphatidylcholine production by the alveoli and endothermy/homeothermy may have been due to the pleiotropic effects of catecholamines, stimulating both surfactant secretion by the alveoli, and coordinately increasing the unsaturated fatty acid composition of peripheral cell membranes, thereby increasing oxygen uptake by increasing membrane fluidity. The progressive phylogenetic increase in the percentage of dipalmitoylphosphatidylcholine in lung surfactant is indicative of the constitutive change in adaptation to endothermy/homeothermy. These fundamental changes in lipid composition in service to metabolism are

exaptations of the events that initiated eukaryotic evolution. Considering the severe conditions generated by Romer's Gap [6], during which vertebrates were virtually wiped off the face of the Earth, it should not come as a surprise that such deep homologies evolved during this existential phase of vertebrate evolution.

Hibernation as Reverse Evolution

The obverse effect of hibernation or torpor on lung surfactant lipid and cell membrane fatty acid composition validates the causal relationship between physiologic stress, catecholamines, and endothermy/homeothermy. Such low physiologic stress conditions lead to diminished catecholamine production, causing both increased surfactant cholesterol content, rendering it less surface active, and lower unsaturated fatty acid content of cell membranes, adaptively reducing oxygen uptake.

Lung surfactant facultatively accommodating ambient temperature is not unprecedented. In a study of Map turtles maintained at different ambient temperatures, it was found that lung surfactant composition changed adaptively. Therefore, the ability to optimize lung alveolar physiology at various environmental temperatures may have been the precursor to endothermy/homeothermy. Empiric evidence for the causal interrelationships between body temperature, surfactant composition, and catecholamine regulation of surfactant secretion supports this hypothesis.

Accommodation of environmental temperature by cholesterol may reflect an exaptation for the fundamental enabling effects of cholesterol at the origins of eukaryotic evolution. Evolution of the alveolar lipofibroblast in mammals is evidence that this is not merely an association. These adipocyte-like homologs, located within the alveolar wall next to alveolar epithelial type II cells that produce surfactant provide a reservoir of surfactant phospholipid substrate for increased surfactant phospholipid production under physiologic demand for oxygen via the stretch-regulated mechanism described earlier. Furthermore, when cholesterol synthesis by alveolar type II cell is experimentally deleted in the developing mouse lung alveolar type II cell by

removing the *Scap* gene, the lung tissue compensates by increasing the number of lipofibroblasts. This epistatic mechanism is apparently due to an increase in PPARγ expression by these cells, resulting from endoplasmic reticulum stress, reprising how peroxisomes initially evolved. Such atavistic traits can be exploited for the diagnosis and treatment of disease, as well as understanding what constitutes "health."

In further support of the hypothesized of intermittent hypoxia as the cause of endothermy/homeothermy, there are other enigmatic changes that occurred during vertebrate adaptation to land that are functionally consistent with this mechanism, offering a narrative for these events: *PTHrP* appears in both the mammalian pituitary and adrenal cortex, thus amplifying the fight-or-flight mechanism; Wurtman has observed the novel appearance of complex vascular arcades in the mammalian adrenal medulla, which amplify the production of catecholamines under stress conditions, as follows. In response to adrenocorticotrophic hormone stimulation, glucocorticoids produced in the adrenal cortex pass down through the vasculature of the adrenal medulla, stimulating the rate-limiting step in catecholamine biosynthesis, phenylethanolamine-*N*-methyl transferase, enhancing adrenaline production for the stress reaction. The expansion of the adrenomedullary microvasculature in association with increased *PTHrP* signaling in the adrenal cortex may not be serendipitous since *PTHrP* is angiogenic, providing a mechanistic explanation for this phenomenon. The coordinate physiologic effects of *PTHrP* on the adrenal cortex and medulla may have facilitated the structural integration of the independent cortical and chromaffin tissues of fish in transition to the amphibian corticomedullary configuration.

It is also feasible that this complex cascade of physiologic stress-mediated cellular mechanisms caused the evolution of the kidney glomerulus, which is virtually absent in fish but is ubiquitous in amphibians, reptiles, mammals, and birds. *PTHrP* is the mediator of fluid and electrolyte balance in the glomerulus, secreted by the epithelial podocyte lining, binding to its receptor on the mesangium, which regulates the amounts of fluid and electrolytes entering the kidney tubules; the distension of the glomerulus is sensed by the podocyte, which then transduces that signal for fluid and electrolyte balance via *PTHrP* signaling

as is the case for the lung. Here too, there arises a functional homology between seemingly structurally and functionally disparate tissues and organs based on descriptive biology, representing the pleiotropic distribution of the same cellular–molecular trait for both breathing and for fluid and electrolyte balance. Since epinephrine inhibits loss of water and salt from the kidney in adaptation to land, this trait may also have evolved under the influence of increased catecholamine production due to physiologic stress.

In further support of this physiologically integrated ontogenetic and phylogenetic scenario for the evolution of land vertebrate physiology, it has been observed that the genome decreased by about 80–90% after the Cambrian Extinction. This phenomenon may have resulted from the onset of endothermy since ectotherms require multiple isoforms for the same metabolic enzyme in order to function at variable ambient temperatures, whereas endotherms largely require only one metabolic isoform to function optimally. This reduction in metabolic enzyme heterogeneity in endotherms would have contributed to the dramatic decrease in post-Cambrian genomic size.

Predictive Power of the Cellular–Molecular Approach to Evolution

The cellular–molecular approach to evolution is highly predictive in comparison to the conventionally dogmatic descriptive view of biology that we have held for thousands of years, starting with the unicellular perspective on the life cycle as the primary level of selection, and the necessity of returning to it as the adaptive strategy for epigenetic inheritance. The recognition that the cell membrane is the homolog for all complex physiologic traits forms the basis for understanding the First Principles of Physiology. By focusing on the mechanistic transition from the unicellular state to the multicellular organism during both ontogeny and phylogeny, such seemingly insoluble properties of life as pleiotropy, the stages of the life cycle, and the aging process can all be understood as one continuous process in service to emergence and contingence.

As an example of the predictive power of the cellular approach to evolution, it has recently been hypothesized that among amniotes, the alveolar lung of mammals may have been the earliest adaptation for land life, subsequently simplifying in snakes and lizards. As interesting as this idea is, there is no mechanistic basis for such speculation. In fact, it runs counter to the ontogeny of the mammalian lung, which begins as simple sacs that become progressively more structurally complex, consistent with the phylogeny of the lung evolving from the swim bladder. It has previously been pointed out that there are systematic errors made in showing associations in evolution without offering a mechanistically causal relationship to environmental factor(s), particularly at the cellular–molecular level in an attempt to determine relationships to other related evolutionary mechanisms, given the complex nature of this process. In that spirit, a hypothetical role of physiologic stress in mammalian lung evolution to other amniotes with "simple" lungs has been applied. The simple sac-like lungs of other amniotes are associated with a lack of an adrenaline response to corticoid-mediated stress due to the fundamental difference in the configuration of the adrenal glands in mammals versus other amniotes; it is helpful here to keep in mind that the fish adrenal is composed of two separate organs for the elaboration of corticoids and catecholamines. In mammals, the adrenal cortex lies on top of the medulla as a separate structure, and the corticoids secreted by the cortex pass down through the medulla, amplifying adrenaline production by stimulating phenylethanolamine-*N*-methyltransferase, the rate-limiting step in adrenaline synthesis. In all other amniotes, the chromaffin cells that synthesize catecholamines are interspersed within the cortical tissue, and the relationship between stress and adrenaline production is not well delineated. Clearly, non-mammalian amniotes evolved different mechanisms to cope with the physiologic stresses of land adaptation, and seemingly as a consequence, their adaptation for breathing as well. The comparators are birds, which have a "stiff" lung composed of large air sacs. The lungs are attached to the dorsal wall of the thorax during embryogenesis. Furthermore, air entering the lung flows in only one direction, unlike the reciprocating nature of the mammalian lung, indicating a fundamentally

different way of adapting to air breathing in birds. Embryonic alligators also exhibit the attachment of the lung to the chest wall during embryogenesis (personal observation), and in the adult (Thomas Owerkowicz, personal communication) in association with unidirectional air flow, in further support of the speculation that the fixing the lung to the chest wall during development is in service to the unidirectional flow of air. This supposition is further supported by the fact that birds have blood glucose levels 10–15 times higher than mammals, suggesting that instead of secreting fatty acids from fat stores in response to adrenaline for metabolic "fuel" on an "as-needed" basis via the fight-or-flight mechanism used by mammals, birds are constantly in a "metabolically on" mode.

Moreover, it is noteworthy in the context of metabolic evolution that both birds and humans are bipedal, which may have been a consequence of their both being endotherms. Being upright is metabolically costly, but by increasing their body temperatures in adaptation to land, both birds and humans have become much more metabolically efficient – cold-blooded organisms require multiple isoforms of the same metabolic enzyme to survive at ambient temperatures, whereas endotherms usually have only one isoform. Bipedalism may have resulted, freeing the forelegs to evolve into wings and hands with prehensile thumbs through common genetic motifs.

The hypothesized evolutionary physiologic interrelationship between stress, metabolism, and endothermy may underlie the effect of meditation on hypometabolism. It has long been known that Yogis have the capacity to regulate their metabolism at will, and formal study of this phenomenon has validated it scientifically. Functionally linking to ever-deeper principles of physiologic evolution through meditation and bio-feedback may prove to be of wider benefit in healing, both conventional and self-healing alike.

Conclusions

By focusing on the necessity and utility of lipids in initiating and facilitating the evolution of eukaryotes, a cohesive evolutionary strategy becomes tenable. In fostering metabolism, gas exchange,

locomotion, and endocytosis/exocytosis, cholesterol in the cell membrane of unicellular eukaryotes formed the basis for what was to come. The basic difference between prokaryotes and eukaryotes is the soft, compliant cell membrane of the latter, interacting with the external environment, adapting to it by internalizing it using the endomembrane system as an extension of the cell membrane. This iterative process was set in motion by competition with prokaryotes, which can emulate pseudo-multicellular behaviors like Biofilm and Quorum Sensing. All of the examples cited in this paper – peroxisomes, the water–land transition, lipofibroblasts, endothermy/homeothermy – are functional fractals of the originating principle of lipids in service to the evolution of eukaryotes.

Following the course of vertebrate physiology from its unicellular origins instead of its overt phenotypic appearances and functional associations provides a robust, predictive picture of how and why complex physiology evolved from unicellular organisms. This approach lends itself to a deeper understanding of such fundamentals as the First Principles Physiology. From these emerge the reasons for life cycles and why all organisms always return to the unicellular state, pleiotropy, and homeostasis. A coherent rationale is provided for embryogenesis and the subsequent stages of life, offering a context in which epigenetic marks are introduced to the genome.

From the beginning of life, there has been tension between calcium and lipid homeostasis, alleviated by the formation of calcium channels by exploiting those self-same lipids, yielding a common evolutionary strategy. The subsequent rise in atmospheric carbon dioxide, generating carbonic acid when dissolved in water, caused increased calcium leeching from rock. Calcium is essential for all metabolism, and it is through calcium-based mechanisms that the inception of life is marked by a calcium spark kindled by sperm fertilization of the ovum, a process that sustains the processes of life until the time of death; perhaps the aura that near-death experiences have chronicled is that very same calcium spark.

A cohesive, mechanistically integrated view of physiology has long been sought. LL Whyte described it as unitary biology, but

the concept lacked a scientifically causal basis, so it remained philosophy. But with the advent of growth factor signaling as the mechanistic basis for molecular embryology in 1978, Whyte's vision of a singularity may now be realized.

Throughout this chapter, the contrast between conventional descriptive physiology and the deep mechanistic insights gained by referring back to the epistatic balance between calcium and lipids, mediated through homeostasis, has been highlighted. It is emblematic of the self-organizing, self-referential nature described for the origin of life itself. Using this organizing principle avoids the perennial pitfalls of teleology, conversely providing a way to resolving such seeming dichotomies as genotype and phenotype, emergence and contingence, unicellular organisms, and vast multicellular organisms. Insight into the fundamental interrelationship between calcium and lipid homeostasis was first chronicled in *Evolutionary Biology, Cell–Cell Communication and Complex Disease* [7]. Further research will solidify the utility of focusing on the advent and roles of cholesterol in eukaryotic evolution, extending from unicellular to multicellular organisms and provide novel insights into the true nature of the evolutionary continuum in an unprecedented predictive and reproducible manner.

This understanding of the "how and why" of evolution provides the unprecedented basis for a central theory of biology, which is long overdue. Many have given up on the notion of a predictive model for biology akin to those for chemistry or physics. This is largely due to the failure to realize that contemporary biology is descriptive, i.e. that describing a mechanism is not the same as actually determining causation based on founding principles, such as quantum mechanics and relativity theory. This may seem surprising in the wake of the publication of the human genome, which is only 19% of the predicted size. That alone should have generated criticism of the prevailing way in which biology is seen as a *fait a complete*, characterized by correlations and associations. John Ioannidis has declared that "'most published research findings are false.". This may be because we are using a descriptive framework, which generates associations and correlations, but ultimately will not allow for predictions.

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10

Endogenous and Exogenous Mechanisms for Healing

Summary

The key to understanding evolution as the integration of ontogeny, phylogeny, and injury-repair is through homeostasis. Homeostasis is the culmination of the process of cell–cell signaling for morhogenesis during embryonic development; as such, homeostasis acts to monitor changes in the cellular environment, both locally between cells and at the organismal level for all cells as allostasis. Relatively small changes can be compensated for by modulating key steps in any given physiologic trait; larger fluctuations may cause damage to the pathways involved both within and between cells. Such injuries are compensated for by scarring, or fibrosis, characterized by the default of differentiated interstitial fibroblasts to myofibroblasts, and the laying down of extracellular matrix in a wide variety of tissues, ranging from the lung to the kidney, liver, skin, vasculature, and brain. Key to understanding these properties is that the endodermal cells produce factors that down-regulate the myofibroblast phenotype, up-regulating the differentiated fibroblast phenotype. The hallmark of the myofibroblast is the expression of the Wingless/int or Wnt pathway and the expression of alpha-smooth muscle actin.

These mechanistic interrelationships for monitoring and rectifying homeostasis are scalable from individual cells to tissues, organs, and organ–organ interactions. The premise of this chapter is that the mechanisms of injury-repair are based on evolutionary

Evidence-Based Evolutionary Medicine, First Edition. John S. Torday, Neil Blackstone, and Virender Kumar Rehan

cellular–molecular principles of development and phylogeny. This insight can be used to effectively predict, prevent, and treat chronic disease.

Introduction

The key to understanding evolution, ontogeny, phylogeny, and injury-repair is homeostasis. Homeostasis acts to monitor changes in the cellular environment, both locally between cells and at the organismal level as allostasis. Relatively small changes in homeostasis can be compensated for by modulating key steps in the cellular–molecular pathways that determine homeostasis for any given physiologic trait; larger fluctuations may cause damage to the pathways involved, both within and between cells. Such injuries must be compensated for by scarring, or fibrosis, i.e. the default of interstitial fibroblasts to a myofibroblasts in a wide variety of tissues, ranging from the lung to the kidney, liver, skin, vasculature, and brain. The premise of this chapter is that the mechanisms of injury-repair are based on evolutionary cellular–molecular principles of development and phylogeny.

Endogenous Mechanisms for Healing

A Fine Homeostatic Balance between the Differentiated Interstitial Fibroblast and the Myofibroblast

The Wingless/int (Wnt) signaling pathway constitutes a large family of highly conserved, secreted glycoproteins, which function as growth factors that are essential to organogenesis. The canonical Wnt proteins bind to frizzled receptors, causing β-catenin inhibition and subsequent transcription of *TCF/LEF* target genes. Genes in the Wnt signaling pathway regulate cell fate and differentiation during embryogenesis, modulate cell proliferation, and are involved in homeostatic functions in adult tissues [1–3]. To date, 19 Wnt proteins have been identified in humans with a vast array of biologic functions allowing for

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redundancy in the pathway with compensation for the loss of certain Wnt ligands by other genes in the pathway.

Parathyroid hormone-related protein (PTHrP) expression is necessary for differentiation of mesenchymal lipofibroblasts, which induce epithelial type II (TII) cell differentiation, both of which are necessary for alveolarization. PTHrP deficiency may be associated with bronchopulmonary dysplasia (BPD), characterized by truncation of alveolarization among preterm infants. This is supported by the baboon model of BPD (failure of alveolarization) that manifests PTHrP deficiency. We provide evidence that TII cell PTHrP expression is down-regulated by alveolar over-distension, resulting in the transdifferentiation of lipofibroblasts to myofibroblasts, characterized by progressive loss of PTHrP receptor expression and triglyceride content, and sequential up-regulation of alpha-smooth muscle actin (αSMA), typifying fibrosis. PTHrP reverses the down-regulation of the PTHrP receptor and up-regulation of αSMA, reverting myofibroblasts to a lipofibroblast genotype. When TII cells are cocultured with lipofibroblasts, they proliferate and differentiate, expressing surfactant protein-B; in contrast, TII cells cocultured with myofibroblasts fail to develop, mimicking the failed alveolarization associated with BPD. Treatment of myofibroblasts with 15-deoxy-delta 12, 14 prostaglandin J(2) [PGJ(2)] stimulates adipocyte differentiation related protein (ADRP) expression, reconstituting the lipofibroblast phenotype. PGJ(2)-treated myofibroblasts promote TII cell growth and surfactant protein-B expression, indicating that failed alveolarization due to transdifferentiation is reversible. We conclude that alveolar overdistension can cause fibroblast transdifferentiation, resulting in failed alveolarization.

Similarly, PTHrP is produced by the endodermally derived podocytes that line the glomerulus. During kidney development PTHrP induces the mesenchymally derived mesangial cell phenotype by differentiating the myofibroblasts that surround the microvasculature of the kidney tubules. The mesangium mediates the flow of protein and electrolytes from the glomerular space into the tubules. Like the role of PTHrP in the alveolus in stretch-regulation of surfactant to prevent atelectasis, PTHrP in the glomerulus is also stretch-regulated by the fluid in the glomerulus, determining the volume of kidney filtrate that passes

out of the kidney. Over-distension of either the alveolus or glomerulus results in down-regulation of PTHrP, causing loss of both lipofibroblast and mesangial cell differentiation, defaulting to myofibroblasts.

Universality of Wnt/β-catenin in Myofibroblast Proliferation and Scarring: DKK, Shh, Alphabet Soup

By tracing the signaling pathway for the dedifferentiation of the lipofibroblast, the common "language" of physiologic homeostasis and pathologic dyshomeostasis can be realized [4]. The lipofibroblast phenotype is induced by PTHrP signaling from the alveolar type II cell during embryonic development. This pathway is triggered by fluid distension of the alveoli, stretching the alveolar wall and up-regulating PTHrP expression by the endodermal epithelial cells. The PTHrP binds to its cognate receptor on mesodermal cells, stimulating cyclic adenosine monophosphate (cAMP) production, which inhibits the Hedgehog-Wnt pathway, composed of patched, frizzled, disheveled, and glioblastoma, the latter being inhibited by cAMP. The same cascade occurs in the glomerulus, causing failed glomerular filtration of fluids and electrolytes.

Pathologically, elements of the Sonic Hedgehog (Shh) have been detected in chronic lung disease, liver fibrosis, and glomerular sclerosis. Thiazolidinediones (TZDs) have been used to either prevent, palliate, or cure both chronic lung and kidney disease.

Prostanoids, Homeostasis, and Regeneration

Lipids form the basis for ancestral vertebrate evolution [5], initially forming lipid-based micelles, or primitive cells when suspended in water. Subsequently, they evolved to regulate the concentration of calcium in the cytoplasm, to form lipid rafts for paracrine cell–cell signaling, and ultimately the endocrine system itself. Prostaglandins are lipid derivatives that are important in cellular homeostasis. Among them is prostaglandin E_2 (PGE_2), which regulates endogenous lipid metabolism. In particular, it is one of the paracrine mechanisms that determines lung-surfactant lipid homeostasis within the alveolus.

Study of the regulation of lung surfactant in isolated and recombined epithelial type II cells (TII) and lipofibroblasts (LIFs) in cell culture has revealed the necessity for physiologic interactions between these cells for the maintenance and reestablishment of homeostasis. PTHrP production by the TII cells stimulates the uptake of triglycerides (TGs) by the LIFs, mediated by ADRP. Release of the TGs by the LIFs is mediated by PGE_2 production by the TIIs. Each of these effectors of the neutral lipid trafficking of TGs as substrate for surfactant production is mediated by their cognate receptors on the surface of the target cell, up-regulating a second-messenger signaling cascade, namely the PTHrP receptor on the LIF, the leptin receptor on the TII cell, and the PGE_2 EPII receptor on the LIF.

Interruption of any or all of these cell–cell interactions causes loss of the LIF phenotype, resulting in ontogenetic/phylogenetic regression back to the myofibroblast phenotype, constituting loss of the evolved homeostatic control of the alveolus mediated by lung surfactant, resulting in mesenchymally mediated fibrosis. The loss of the LIF phenotype is due to decline and loss of peroxisome proliferator activated receptor gamma (PPARγ), which is necessary for the LIF Phenotype. Accordingly, the key to the reconstitution of the LIF phenotype is the restoration of PPAR**γ** expression. Sime et al. [6] have also shown that PGE_2 protects the lung fibroblast against transforming growth factor beta (TGFβ)-induced lung fibrosis, likely through the same homeostatic mechanism of action. However, the latter study did not attribute the cytoprotective effect to an evolved physiologic property of PGE_2 paracrine signaling. Garrison et al. [7] have similarly shown rescue of the myofibroblast phenotype by treatment with exogenous PGE_2, corroborating its role in maintaining and sustaining the LIF phenotype.

PGJ(2)

PGJ(2) [15-deoxy-delta 12,14- PGJ(2)], an endogenous ligand of PPARγ, has multiple cellular functions. Among these, it has been found to accelerate fetal lung development and prevent the deleterious effect of nicotine on asthma. In both cases, the effect of PGJ(2) is mediated by PPARγ, establishing the physiologic significance of this prostanoid in the homeostatic control of the lung alveolus.

The interrelationship of neutral lipids and alveolar homeostasis runs deep in vertebrate ancestral evolution, harkening back to the advent of cholesterol in response to the rising levels of oxygen in the atmosphere. Konrad Bloch, the discoverer of the cholesterol synthetic pathway, had hypothesized that since it requires 11 atoms of oxygen to synthesize one molecule of cholesterol, that the latter was a "molecular fossil," reflecting the rise in atmospheric oxygen.

The utilization of cholesterol by unicellular eukaryotes marked the beginnings of vertebrate evolution. The insertion of cholesterol into the cell membrane thinned it out due to the physicochemical effects of cholesterol on its phospholipid elements. This resulted in increases in gas exchange, metabolism, and locomotion (cytoplasmic streaming), the three keys to vertebrate evolution [8]. This cardinal relationship between lipids and membrane structure-function likely evolved from bacteria, since they also respond to oxygen by increasing sterol production – in both prokaryotes and eukaryotes, oxygen stimulation of sterols is mediated by hypoxia inducible factor-1 (Hif-1) [9], bespeaking the evolutionary homology between these processes. The role of lipids in vertebrate evolution subsequently fostered cell–cell signaling by forming the cell-surface lipid rafts where receptor-mediated cell–cell signaling resides. And de Duve has hypothesized that peroxisomes evolved to protect the cell from rising oxygen in the atmosphere by buffering the cytoplasmic calcium released from the endoplasmic reticulum due to stress, reprising the use of lipids as antioxidants.

That mechanism ultimately evolved into the differentiation of myofibroblasts into LIFs in the mammalian lung, protecting the alveolus against oxidant injury yet again. In so doing, it also facilitated the coordinate uptake and transit of neutral lipids from the LIF to the TII for lung surfactant production under control by stretch, or ventilation–perfusion (v/q) matching. This stretch-regulated mechanism for on-demand surfactant production was critical for adaptation to air breathing. It is the result of coordinate stretch stimulation of the PTHrP–PTHrP receptor, leptin–leptin receptor, and PGE_2–PGE_2 receptor on the TII and LIF, accordingly.

Damage to any or all of these elements of the v/q matching mechanism for lung-surfactant homeostatic control results in

loss of structure and function of both the TII and LIF to one degree or another, depending upon the duration and severity of the injury involved. It minimally causes default of the LIF to its myofibroblast origins, developmentally and phylogenetically, resulting in decreased gas exchange due to thickening of the alveolar wall and loss of surfactant production. In its most severe form, it causes atelectasis and "simplification" of the alveolar bed, or chronic lung disease. In its most extreme clinical form as emphysema, the lung parenchymal histology appears to be more like that of a frog, with large alveolar-like faveolae lined by muscle instead of LIFs. At the molecular level, the PTHrP signaling pathway is supplanted by its Wnt/β-catenin antecedent, both developmentally and phylogenetically. It has been shown experimentally that this reversion to an earlier developmental/phylogenetic form of the lung can be predicted and prevented using PPARγ agonists such as PGJ_2 and Rosiglitazone, exploiting the evolutionary basis for physiologic homeostasis.

ApoE4

Apolipoprotein E (ApoE) is an apolipoprotein found in chylomicrons and intermediate-density lipoproteins. It is necessary for normal catabolism of triglycerides. Systemically, ApoE is produced by the liver and macrophages, and it mediates cholesterol metabolism. In the central nervous system ApoE is produced by astrocytes, acting to transport cholesterol to neurons mediated by ApoE receptors. It has been implicated in Alzheimer's disease and cardiovascular disease.

ApoE is polymorphic, having three major alleles – ApoE-ε2, ε3, and ε4. These alleles differ from one another by one or two amino acid residues at positions 112 and 158. Such modifications affect the structure and physiologic function of ApoE.

ApoE ε2 binds poorly to cell surface receptors, whereas ε3 and ε4 bind tightly. ε2 is associated with risk of atherosclerosis. ε3 is not known to be associated with any disease, whereas ε4 has been implicated in a wide variety of pathologies, ranging from atherosclerosis, Alzheimer's disease, decreased cognition, reduced hippocampal volume, cerebrovascular disease, telomere shortening, and reduced neurite outgrowth. An apparent clinical benefit of ε4 is its association with higher levels of vitamin D.

Physiologically, ApoE ferries lipoproteins, fat-soluble vitamins, and cholesterol around the body via the circulatory system. It is primarily synthesized in the liver, along with smaller amounts in the brain, kidneys, and spleen. In the nervous system, non-neuronal astrologia and microglia are the primary sites of ApoE production, whereas the neurons express the receptors for ApoE. There are seven known mammalian receptors for ApoE that belong to the evolutionarily conserved low-density lipoprotein (LDL) receptor family.

Initially, ApoE was identified with lipoprotein metabolism and cardiovascular disease. ApoE abnormalities result in dys-beta-lipoproteinemia, or type III hyperlipoproteinemia, resulting in elevated plasma cholesterol and triglyceride levels due to decreased chylomicron, very low density lipoprotein, and low-density lipoprotein remnant clearance.

ApoE has also been implicated in immunoregulation, including suppression of T cell proliferation, macrophage function regulation, presentation of lipid antigen to natural killer T cells, and modulation of inflammation and oxidation.

ApoE polymorphisms have provided the major rationale for identifying risk for late-onset Alzheimer's disease, and prediction of recovery of cognitive function post-brain injury. However, Guerrant's research group [10] have found that Apoε4 protects children in the developing world against diarrheal impairment of growth, cognition, and school performance. In the aggregate, the pleiotropic over-expression of Apoε4 in the gut prevents children against diarrheal disease but increases the adult incidence of Alzheimer's disease. Therefore, Apoε4 increases reproductive success at the "expense" of late-life morbidity and mortality.

Evolutionary versus Traditional Medicine

Needless to say, recognizing the evolutionary principles behind the maintenance of physiologic homeostasis and the pathophysiologic consequences of their loss is advantageous. It offers ways to objectively determine if the individual is healthy based on homeostatic principles, and conversely it offers preemptive means of preventing the loss of homeostasis, and diagnosing and treating disease based on molecular indicators characteristic of

loss of homeostatic control. Such a continuum of health and disease instead of health in the absence of disease and vice versa is far more desirable than having to rely on "signs and symptoms" because by the time such hallmarks of disease appear, damage has already been done. Clearly, the earlier loss of homeostasis can be detected, and the more sensitive and specific its measure, the more effective the intervention will be in terms of medication and time for healing. Such a holistic approach to medicine is far superior to "personalized medicine," which is merely a more accurate ex post facto "guess" as to the nature and treatment of disease. Moreover, the cellular–molecular approach would alleviate the iatrogenic side-effects of medications by specifically targeting the cause of disease.

Exogenous Mechanisms for Healing Using Evolutionary Principles

Summary

Given that PPARγ agonists, statins and target of rapamycin (TOR) agonists all prevent or heal fibrosis, we will focus on these classes of compounds as those that maintain or reconstitute homeostasis.

Cholesterol and Homeostasis

The cholesterol biogenetic pathway was discovered by Konrad Bloch in the 1950s. He had hypothesized that it was a "molecular fossil" since it takes 11 atoms of oxygen to synthesize one molecule of cholesterol.

Cholesterol was key in the evolution of eukaryotes. Its insertion into the phospholipid bilayer facilitated metabolism, respiration, and locomotion because it thinned the cell membrane. Metabolism, respiration, and locomotion are the three most important features of vertebrate evolution.

Pathophysiology of Hypercholesterolemia

Elevated levels of circulating cholesterol have been associated with atherosclerosis for many decades. Specific forms of circulating

cholesterol, namely LDL cause damage to the endothelial lining of blood vessels, whereas high-density lipoproteins appear to be beneficial.

Statins as Anti-Inflammatory Agents

Statins inhibit cholesterol synthesis, lowering circulating levels of cholesterol. Since elevated cholesterol leads to oxidized lipids that are toxic to endothelial cells, the reduction in cholesterol levels has had a ubiquitous effect on such diseases as atherosclerosis, coronary heart disease, osteoporosis, and rheumatoid arthritis. This plethora of benefits from the reduction in circulating cholesterol comes as no surprise when considered in light of the importance of cholesterol in vertebrate evolution, beginning with its role in the cytoprotection of the unicellular eukaryotic cell membrane.

Given the fundamental importance of cholesterol in vertebrate evolution, it is no wonder that hypercholesterolemia has such devastating effects on physiology, and that statin lowering of serum cholesterol has such ubiquitous beneficial effects on health.

PPARγ and Homeostasis

Peroxisomes were discovered by Christian de Duve as a way for vertebrates to cope with oxidant stress. Although the evolutionary origin of peroxisomes is controversial, they were critical in the ability of unicellular eukaryotes to survive oxidant injury. As oxygen levels in the atmosphere rose, they caused endoplasmic reticulum stress, releasing calcium into the cytoplasm. In response, the cell formed or adopted peroxisomes, which "buffer" the excess calcium in the cytoplasm using neutral lipids. Since PPARγ agonists induce the formation of peroxisomes, it is this ancient atavistic mechanism for the prevention of oxidant injury that has ubiquitous effects in preventing inflammatory diseases ranging from the lung to the gut to the liver to the brain.

Lung Fibrosis

The fundamental principle involved in alveolar fibrosis is the interstitial fibroblast phenotype. Myofibroblasts dominate this structure phylogenetically in the swim bladder, as well as the lungs of amphibians and reptiles. The mammalian lung possesses

lipofibroblasts, which play an active role in protecting the alveolar epithelial cells against oxidant injury, and in transporting neutral lipid from the microcirculation to the alveolar epithelial type II cells for surfactant production. During lung development, PTHrP produced by the alveolar epithelial type II cell induces the differentiation of the myofibroblast to the lipofibroblast by inhibiting the Wnt/β-catenin pathway, resulting in cAMP production, inducing the lipofibroblast phenotype, characterized by ADRP, which is necessary for the uptake and storage of neutral lipid.

Injury to the alveolar epithelial type II cell in the form of infection, oxidant injury, or physical trauma causes loss of PTHrP production, leading to dedifferentiation of the lipofibroblast to a myofibroblast, or fibrosis. Treatment of the lung with a PPARγ agonist will either prevent or reverse the loss of the lipofibroblast phenotype, thus preventing lung fibrosis.

Gut Fibrosis

Intestinal fibrosis commonly occurs as a complication of inflammatory bowel diseases such as Crohn's Disease and ulcerative colitis. Three out of every four Crohn's patients undergo corrective bowel surgery for intestinal strictures. A recent study had shown that once intestinal fibrosis is initiated it is self-propagating. Therefore, anti-fibrotic treatment might be effective in preventing fibrotic disease. Fibrosis is the result of local chronic inflammation, resulting in the deposition of extracellular matrix (ECM) proteins. Such ECM proteins as collagen and fibronectin are produced by myofibroblasts, the key effectors of intestinal fibrosis. MYFs also produce excess αSMA fibers that render the cells more contractile. The fibrogenic activation of MYFs AQ2 is determined by both mechanical stress combined with the numerous cytokines (interleukins, PDGF, prostanoids, etc.), the most powerful being TGFß. TGFß-induced ECM and αSMA are modulated by Smad-dependent and Smad-independent TGFß signaling pathways. Smad-dependent TGFß signaling is transduced by phosphorylation of Smad2 and Smad3, in combination with Smad4. Non-Smad dependent TGFß signaling is mediated by phosphorylation of extracellular signal regulated kinase, or ERK, c-Jun N-terminal kinase, p38 mitogen-activated protein kinase, Akt and myosin light chain 2, or Rho signaling.

Previous studies have shown that PPARγ agonists such as TZDs have anti-fibrogenic properties in several tissues, including the lung, skin, kidney, eye, and heart ( ). The PPARγ agonists prevent or inhibit fibrogenesis by regulating both Smad-dependent and Smad-independent TGFß signaling pathways. Studies of the efficacy of TZDs for the prevention and treatment of inflammatory bowel disease are ongoing ( ).

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TOR and Homeostasis

The *TOR* gene is central to homeostatic control of a host of cellular metabolic functions ranging from oxygen to nitrogen, ions, heavy metals, and other biological entities (bacteria). The *TOR* gene was discovered in response to exposure to rapamycin and was found to have a multitude of pro-homeostatic effects.

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11

Systems Biology as Recapitulation of Ontogeny and Phylogeny

Summary

Evolutionary systems biology is devised to determine mechanisms of evolution that are testable, integrated, and dynamic. To date that systems focus is predicated on a search for multidimensional fitness landscapes that might predict fitness changes caused by transitions between potential states of individuals and/or their environments, and evolutionary paths of changing populations of such individuals as they travel through state-space.

However, the only way that true progress can be made in systems biology would be to distance ourselves from selection as the only significant driver in evolutionary systems, and replace our notions about space-time in the macro sphere with a cellular stance.

Introduction

The purpose of evolutionary systems biology is to devise mechanisms of evolution that are testable, integrated, and dynamic. However, to date, that systems focus is predicated on a search for multidimensional fitness landscapes that might predict fitness changes caused by transitions between potential states of individuals and/or their environments, and evolutionary paths of changing populations of such individuals as they travel through state-space.

Evidence-Based Evolutionary Medicine, First Edition. John S. Torday, Neil Blackstone, and Virender Kumar Rehan

However, the only way that true progress can be made in systems biology would be to distance ourselves from selection as the only significant driver in evolutionary systems, and replace our notions about space-time in the macro sphere with a cellular stance. Space-time was first revised in physics by acknowledging that the Universe evolved from the Big Bang, the products of that explosion emanating from a point source offering the opportunity to understand the origins of stars, planets, and black holes as a continuum rather than as isolated anecdotal descriptions of how and why such elements of the Cosmos came into existence. A "reset" for the conventional biological view of space-time placing evolutionary development on a continuum with physics using the concept of "biological relativity" drew an analogy between the Big Bang as a point source for cosmic development and the continuous recapitulation of the zygotic unicellular form as its own biologic point source for eukaryotic development.

By viewing biological development and evolution as continuously emanating from the unicellular state, proceeding though iterative maintenance of cellular homeostatic equipoise, insights can be gained for understanding the origins and fate of life.

A Paradigm Shift in Evolution

Decades studying lung development centered on surfactant biology have allowed us to trace the arc of gas exchange from the unicellular origins of life to the mammalian lung. The recognition that cholesterol facilitated vertebrate physiology, enabling primitive unicellular metabolism, respiration, and locomotion is the fundament of vertebrate evolution, bearing in mind that the effects of cholesterol on these properties of the cell membrane are the result of its increased compliance, which is homologous with its later exaptation as the most primitive lung surfactant. The trajectory of multicellular eukaryotic development from the assembly of the first cell forward as a self-referential entity grants a consistent evolutionary narrative. This is based on its primary feature: the distinction of the external and internal environments using a semipermeable membrane. From that point, through serial exaptations, fully functioning cell membranes

containing cholesterol, linked to lipid rafts, become the basis for the endocrine system. The mechanistic basis for complex physiology is thus understood as a path requiring cellular solutions to environmental stresses disciplined by selection. But equally importantly, it offers the opportunity to formulate testable hypotheses untenable by descriptive biology. For example, Bloch [1] hypothesized that the synthesis of cholesterol occurred because of the rising levels of oxygen in the atmosphere, leading to his discovery of its biosynthetic pathway. Yet, assuming that cholesterol is necessary, the "solution" to oxidant stress is reasoning after the fact. On the other hand, if evolution is seen as a continuum of interlinked exaptations from the earliest protocell forward, there would have been an earlier event in which lipids were used to cope with the environment as a necessary foundational scenario for the first instantiation of life. That passage requires lipids forming micelles to distinguish between the internal and external milieus. By viewing this progression in the forward direction, the arc of vertebrate evolution from the first protocell to complex physiology can now be understood as a causally linked chain of events.

This a priori way of thinking about vertebrate evolution can therefore be seen as predictive, and as a more effective tool than a posteriori Darwinian evolutionary deconstructions that are descriptive and have not proven to be predictive. In that vein, in a series of publications, it has been shown that many dogmatic assumptions about such biologic traits as the cell, the life cycle, homeostasis, heterochrony, and pleiotropy can all be understood in mechanistic terms that are internally consistent with one another, scale-free, and predictive. Each is based upon serial exaptations from the unicellular state forward.

With such a backdrop, the impact of the physiologic stresses of the water–land transition on vertebrate evolution – including the necessary adjustment to terrestrial gravity, the need to breathe air, and the requirement for barriers against ion and water loss – can all be understood as mechanistic exaptations, leading to endothermy as cellular accommodations for these new stresses. In a similar manner, other aspects of vertebrate evolution such as the vertical integration of bipedalism, specialization of the forelimbs, and the advent of higher consciousness in birds and mammals can be predicted through cellular

mechanisms based on scientific evidence. Indeed, the arc from self-organization, negentropy, and homeostasis forming the protocell vertically integrates the "consciousness" of the physical environment with what we think of as biologic consciousness, both structurally and functionally.

Ernest Rutherford famously said that "All science is either physics or stamp collecting" [2], and because no one has yet been able to explain the scientific interrelationship between physics and biology, biology has been castigated as a "lesser" science. Now, however, we can understand the interrelationship between physics and biology, but only when and if the latter transitions to a mechanistically based science. Short of that major paradigm shift, we continue to delude ourselves into thinking that we understand the underlying nature of biology. The epitome of this paradox is the failure to predict the size of the human genome; the realization that we hominids have only about 19 000 genes instead of the predicted 100 000-plus genes that would have been expected based on analogy with the genomes of what are thought of hierarchically as less complex organisms.

Endothermy as "Proof of Principle" for the Evolution of Serial Exaptations

The predictive nature of the cellular–molecular approach to physiologic evolution is underscored by considering the emergence of endothermy. This adaptation occurred in the context of the physiologic stress caused by the water–land transition, brought about by the rising levels of atmospheric carbon dioxide causing a greenhouse effect. Numerous prior attempts to breach land based on the fossil record are hypothesized to have caused the evolution of endothermy – the evolving lung would have periodically been inefficient for gas exchange, causing hypoxic stress that stimulated the hypothalamic–pituitary–adrenal axis increase, thereby alleviating the oxygenating constraint on the lung by stimulating alveolar surfactant production, increasing the distensibility of the alveoli. In tandem, catecholamines would have caused lipolysis, releasing fatty acids from peripheral adipocytes, which led to increased metabolism, and thus

increased body temperature. Both lung and adrenocortical physiology are exaptations of lipid metabolism, and the fact that this facilitated the internal regulation of body temperature can be seen as a continuation of the cellular imperative to circumvent the Second Law of Thermodynamics.

Endothermy Defies Physics, Fostering Migration

Arguably, endothermy gave rise to bipedalism, specialization of the forelimbs and higher consciousness in birds and mammals. This chain of events is consistent with a paradigm shift in the way phenotype should be considered. Instead of being seen as merely the description of the consequences of reproduction, phenotype is an efficient mechanism for maximizing the acquisition of epigenetic marks passed on to the primary zygotic unicellular form.

Therefore, it should not be totally surprising that higher consciousness, as its own form of phenotype, is its impulse for the phenotypic engagement of macroorganisms with a complex outward environment that requires continuous adjustment. In support of this supposition, *DRD4*, the "risk taking gene" has been implicated in human migratory habits that have led to the global dispersal of hominins instigated by climate change.

The book *American Mania: When More Is Not Enough* [3] makes the case for the dopamine receptor DRD4-7 being the cause for primate migration out of Africa because it is associated with risk taking. At the time of the migration, the world was significantly colder than it is now. Based on geological evidence, these predominating cold conditions lasted until about thirteen thousand years ago when the world began to experience significant warming periods. Prior climatic conditions fostered intermittent bursts of widely varying temperatures that included warm periods interspersed with other eras when glaciers covered North America, and arctic conditions came and went in rapid succession. During this period, land masses were interconnected by bridges, facilitating human dispersal both northward and eastward. Migrant behavior is of considerable biological importance because it leads to "gene dispersal" and a potential for reproductive advantage. However, "out-migration"

is dangerous (risky) even as it opens up new opportunities. In most primate species, some animals will ultimately leave the group of their birth and seek another habitat. Commonly, it is the males, but for some – in chimpanzees, gorillas, and spider monkeys for example – it can be the females. Most out-migration occurs in adolescence, when risk taking increases. Competition for scarce resources is a significant factor that interacts with risk-taking predisposition of those who migrate. Social rank is another prime determinant for which animals leave the troop. In bad times, when there is not enough food to go around, the high-ranking animals usually stay in place and the aggressive lower-ranking animals are those most likely to leave the troop. Such dispersion does not happen regularly or in every generation, but when it does occur, it has a major impact on future generations by weeding out the parent troop and potentially seeding new ones.

During the Miocene – 20 million years ago – global cooling began, and it was under these challenging circumstances, as the food supply dwindled and competition for survival increased, that our direct forebears emerged. It is known from the fossil record and genetic studies that hominins, gorillas, and chimpanzees all descended from common ancestors. These small ape-like creatures were distinguished by walking upright and lived late in the Miocene period, some five to seven million years ago.

Novelty seeking, curiosity and impulsive behavior are interrelated. Fairbanks has found that the most impulsive risk-taking males in her primate colony are those who have the lowest levels of the serotonin breakdown product 5-hydroxyindolacetic acid (5-HIAA) in their cerebrospinal fluid (serotonin modulates behavior, opposing the curiosity-provoking dopamine superhighway and the alerting drive of norepinephrine). In some individuals or subspecies, serotonin only weakly opposes the dopamine drive, so they may not be genetically "programmed" for migratory behavior.

Jay Kaplan [4] has found that those males that remain within a troop beyond puberty have higher levels of 5-HIAA in their cerebrospinal fluid. In baboons in the Rift Valley, in whom dispersal occurs around puberty, there is an inverse relationship between serotonin levels and dispersal, again suggesting

a strong role of dopamine drive in migratory behavior and reinforcing that response to physiological stress drive and environmental curiosity, and are then subject to reciprocal environmental pressures.

Therefore, when vertebrate physiology is placed in a proper mechanistic frame, it is apparent that there can be vertical integration of lipid metabolism that leads to endothermy, and then links to risk-taking behaviors. The entire arc is clarified as sets of serial exaptations under the influence of cellular problem-solving, epigenetics, niche construction, and selection. The unifying factor that connects this complex system is understanding that the cell was the first niche construction, and it remains the epicenter of evolutionary development to this day.

Conclusions

The fundamental interrelationships and principles of biology must be embraced for a number of reasons. First, biology must correctly identify fundamental mechanisms if it is to be a full-fledged predictive science. Fully comprehending our evolutionary path is a requisite for such an understanding. Secondly, biology is the scientific basis for medicine. Absent a cardinal basis for biology, medicine will remain a series of derivative associations and correlations without the predictive capacity that would represent our greater good. Further yet, there are many emerging technologies whose consequences are ill understood: artificial reproduction, cloning, artificial intelligence, or gene editing. If evolutionary mechanisms have been misconstrued and those elemental First Principles of Physiology that underlie evolutionary development are not considered, our long-term survival is jeopardized by unintended consequences. In this regard, it should be considered that the higher consciousness licensed by the cellular form and lipid membranes have led us to a salient moment in which our ethical capacity fully lags our technological prowess. Therefore, the considered emphasis of a deeply integrative systems approach to biology and evolution is not only desirable on a scientific basis, but a requirement for our proper ethical stewardship of our planet.

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12

Terminal Addition as Physiologic Homeostasis and Regeneration, or Evolutionary Medicine

Summary

The addition of epigenetic inheritance to our understanding of evolutionary development permits a reboot for our understanding of Terminal Addition. It is not simply the arithmetic extension of structure and function. It is a continuous historical mechanism for cellular–environmental complementarity. Within this context, evolutionary terminal additions can be identified as environmental induction of episodic adjustments to cell–cell signaling patterns that yield the cellular–molecular pathways leading to novel developmental forms. Phenotypes derive, thereby, through cellular mutualistic/competitive niche constructions in reciprocating responsiveness to environmental stresses and epigenetic impacts. As such, Terminal Addition progresses according to a logic of cellular needs confronting environmental challenges over space-time. A convergence of evolutionary development with Terminal Addition can be accomplished through a mutual focus on cell–cell signaling, molecular phylogeny, and a broader understanding of epigenetic phenomena among eukaryotic organisms. When seen in this manner, Terminal Addition plays an important role in evolutionary development, and chronic disease might be considered as a form of "reverse evolution" of the self-same processes.

Evidence-Based Evolutionary Medicine, First Edition. John S. Torday, Neil Blackstone, and Virender Kumar Rehan

Introduction

The evolutionary principle of Terminal Addition forms the molecular basis for physiologic homeostasis, compartmentation, and regeneration. Conventionally, Terminal Addition refers to growth and patterning in a posterior subterminal growth zone. This leads to the further premise of ontogeny recapitulating phylogeny [1], the embryonic development of an organism repeating the adult features of ancestral organisms. These dual concepts of Terminal Addition have led to a general misapprehension that their applicability should be judged by the manner in which visible forms from the fossil record can match that narrative. Consequently, the general concept has fallen into disrepute. The lack of correspondence between genotype and phenotype has tended to undermine the validity of this idea. Yet, there is a logic to considering Terminal Addition as an evolutionary mechanism insofar as evolution is a time-weighted sequential narrative. Therefore, a reappraisal of Terminal Addition through new approaches to the nature of phenotype is warranted through a fresh appraisal of the primacy of the unicellular form, and a more complete understanding of the cellular nature of eukaryotic, holobionic life.

First, many bilateral animals do extend their growth from the posterior generative zone, and this can be considered a form of Terminal Addition. More importantly, it is argued that the efficacy of adding a new trait determined by a sequence of signaling pathways at the end of a sequence of compatible and evolved signaling pathways is a more sensible approach to evolutionary development than interpolating any of them within that series, potentially undermining preexisting adaptations. The cellular–molecular modularization of this process, seen as Terminal Addition, can thereby be seen as underpinning the developmental, phylogenetic, and regenerative successes of evolution. Moreover, this perspective is consistent with the proximate nature of cell–cell interactions when growth factors are produced by the mesoderm, while their receptors reside on neighboring endodermal and ectodermal cells, governing the direction and magnitude of their communication.

Recognizing cell–cell signaling mechanisms as an effective means of evolutionary development offers a critical understanding of developmental physiology in a manner that bears pertinent

application to the diagnosis and treatment of chronic disease. Furthermore, an understanding that Terminal Addition affects how and why genes are repurposed offers a novel way of thinking about pathophysiology.

Terminal Addition is central to Haeckel's Biogenetic Law, as reflected by his alphabetizing the stages of embryogenesis [2]. His "ontogeny recapitulates phylogeny" was dismissed by evolutionists because there was no empiric evidence to further support or sustain that line of investigation at the time. However, more recently, cellular–molecular data consistent with this theory have emerged. Thus, a reconsideration of the nature and implications of Terminal Addition is warranted.

It has been argued that Terminal Addition was the basal condition in Bilateria, accounting for the rapid Cambrian evolution of novel bilaterian morphologies. The defense of this assertion has been made through the evident fossil record and by inferences of homology of Terminal Addition across bilaterian metazoans. Yet, the fossil record is incomplete and some homologies are subject to a wide variety of interpretations. But if evolution is a process, why do we tend to dwell on phenotypes and genes? If instead, the focus is placed on "process," the problem remains as an issue of which one to select. By examining the various individual processes of biology – development, physiology, homeostasis, regeneration, and aging – only limited aspects of evolution are being appraised rather than an integrated whole. Clearly then, any process of evolution that can be imputed and held to account for what is biologically evident must lie somewhere between the gene and the phenotype. It must exist within the exact manner in which they interrelate. It is argued here that throughout individual development along any evolutionary timeline, that coordinate space is occupied by cell–cell signaling mechanisms mediated by soluble growth factors that can be identified as the mechanistic basis for understanding Terminal Addition.

Conflicting Viewpoints

Biologists have puzzled over the evolutionary origins of life for far more than the 150 years since the challenge of The Origin of Species. Darwin delineated the problem with focused deliberation

and provided a metaphoric mechanism through Natural Selection. Ever since, vigorous attempts have been made to understand the operative causes of descent with modification that generate "forms most beautiful" that have resulted in the primary concepts of evolutionary conservation. Initially, embryologists took the lead back in the nineteenth century, trying to reconcile development with phylogeny. Haeckel formulated his Biogenetic Law, and Spemann offered his concept of the Organizing Principle for development. These efforts were thereafter usurped by geneticists for lack of empiric evidence from their embryologist brethren; Spemann could not isolate the "organizer," and Haeckel could not provide empiric evidence as to how ontogeny recapitulates phylogeny. In a concerted effort to integrate these concepts, the genetic approach subsequently merged with Darwinian evolution as the Modern Synthesis in the first third of the twentieth century. More recently, the role of development in evolution and the study of the conservation of form have reemerged as evolutionary-developmental biology, or Evo-Devo.

There have been a number of persistent controversies regarding the advancement of the developmental characteristics that Haeckel attempted to illuminate with his Biogenetic Law. Although few regard that specific rule as credible, there are aspects that relate to single characteristics, as opposed to entire stages, that have gained some support. In an attempt at reconciliation, one direction has been pursued through the concept of the highest conservation of form and function. Some molecular studies suggest that the strongest conservation is at the early stages of embryogenesis, which can be viewed as a funnel-like model that constrains development at an early state and accounts for any initial superficial similarities.

Others have investigated homologous structures and studied ancient similarities in patterning mechanisms among diverse organisms. These homologies suggest a continuity of morphological features over time from a common ancestor. However, that path has been incompletely rewarding, and it is now believed that such a search must proceed through "deeper" homologies in which the physical similarities may not be evident but can instead relate to genetic relationships and their complex regulatory circuits and transcriptional effects.

The disparities become quite apparent when the evolution of segmentation is considered. Conflicting sources of data can lead to a variety of differing conclusions. Molecular similarities suggest one set of developmental pathways. Data from paleontology suggest other differences, whereas comparative morphology drives in yet another direction. One suggested resolution is to investigate parallel co-option of gene regulatory networks, permitting a modular type of segmental body organization with subsequent divergent phenotypic radiations as variations on a common theme. Some researchers suggest that a path toward a unifying position could emanate from our current knowledge of metamerism (a linear series of body segments termed somites or metameres that are fundamentally similar in structure, although some perform special functions). It is thought that this form of serial anatomy is governed by a segmentation clock that times its evolutionary appearance independently of ancestry. It was Gould who had first suggested there was some validity to an explanation of the relationship between ontogeny and phylogeny through embryonic ancestral recapitulation via developmental timing, especially as there were underlying mechanisms of heterochrony that would permit its acceleration or retardation that might yield differing forms.

Yet, even after so many decades, none of these varied mechanisms have widespread acceptance. Therefore, a mechanism that provides a bridge across this disparate landscape would be desirable. It is proposed that a focus on cell–cell signaling as an elucidating manifestation of Terminal Addition provides an appropriate justification for the concept's validity.

Terminal Addition as a Perpetual Cellular Link with the Environment

It can be advanced that recent progress in our contemporary understanding of evolutionary development permits a reframed appraisal of Terminal Addition as continuous historical cellular–environmental complementarity (Figure 7.3). Thereby, evolutionary terminal additions can be identified as episodic adjustments to cell–cell signaling.

Molecular cell embryology supports this goal by tracing development from the fertilized egg to the multicellular organism, and then, back again to the unicellular zygote during the life cycle in an iterative manner from one generation to the next. This body of knowledge offers insight into the formation of the embryo, and how it grows, differentiates, and adapts to its environment. It is now known that the communication between cells in the developing conceptus is mediated by soluble growth factors, like the Spemann organizer, binding to its cognate receptors on neighboring cell-types to signal their presence and level of growth and differentiation. The target cell binds growth factors from its cell neighbors, triggering its developmental and resultant homeostatic programs. These growth factors are highly conserved throughout evolution and have been expressed in continuity from their unicellular origins forward across multicellular organisms, offering the opportunity to trace the molecular origins of vertebrates. This approach also leads to an understanding of how these patterns of cell–cell communications determine physiology, and why the breakdown in cell–cell communication leads to either adaptive strategies on the one hand, or maladaptive outcomes as another.

Terminal Addition as Layered Cell–Cell Signaling

It is asserted that evolutionary terminal additions can be identified as environmental induction of episodic adjustments to cell–cell signaling. This interaction yields cellular–molecular pathways that lead to differing developmental forms as a derivative manifestation of mutualistic/competitive cellular niche construction. This approach is not without precedent and can be used as a functional genomic approach to evolution via Terminal Addition. Horowitz [3] formulated a similar approach to the evolution of biochemical pathways by assuming a retrograde mode of evolution. That approach describes the functional phenotype for the evolution of a biosynthetic pathway, like the pathway labeled "Genetic" depicted at the bottom of the schematic in Figure 7.3. In contrast to that, the cellular–molecular paracrine

mechanism depicted as cell–cell interactions (Figure 7.3, top), underpinned by a series of ligand–receptor interactions (Figure 7.3, middle) that evolved in response to a series of external (atmospheric oxygen, stretch) and internal (metabolic demand, tissue oxygenation, alveolar surface tension, blood pressure) selection pressures would have caused the evolution of the homeostatic mechanisms that determined those biosynthetic pathways from phenotypes to genes, i.e. cell auto-engineering. Subsequent selection pressure for such ligand–receptor-mediated gene regulatory networks would have generated both evolutionary stability and novelty through such well-known mechanisms as gene duplication, gene mutation, redundancy, alternative pathways, compensatory mechanisms, and balancing selection pressures. Such phenotypic changes are consistent with reproductive success and are depicted as a progression of interlocking arrows (Figure 7.3, bottom). These genetic modifications were manifested by the structural and functional changes in the gas exchanger, primarily by the thinning of the blood–gas barrier in conjunction with adaptive phylogenetic changes in the composition of the surfactant, as described by Daniels and Orgeig [4]. The reverse engineering of these phenotypic changes in the blood–gas barrier form the basis for a molecular genetic approach to lung evolution. It should be noted that this path provides a novel emergent and contingent mechanism for evolution. More importantly, this model of cellular–molecular evolution predicts the evolution of other physiologic mechanisms by integrating reproduction into the selection pressure process– specifically, at each proximal step in the retrograde evolution of surfactant, its physiologic roles, either as a newly evolved step or as a functionally interrelated aspect of integrated physiology, would have been constrained by the immediate and related mechanisms that prepare the embryo for its homeostatic adaptation to extrauterine life.

For example, the relationship between stretch-regulated parathyroid hormone-related protein (PTHrP) signaling and surfactant production interrelates functionally (and genomically) with its complementary roles in bone development, skin maturation, the birth process, and glomerular physiology. Taking this one step further, that is to say by tracing its evolution backward, this same cellular–molecular processing from proximate to

ultimate physiologic characteristics has been canonically repeated, perhaps rooted in its unicellular origins – the cellular–molecular mechanism of lung evolution based on the evolution of the surfactant dovetails with fundamental mechanisms of membrane evolution put forward by Konrad Bloch [5], and by Thomas Cavalier-Smith [6]. Bloch demonstrated that cholesterol evolved in response to the rise of oxygen in the atmosphere, speculating that its biologic advantage was due to the reduced fluidity, or increased microviscosity resulting from the addition of cholesterol to the cell membrane phospholipid bilayer. The discovery of hopanoid triterpene derivatives in some prokaryotes in the form of "molecular fossils" of ancient times has led to the suggestion that these relatively rigid, anaerobically evolved, amphiphilic molecules play a membrane reinforcing role in some prokaryotes similar to that exhibited by aerobically evolved sterols such as cholesterol in eukaryotes. Bloom et al. [7] hypothesized that the biosynthesis of cholesterol in the newly established aerobic atmosphere alleviated this constraint on the evolution of eukaryotes. The observation by Cavalier-Smith [6] that "there are twenty-two characters universally present in eukaryotes and universally absent from prokaryotes" presented a detailed argument that the advent of exocytosis (and endocytosis) most likely provided the driving force for the evolution of eukaryotes into their present form. In turn, the advent of cholesterol might have constrained cytosis. Therefore, there is a cellular–molecular continuum from the evolution of cholesterol for the compliance of the plasma membrane of unicellular eukaryotes, to endocytosis/exocytosis in eukaryotes, to the efficient functioning of the swim bladder resulting from the secretion of cholesterol as a lubricant, to lung surfactant reducing surface activity.

The characterization of the above cellular–molecular events as Terminal Addition is borne out by the epithelial–mesenchymal interactions involved in the development and phylogeny of the lung alveolus. This pathway begins with PTHrP, produced and secreted by the alveolar epithelium binding to its cognate receptor on the surface of the mesenchyme, triggering a cyclic adenosine monophosphate cascade that causes differentiation of the lung myofibroblast into the lipofibroblast. The lipofibroblast, in turn, produces and secretes leptin, which binds to its cell surface receptor on the epithelial type II cell, stimulating the

production of lung surfactant. Leptin also elicits the transit of fatty acid from the lipofibroblast to the alveolar type II cell, mediated by the production of prostaglandin E_2 by the alveolar type II cell. In the context of Terminal Addition, each of these intermediary steps in lung alveolar development comply with the phylogeny of the alveolus from fish to amphibians, reptiles, mammals, and birds.

As experimental evidence for the causal evolutionary nature of this mechanism, when cholesterol synthesis by the alveolar type II cell is deleted [8], it decreases the surface-tension reducing capacity of the surfactant, resulting in stress on the alveolus. To compensate for this effective surfactant deficiency, the alveolus "recapitulates" the evolutionary formation of lipofibroblasts during development to compensate for the loss of surface-activity and prevent alveolar collapse. Thus, the steps in alveolar evolution from the swim bladder of fish to the advent of the lipofibroblast in preventing oxidant injury is reprised both developmentally and phylogenetically as originally described by Haeckel. It is an important outgrowth of this in-depth understanding of the underlying cellular pathways that a group of bioactive molecules appear to have similar developmental roles in some insects, crustaceans, and chelicerates. This provides evidence of homologies of linked molecular action and signaling pathways across these arthropods that have deeper evolutionary meaning than morphologic overlap.

Epigenetic Impacts and Terminal Addition

It can be supported that the general processing of these terminal additions is through epigenetic means as a mechanism whereby environmental stresses are accommodated, first at the somatic level and then the germ line. Therefore, in order to properly understand Terminal Addition, it is first necessary to clarify some aspects of evolutionary development that have been persistently mistaken. Despite any conventional visual appraisal, the phenotype is a means by which macroorganisms acquire epigenetic experiences for the perpetuation of the dominant eukaryotic unicellular form. For eukaryotes, the phenotype is the means by which terminal additions are added to holobionts

through self-referential consensual cellular collaborations as niche construction. The phenotype is therefore purposed toward environmental exploration that returns from phenotype to be indirectly experienced through obligatory recapitulation to the unicellular form. The unicellular zygote adjudicates epigenetic impacts and, therefore, has a crucial role in the addition of appropriate forms of Terminal Addition or their heritable exclusion.

Physiologic Stress, Vascular Shear Stress, Radical Oxygen Species, and Mutation within Constraints = The Mechanism of Terminal Addition

Physiologic stress has played a role in vertebrate evolution since its inception in the unicellular state. The rise in atmospheric oxygen brought about the synthesis of cholesterol, whose presence in the cell membrane fostered the evolution of eukaryotes, serving metabolism, respiration, and locomotion. Subsequently, cellular stress led to the evolution of the peroxisome, protecting the cell against calcium toxicity. Eons later, fundaments of vertebrate evolution, were recapitulated during the water–land transition in support of lung evolution from its ancestral fish swim bladder. Cellular–molecular pathways evolved for the efficient production of lung surfactant, mediated by endodermal–mesodermal interactions to be effected by soluble growth factors and their receptors. The impetus for such structural–functional remodeling events was due to the physiologic stress on the microvasculature causing shear stress, generating radical oxygen species known to cause gene mutations and duplications. It is important to note that these modifications of preexisting structures occurred within the boundaries of their physiologic constraints, and thereby the trial and error was limited within those constraints, permitting the narrowing of cellular choices from which novel structures might be fashioned.

Each of the documented three gene duplications that occurred during the water–land transition were for receptor genes – the *PTHrPR*, the β adrenergic receptor (*βAR*), and glucocorticoid

receptor (*GR*). It is probably not a coincidence that all three duplications were for the receptor rather than for the ligand, and this becomes support for the contention that receptor-mediated pathways evolved by Terminal Addition, with the downstream mechanisms evolving to provide iterative homeostatic stability over the course of phylogeny and ontogeny (Figure 7.3).

Moreover, it can be contended that duplication of the receptor component has far more bioenergetics efficiency than augmentation of the ligand since the receptor has an inherent amplification effect. Although the structural homologs of these signaling pathways are the usual focus of attention in describing Terminal Addition (see Figure 7.3, top panel), it is actually the underlying cellular–molecular components that are the operative players. The growth factors themselves are elaborated by one cell type, whereas the growth factor receptors are elaborated by a neighboring cell-type derived from a different embryonic cell line. The receptor then elaborates a "second messenger" that communicates to the nucleus of the cell, binding to DNA polymerase to produce RNA, which then stimulates the biosynthesis of a peptide that facilitates the metabolic function of the pathway involved.

Homeobox Genes, Colinearity, and Terminal Addition

The classic example of Terminal Addition is the homeobox genes, which determine the axes of the body. The relationship between the colinear formation of the body axes and the genes that determine this process and their conservation over evolutionary development is itself a sturdy support for the validity of the presence of a basic mechanism of Terminal Addition. Given the premise that Terminal Addition is a natural result of the mechanism of cell–cell signaling, the congruence of the homeobox genes with their spatiotemporal effects on the generation of the body plan should be self-evident rather than enigmatic. Such an explanandum for Terminal Addition reiterates the predictive power of the cellular–molecular approach to evolutionary biology.

The Alveolar Lipofibroblast as Terminal Addition

The alveolar lipofibroblast (LIF) evolved to protect the lung alveolar surface against oxidant injury. The precedent for that property was that cultured muscle cells differentiate into adipocytes in 21% oxygen but not in 6% oxygen. Subsequently, the expression of adipocyte differentiation related protein by LIFs was shown to mediate neutral lipid uptake and storage, facilitated by the presence of *PTHrP* and prostaglandin E_2 receptors mediating stretch-regulated surfactant production, both developmentally and phylogenetically. In further support of the "terminal" nature of the LIFs, when the lung is injured by oxidants or over-distension the LIFs regress to their atavistic myofibroblast progenitors, promoting fibrosis as a way of stabilizing the homeostatic structure and function of the alveolus. Conversely, treatment with a peroxisome proliferator activated receptor gamma (PPARγ) agonist (the determinant of the adipocyte phenotype) prevents such injuries by maintaining the LIF phenotype, demonstrating the terminal nature of this mechanism. It is a significant observation that the response to injury that is illustrated through these mechanisms can be appropriately construed as "backwardization" of the more developed cellular response toward its ancestral form. In that sense, it can be viewed as a type of "reverse evolution." When this relationship is recognized, a pathway toward understanding aspects of chronic diseases of the lung and other organs is elucidated.

AO1

The Participation of Glomerular Mesangial Cells

The mesangial cells of the glomerulus, like the LIFs, are specialized fibroblasts that act to facilitate homeostasis. The simple microvascular glomus of the fish kidney evolved into the stretch-regulated glomerulus of land-adapted vertebrates. When the glomerulus is distended by fluid, it stretches the podocytes lining it, stimulating *PTHrP* production by these cells, regulating fluid and electrolyte secretion by the mesangial fibroblasts surrounding the kidney tubules. Similar to the effect of over-distention of the alveolus causing fibrosis, over-distension of the glomerulus causes sclerosis. In doing so, it is acting to maintain

homeostasis, reflecting the terminal nature of the PTHrP-mesangial signaling mechanism. The homology between the alveolus and glomerulus begins in utero, where both structures contribute to amniotic fluid production.

PTHrP Effects on the Anterior Pituitary, Adrenal Cortex, and Adrenal Medulla

PTHrP appears in the anterior pituitary of mammals and birds, where it stimulates adrenocorticotrophic hormone (ACTH) production, the end-product of the anterior pituitary. In the adrenal cortex, *PTHrP* amplifies the effect of ACTH on glucocorticoid production. In tandem, it has also been observed that the microvasculature of the adrenal medulla is expanded in rats, amplifying the effect of glucocorticoids on phenylethanolamine-*N*-methyltransferase, the terminal step in epinephrine synthesis from norepinephrine. Since *PTHrP* is angiogenic, this mechanism may be the net effect of *PTHrP* production by the adrenal cortex.

Catecholamines, Lung, and Heart Biology

The lung and heart have evolved in tandem, likely due to the duplication of the *βAR*, facilitating the adaptation to land by both structures. Lung evolution that allowed for the independent regulation of the pulmonary and systemic blood pressures was mentioned earlier. In the case of the heart, catecholamines provide homeostatic regulation of heart rate, determine embryologic heart chamber development, and facilitate heart regeneration. Such conjoint sequencing can best be understood as occurring within the framework of Terminal Addition.

Oxytocin, Endothermy, and the Retina

It has been hypothesized that endothermy evolved as a consequence of the water–land transition [9], facilitated by the gene duplications that occurred during that era, namely the *PTHrPR*,

βAR, and the *GR*. More recently, it has been discovered that deleting the oxytocin gene in mice results in failure to thermoregulate, indicating that this is the constitutive adaptation for body temperature control. It is notable that, in turn, oxytocin promotes retinal development late in the development and phylogeny of the eye. It is argued that such a sequence of historical evolutionary events is an illustration of Terminal Addition in adaptation to land life.

Central Nervous System

For many years, progress in understanding the evolution of the central nervous system (CNS) was at a standstill as there was no evidence for its presence in invertebrates. That changed when Nick Holland pointed out that the CNS of worms was in its skin, thus providing an evolutionary link between invertebrates and vertebrates. Hughlings Jackson had speculated a hierarchical relationship between the lower, middle, and higher centers of the CNS to account for its functional evolution. The lowest level, for control of movement, was represented by the medulla and spinal cord. The middle level consisted of the motor area of the cortex, and the highest motor level was localized to the prefrontal cortex. The validity of this perspective is provided by the observation that when patients recover from general anesthesia they do so in the phylogenetic sequence from the most primitive to the most advanced level of consciousness. It is important in the context of cell–cell signaling and Terminal Addition to note that the CNS develops under these same principles.

Terminal Addition, "Reverse Evolution," and Evolutionary Medicine

Jean Guex [10] has made the case for reverse evolution in ammonites. It is argued that this mechanism could only have occurred if Terminal Addition were the underlying principle of morphogenesis. Guex has provided experimental evidence for environmental stress causing reversion to an earlier stage of

evolution in these extinct mollusks. Further evidence for reverse evolution in vertebrates comes from the pathophysiology literature that shows the systematic breakdown in structure and function consistent with reverse ontogeny and phylogeny. Moreover, it has been shown that specific agents can be exploited to "drive" the process back to its evolved state, thereby forming the basis for one aspect of evolutionary medicine that can be based on mechanistic principles of cellular–molecular biology.

Discussion

Evolutionary Biology has a long and contentious history, and it has only been recently that there has been a scientific effort to understand its evolutionary origins through a series of First Principles of Physiology. These First Principles of Physiology have previously been described, consisting of negentropy, chemiosmosis, and homeostasis. Indeed, it can be stated that all Terminal Addition extends forward based upon these discrete cellular essentials.

Terminal Addition: The Fundament of Haeckel's Biogenetic Law

Haeckel based his Biogenetic Law on three principles. The first principle was correspondence. Each stage of development in higher organisms corresponds to adult stages of lower animals. The second principle was that phylogenesis occurs by addition of new traits to the terminus of normally developing structures. He based this principle on his observation that the early stages of the embryological development of different species look similar to one another due to developmental constraints during early development. He reasoned that such constraints abate toward the end of development, allowing for the addition of new traits, or evolution. The third principle was based on the principle of truncation, arguing that if new traits were continuously being added on in an evolutionary chain, older traits had to develop faster.

It could be argued, then, that if this type of process had evolutionary credibility, then there should be evidence for its reverse capacity under some circumstances, and then, if such was the case, it might impact human health. It can be considered, then, that this "cell's eye view of evolution" predicts some aspects of the aging process. It might be speculated that aging is development in the reverse direction. The logic is plain: development forms through cellular communications, aging can therefore be considered the systematic breakdown in those very same cellular communications.

Along that line of reasoning, if the function of the evolutionary process is to mediate the adaptation of species over generations to an ever-changing environment, then reproduction may be seen as the intergenerational optimization of the communication of such knowledge, providing an additional rationale for understanding the aging process. If biologic systems initially evolved by reducing entropy within cellular boundaries, and subsequently devised the means for conveying that knowledge from generation to generation through replication and reproduction, relative negentropy as its living circumstance is sustained as a circumvention of the Second Law of Thermodynamics and therefore must be considered costly. As a result, the vigorous cost shift in bioenergetic expenditure toward its reproductive strategy counterbalanced by a loss of bioenergetics in late life. The common denominator is the loss of cell–cell communication, culminating in death. Even within this constraint, or perhaps because of it, organisms accumulate genetic information from previous generations and then communicate it to the next generation through reproduction. The key is to following the flow of biologic information through communicative processes, which in their most applicable manifestation are through cell–cell signaling and its results.

Somewhere between Gene and Phenotype Lies the Process of Evolution

It has been challenging to determine just what process lies between the gene and the phenotype. In the past, that mechanistic black box has been bridged by the use of such metaphors

as natural selection, survival of the fittest, descent with modification, selection pressure, genetic assimilation, and adaptation. But these catch phrases do not reveal how such mechanisms actually work. In the era of genomics, we must determine the nature of such mechanisms for a number of reasons: (i) to demonstrate the legitimacy of evolution; (ii) to provide a predictive model for biology and medicine; and (iii) to discover our. AQ2 In support of those pursuits, a cellular–molecular context for evolution provides a working model for a continuum of biology throughout the lifecycle. Therefore, a cohesive narrative that can bridge the perception of a divide between microevolution and its macro form, contingency and emergence, or gradualism as opposed to punctuated equilibrium must be considered of value. A concentration of cell–cell signaling mechanisms in support of cellular homeostasis affords a necessary narrative shift away from a dominant gene centered one.

Despite the academic revolution forged by merging developmental biology and evolution through Evo-Devo, little actual hypothesis testing experimentation has been done to determine the validity of evolution theory. It is argued that progress can only be made if the singular concentration of neo-Darwinism on genomics is foresworn for a concentration on cellular dynamics, cellular requisites, and their embodied communicative skills.

As an example, the classic representation for vertebrate evolution is as phyla, from fish to mammals. Biologists are accustomed to regarding the adults in each phylum as being the representatives of each group. Yet, evolution encompasses the entire life cycle, from embryo to adult, although our natural inclination might be to concentrate on our own adult form as a primary focus, there is no prima facie that this is actually the case. It can be vigorously defended that the purpose of reproduction among multicellular eukaryotes is its obligatory return to its unicellular state within the zygotic form.

The description of phyletic evolution on the left of the schematic in Figure 12.1 allows us to organize known biology into a first approximation of evolution. In order to make this conceptual transition to a format in which evolutionary mechanisms can be tested, focus must be on the embryonic stage within each phylum, comparing the cellular and molecular processes that give rise to structures and functions. The lung is such an example. The basic

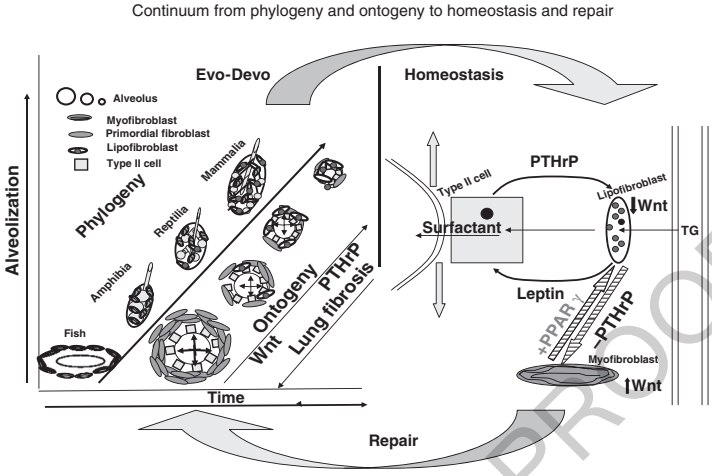

Figure 12.1 Lung Biologic Continuum from Ontogeny–Phylogeny to Homeostasis and Repair. The schematic compares the cellular–molecular progression of lung evolution from the fish swim bladder to the mammalian lung (left portion) with the development of the mammalian lung, or Evo-Devo, as the alveoli become progressively smaller (see legend in upper left corner), increasing the surface area–blood volume ratio. This is facilitated by the decrease in alveolar myofibroblasts and the increase in lipofibroblasts, due to the decrease in Wingless/int (Wnt) signaling and increase in *PTHrP* signaling, respectively. Lung fibrosis progresses in the reverse direction (lower left corner). Lung homeostasis (right portion) is characterized by *PTHrP*/leptin signaling between the type II cell and lipofibroblast, coordinately regulating the stretch regulation of surfactant production and alveolar capillary perfusion. Failure of *PTHrP* signaling causes increased Wnt signaling, decreased PPAR γ expression by lipofibroblasts, and transdifferentiation to myofibroblasts, causing lung fibrosis. Repair (arrow from homeostasis back to ontogeny–phylogeny) is the recapitulation of ontogeny–phylogeny, resulting in increased PPARγ expression. (Reproduced from Torday and Rehan [11].) (*See insert for color representation of the figure*.)

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mechanism of lung morphogenesis changes progressively from fish to humans, mediated through PTHrP signaling intensity as it is amplified from the swim bladder of fish, toward the lungs of frogs, alligators, birds, and humans. The stepwise increase in *PTHrP* signaling increases surfactant synthesis, which allows for the adaptive increase in alveolar surface area-to-blood volume ratio that facilitates the increase in gas exchange during vertebrate

evolution, as first described by Clements et al. [12]. This phenomenon has more recently been well documented in a series of publications by Daniels and Orgeig [4].

Recognizing the underlying mechanism of Terminal Addition as stepwise cell–cell signaling in support of environmental responsiveness is important both in understanding the basic principle of evolution and as it applies to the diagnosis and treatment of chronic disease.

It has been noted that if Terminal Addition sources evolutionary development, then recapitulation is its result as a necessary connection toward evolutionary development. Yet, many are not convinced that evolution proceeds by Terminal Addition, since the possibility can be entertained that new processes can intrude at the embryologic stage rather than adding to the adult form. However, in a frame in which epigenetic impacts are the source of terminal additions that affect cell–cell signaling in which phenotype is only its derivative, there is no inconsistency. In effect, the adult elaboration is only a proxy for the essential unicellular state. Therefore, within this proper biological context, there is no privileged level of causation within the adult form in biological development, and information flows are fluid across all levels. Thus, Terminal Addition takes on an entirely different cast. Imperative to that process is cell–cell signaling, which is related to the genome as a part of its transcriptome, but it is not a one-to-one relationship. Therefore, it is no longer surprising that body plans and morphology do not necessarily correspond to gene sequences and the paradox noted by Gerhart and Kirschner [13] whereas, when variation was expected to be found, there was instead often conservation or stasis, can be fully resolved. The critical intermediary of cell–cell signaling across eukaryotic organisms that are always cellular entities resolves the gap.

Therefore, it is defended that conflicting aspects of Terminal Addition can be properly reconciled with evolutionary development through a concentration on cell–cell signaling and their molecular phylogenies on the one hand, and the true nature of epigenetic phenomena among eukaryotic organisms on the other. Crucial to this frame is our new understanding: phenotype is not an end but merely an ever-evolving, effective means of environmental exploration, dedicated to the perpetuation of its unicellular form.

Conclusions

It is argued that a contemporary reappraisal of Terminal Addition should reside within the overarching viewpoint that its appropriate focus threads through an appreciation of the primacy of cellular needs over macro-organic form. Looking at a fossil record for validation of Terminal Addition, either by specific changes to any primordial zone or through genotype–phenotype homologies, will not yield the requisite concordances that have been sought. Instead, evolution should be regarded as the continuous cellular utilization of information through cell–cell signaling, at its specific levels, to solve its problems in steady incremental responsiveness to environmental stresses. This proceeds through perpetual cellular–environmental complementarity and is enacted through progressive layers of cell–cell signaling in historical response to cumulative environmental stresses. Terminal Addition is one active means. Successive alterations of cell–cell signaling mechanisms and their correspondent metabolic pathways are continuous adjustments to epigenetic impacts. Importantly, it is a dynamic process that is never unilateral between cellular levels within any macro-organic whole. Paths open and close and then reopen. Yet, many remain available as a form of "reverse evolution" to be revealed only in response to injury or repair.

Therefore, Terminal Addition represents a significant part of evolution as layered historical continuity is reinforced as cellular memory. Its recapitulation cannot be appraised through macro-organic diagrammatic illustrations or embryological development in the manner of Haeckel. Instead, it can only be properly appreciated through the unfamiliar lens of recapitulation through the obligate eukaryotic unicellular zygotic phase. Despite such unfamiliar contours, that entire process is in conformity with a continuous set of First Principles and modified by serial accommodations and exaptations in continuous adjustment to epigenetic processes, as has been illustrated. The initiating and historical concept of Terminal Addition that had been based on serial macroscopic recapitulation is not tenable in an era in which genetic deletions, silencing, wholesale genetic accretions, or wide-scale duplications are known as episodic epigenetic consequences. Instead, Terminal Addition and its

AQ5

recapitulative aspects can only be understood through the primacy of cellular requirements that express through successive heritable alterations in cell–cell signaling. Clearly, such bioactive signals precede metabolism. Further yet, metabolism must be established prior to phenotype. Consequently, phenotype must now be judged as a distal epiphenomenon, not as evolutionary purpose. Importantly, this is an evolutionary system that is based on cellular responses to stress. At its scale, proactive cellular outcomes precede permanent macro-organic biological expression.

The mechanics of evolution can therefore be measured through Terminal Addition, now understood as layered, accreted cell–cell signaling paths directed toward cellular solutions to epigenetic stresses. Recapitulation does indeed occur but not as historically supposed. It manifests through a shrouded form as the perpetuating unicellular zygote.

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13

Phantom Limbs: Imagination and Epigenetics

Summary

The phenomenon of phantom limbs makes one wonder what selection advantage it offers. Upon reflection, it is actually consistent with and in furtherance of the concept of the "phenotype as agent" as the primary purpose of the organism, functioning to obtain epigenetic marks over the course of its life cycle. Rudimentary limbs evolved during the unicellular stage of vertebrate evolution along with metabolism and oxygenation, particularly when cholesterol appeared in the cell membrane, and as such are functionally interrelated with all of the other biologic traits that evolved over the subsequent course of vertebrate evolution. Physical loss of a limb does not obviate the dedicated need to collect epigenetic marks in conjunction with all of the other physiologic relationships that must be sustained if the organism is to remain evolutionarily competitive with other organisms. The exception proves the rule yet again.

Introduction

The phenomenon of sensing a phantom limb is conventionally only thought of in the context of the traumatic loss, as would be expected given the circumstances. However, it may be a manifestation of the "phenotype as agent," the organism evolutionarily mandated to obtain epigenetic marks, given that its

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evolved state is the net result of structural–functional changes over the history of the organism, beginning in the unicellular state. Since limb development is an essential aspect of that arc, its emergence is a result of earlier events, beginning with the acquisition of cholesterol in the cell membrane of unicellular eukaryotes, promoting metabolism, oxygenation, and most importantly in the context of phantom limb sensation, locomotion. The latter originated as increased cytoplasmic streaming as a result of cholesterol in the cell membrane, over time evolving as limbs in multicellular organisms, linked to metabolism and respiration by cholesterol from the inception of vertebrate evolution. That connection is emphasized because it provides the rationale for the etiology of phantom limb sensation since all of these properties are interconnected and must act in unison to facilitate the collection of epigenetic marks in order for the organism to faithfully monitor its environment and inform itself of any changes via the germ cells, zygote, embryo, and offspring in the next generation.

Background to Phantom Limb Sensation

The prevailing hypothesis surrounding phantom limbs has been that it is due to the inflammation of the severed nerve endings. Given the absence of the limb, this phenomenon was strictly thought of as the brain perceiving pain. However, Ronald Melzack [1] disputed this hypothesis in a paper entitled "Phantom Limbs, The Self And The Brain." He proposed the neuromatrix hypothesis, that the body is composed of a wide network of interconnecting neural networks. Pons et al. [2] subsequently showed experimentally that the somatosensory cortex of the brain reorganizes after loss of sensory input in macaque monkeys. Based on those observations, Ramachandran and Hirstein [3] hypothesized that phantom limb sensations in humans could be due to the sensorimotor cortex, located in the postcentral gyrus, which receives input from the limbs and body. He and his associates demonstrated this hypothesis by showing that stimulation of different parts of the face caused perceptions of being touched on different parts of the missing limb. Flor et al. [4] showed that the pain of limb loss was the result of the cortical

reorganization. In 1996 Knecht et al. [5] concluded that there was no relationship between referred sensations and cortical reorganization within the primary cortical areas. Flor has also found that non-painful referred sensations correlate with a wide neural network beyond the primary cortical areas. Despite such active research into the neural mechanisms of phantom limb sensation, there is no consensus as to the cause.

Relevance of Phantom Limb Sensation to Terminal Addition

In Chapter 12 we developed the concept of Terminal Addition as a continuous historical mechanism for cellular–environmental complementarity, mediated by cell–cell interactions and their downstream signaling cascades. Such relationships refer all the way back to the unicellular origins of the organism, and as such have formed both linear and nonlinear network interconnections with collateral structures and functions in ways that are consistent with the evolution of the organism. As such, any interference with such networks disrupts the agency of the phenotype, endangering the ability of the organism to fulfill its responsibility as communicator of epigenetics. Seen in this context, phantom limb sensation makes sense.

Phantom Limb Sensation as Non-Localization

The notion of non-localization has been discussed at length by Bohm and Hiley [7]. They bring out the fact that the essential new quality implied by the quantum theory is non-locality; i.e. that a system cannot be analyzed into parts whose basic properties do not depend on the state of the whole system. They show that this approach implies a new universal type of description, in which the standard or canonical form is always supersystem-system-subsystem, and this leads to the radically new notion of unbroken wholeness of the entire Universe.

Biology ascribes to the same description. It is not apparent when seen from a synchronic descriptive vantage-point, but

when understood from a diachronic perspective, transcending space and time, it can be understood in the same terms used by Bohm and Hiley [7]. This way of thinking about biology in cellular–molecular terms is exemplified by recalibrating of pleiotropy [8]. In contrast to the stochastic way of conventionally thinking about pleiotropy as the random expression of genes throughout the organism to generate more than one distinct phenotypic trait, it is actually a deterministic consequence of the evolution of complex physiology from the unicellular state. Pleiotropisms emerge through recombinations and permutations of cell–cell communication established during meiosis based on the history of the organism, both developmentally and phylogenetically, in service to the future existential needs of the organism. Functional homologies ranging from the lung to the kidney, skin, brain, thyroid, and pituitary exemplify the evolutionary mechanistic strategy of pleiotropy. The power of this perspective is exemplified by the resolution, for example, of evolutionary gradualism and punctuated equilibrium in much the same way that Niels Bohr [8] resolved the paradoxical duality of light as complementarity. Hence, seen in this way, biology and physics are both non-localized, acting at all scales to form and maintain their integrated entirety.

Limbs and Hearts

The first to mention a heart–limb defect syndrome were Holt and Oram in 1960. Actually, the term "Holt–Oram syndrome" is used to depict skeletal deformities located exclusively in the upper limbs when coexisting with congenital heart diseases, especially atrial or ventricular septal defects. Another heart–limb syndrome is Albright's hereditary dystrophy, which affects the bones of hands and feet, sporadically accompanied by secundum ASD. This syndrome is usually due to mutations in the *GNAS* gene and when coexisting with high parathormone, low calcium, and low 25(OH)D levels, it is referred to as pseudohypoparathyroidism. Here we describe a case of ASD associated with type D and E brachydactylies, and laboratory findings mimicking a pseudohypoparathyroidism syndrome.

This pattern of being is shared among all living things. For example, Brad Davidson [9] has shown that, developmentally, the stem cells for the heart in the tunicate *Ciona intestinalis* are derived from the tail, suggesting that the beating of the tail for locomotion has been exapted for heart beat. Unicellular organisms do not require a heart or a circulatory system, suggesting that the heart evolved in support of fundamental biologic traits like respiration, metabolism, and locomotion in multicellular organisms. That is, the heart is derivative. Exaptations, such as the evolution of the middle ear bones in vertebrates from the jaw bones of early fishes, have generally provided powerful clues to the ancestry of structures and reveal the repeating process of evolution through innovation from preexisting conditions. Similarly, the brain may have a history in response to the demand for central control of the evolving viscera (organ systems for respiration, digestion, barrier function, and movement).

Relationship of Limbs to Bipedalism and the Evolution of Birds and Mammals

The history of physiologic cellular–molecular interrelationships can be traced all the way back to the unicellular state by following the pathway formed by lipids ubiquitously accommodating calcium homeostasis, and its consequent adaptive effects on oxygen uptake by cells, tissues, and organs. As a result, a cohesive, mechanistically integrated view of physiology can be formulated by recognizing the continuum comprising conception, development, physiologic homeostasis, and death mediated by soluble growth factor signaling. Seeing such seemingly disparate processes as embryogenesis, chronic disease and dying as the gain and subsequent loss of cell–cell signaling provides a novel perspective for physiology and medicine. It is emblematic of the self-organizing, self-referential nature of life, starting from its origins. Such organizing principles obviate the pitfalls of teleologic evolution, conversely providing a way of resolving such seeming dichotomies as holism and reductionism, genotype and phenotype, emergence and contingence, proximate and ultimate causation in evolution, cells and organisms. The proposed approach is scale-free and predictive, offering a central theory of biology.

Of Limbs and Consciousness

The selection pressure for the evolution of endothermy/homeothermy was largely due to the combined effect of global warming and the greater metabolic efficiency of warm-bloodedness. It takes several isoforms of the same enzyme to catalyze any given metabolic step in poikilotherms, whereas in homeotherms it only takes one, given the adaptation to multiple temperatures versus one, respectively. The increased efficiency of metabolism allowed for bipedalism in hominins and birds alike, freeing the forelimbs for specialization – flight, tool making, and texting. The dynamic interactions between forelimb evolution and endothermy led to higher consciousness among hominins and birds. It also fostered global range of habitats – witness albatrosses circumnavigating the globe and hominins in outer space. Not only does this allow for greater range of options for habitation, it also exposes birds and hominins to much greater variety of epigenetic marks.

Life as Fractals

As a disclaimer, the following concept of physiology as fractal is not descriptive "turtles all the way down," it is founded on adherence to the First Principles of Physiology, starting with unicellular organisms, all the way up to complex physiology. This way of understanding the evolution of physiology comes from an understanding of the ecological niche in which we evolved and how our bodies respond, through cell–cell communication, and physiological regulation of genes, to the signals provided by the ancestral environment. At the root of this approach is an appreciation for the fractal nature of physiology, founded on the ubiquity of the cell membrane. The self-similarity of physiology at different scales is important because it demonstrates the universality of the underlying self-referential, self-organizing principle involved.

The ongoing discovery of deep homologies in the physiological systems of widely disparate taxa underscores the fractal nature of physiological processes. To start, a fractal is a mathematical pattern – it is the math that underlies the dynamics of natural systems – and it drives the evolution of phenomena via a basic

function that repeats itself across all scales of time and space, producing self-similarity on all levels of inspection. The similarity of ontogeny and phylogeny are not being claimed to have resulted from selection acting independently on different processes (development of a trait versus the evolution of traits). Instead, it is being claimed that the processes of ontogeny and phylogeny are one and the same, operating at different time scales. Upon inspection of molecular traits, ontogenetically (within an individual across time) and phylogenetically (across generations of individuals), they appear in specific sequences on both time scales. The genes expressed early in ontogeny (i.e. immediately following conception) are those that are phylogenetically most ancient. Genes expressed late in development are those that are evolved more recently and have a much narrower phylogenetic distribution. When molecular traits are "stressed" they follow the same trajectory in the reverse direction of ontogeny/phylogeny, suggesting that there is a common origin for all traits going back to the unicellular state. Organismally, this means that the dynamics playing out at the molecular level are self-similar in nature to the actions at the cellular level, which scale up to produce both the organ and the organ system level interactions that culminate in physiology. These fractal interrelationships may reflect the mechanism for the evolution of the internal environment, or physiology, in adaptation to the external environment. The external environment was formed by the Big Bang, which we now know because the Universe refers to that event through phenomena like the background radiation referred to as the Redshift. In contrast to this, physiology mimics the external Universe to form its own internal "Universe," homeostasis being its iterative self-referential framework, an emerging concept in evolution theory.

Reaching further into the past, the evolution of semipermeable cell membranes provides an informative example of how fractal processes influence human beings' nutritional needs in the modern day. The following thoughts may be helpful in thinking about fractal physiology and nutrition. Biology entrained energy via semipermeable membranes, promoting the reduction in entropy that is the "metabolic driver" for evolution as a way of perpetuating that mechanism. For example, the entraining of cholesterol in the plasma membrane facilitated both endocytosis and exocytosis by eukaryotes, and aerobic respiration by thinning

out the membrane, making it more permeable for gas exchange. Another process in this context is chemiosmosis, the theory that forming semipermeable membranes allowed for the creation of ionic gradients that are fundamental to generating the "vital force" of life. The entropy and chemiosmosis mechanisms are complementary in their mutual dependence on the existence of a semipermeable membrane. As these processes evolved, they had to cope with thermodynamics in a hierarchical manner. Cholesterol subsequently was exapted to facilitate the formation of lipid rafts, which are the structural basis for cell–cell signaling, ultimately culminating in the synthesis of steroid hormones to form the endocrine system. That interrelationship has been reiterated in evolution, particularly as vertebrates emerged from water to land, tracing the arc of physiologic evolution fractally from unicellular to multicellular organisms, from simple to complex physiology.

Consciousness, the Epitome of the Continuum from Inanimate to Animate

As indicated above, the case can be made for the interrelationship between the physical and biologic realms based on the "logic" of each. The consideration of consciousness as the interface between the two forms the conduit for the flow of information between the inanimate and animate. This is what is referred to in the literature as the "hard" problem, which has been debated for thousands of years. By providing a level playing field between the atom and the cell, in combination with such concepts and non-locality of both, the bigger venue of consciousness has become soluble.

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14

Man's Place in the Universe

Summary

Human endeavors are anthropocentric, based on our conventional way of thinking of ourselves in the way that the Greeks thought of us, as "The Measure of all Things." Consider da Vinci's "Vitruvian Man" that is used to express this idea that we are the standard by which everything on Earth is determined. He used this physiologic conceptualization of Man in proposing a redesign of the city of Milan to Ludovico Sforza, the Duke of Milan from 1494 to 1499 [1]. da Vinci drew up plans for a utopian city, since Milan had been ravaged by the bubonic plague that had killed one-third of its inhabitants. He proposed a radical concept in which the people of Milan would be disbursed to 10 new towns designed and built along the river. By analogy between the human body and the city, he reasoned that cities are organisms that need to breathe and have fluids that circulate nutrients and waste products.

However, now we know that our physiology evolved from unicellular organisms through cell–cell interactions based on the First Principles of Physiology. Therefore, it behooves us to reconsider our actions and motivations on this planet in ways that are faithful to such principles. This is particularly true since we are no longer necessarily constrained by the same physical factors that we were in the past, with cell phones and drones usurping the need for wires and roads.

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Introduction

Modern social systems are being bombarded daily with huge organizational challenges such as climate change, drought, overpopulation, poverty, famine, and general infrastructural weaknesses. Our traditional approach to such profound problems has been ad hoc coping strategies reflective of our self-perception as beings, going all the way back to the dawn of time. However, there is a means of reenvisioning our modern communities that would be in harmony with its inhabitants based on our deepest understanding of our own physiology. This can be accomplished through an emerging understanding of human physiology that realigns its unicellular origins with processes that originated with the Big Bang of the Universe [2] (Figure 14.1). This novel approach assumes that social structures should ideally foster and optimize human existence. Extending this understanding of

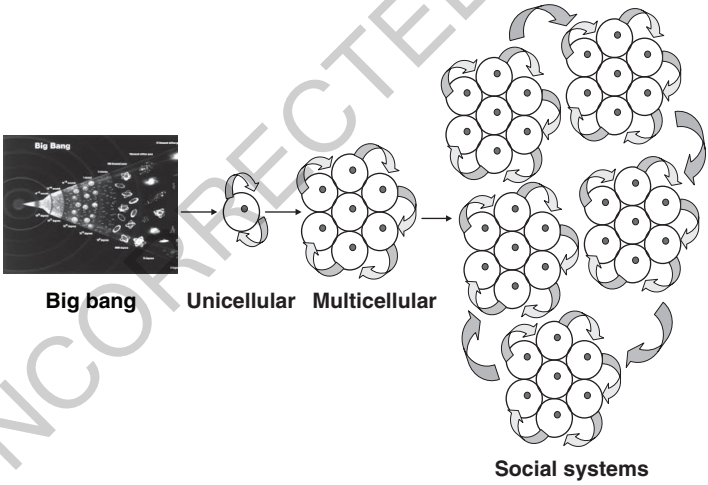

Figure 14.1 From the Big Bang to social systems. On the far left is the Big Bang of the Universe, which scattered the elements based on their atomic mass, creating an informatic hierarchy. Biology exploited the physical environment to generate autonomous cells that used homeostasis to maintain negentropy. Competition between prokaryotes and eukaryotes gave rise to multicellular organisms, which ultimately formed social systems, all based on the First Principles of Physiology. (*See insert for color representation of the figure.*)

our deepest physiology from its atomic origins can suggest how this knowledge could be exploited to optimize and epitomize the universal human social condition.

Anthropomorphisms Subvert the Biologic Imperative to Cooperate

Nowadays, biologists are militant about dissuading us from thinking hierarchically about the evolution of species. Each species has its own set of traits that allow it to adapt to its particular environmental niche, including Man. A classic example of how our highly evolved central nervous system misguides us is The Anthropic Principle, that the Earth's environment is "just right"– oxygen in the atmosphere, ambient temperature, water freezing at 32 °C, minerals. That perception is very deceptive because it suggests some sort of higher power placing us and all other biota on Earth, when in fact we have evolved from the physical environment. For example, by regressing the genetic pathway for the evolution of the mammalian lung against major epochs in vertebrate evolution – salinization of the oceans, the water–land transition, and the Phanerozoic oxygen fluctuations – a pattern of alternating internal and external selection pressures mediated by genetic mechanisms consistent with specific physiologic developmental and phylogenetic adaptations emerges (Figure 14.2). Moreover, this perspective is mechanistically consistent with the Gaia Theory [4], proposing that *organisms* interact with their *inorganic* surroundings on *Earth* to form a *self-regulating*, *complex system* that contributes to maintaining the conditions for *life* on the planet.

Euphysiology

Up until now, social communities have been founded on the "biologic imperatives" for food, shelter, education, religious institutions, trade, and government. Towns and cities were constructed on bodies of water both for agricultural and for sanitation requirements. For example, Roman fortresses were built on the principle of bilateral symmetry, with entrances at all

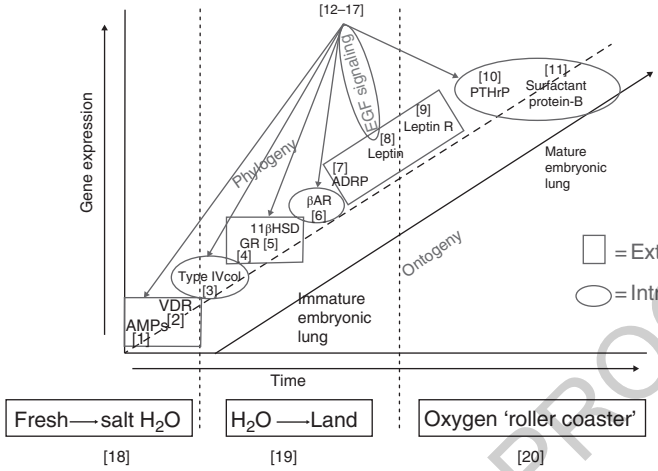

Figure 14.2 Alternating extrinsic and intrinsic selection pressures for the genes of lung phylogeny and ontogeny. The effects of the extrinsic factors (salinity, land nutrients, and oxygen on the *x*-axis) on genes that determine the phylogeny and ontogeny of the mammalian lung alternate sequentially with the intrinsic genetic factors (*y*-axis), highlighted by the squares and circles, respectively. Steps 1–11 appear in the sequence they appear during phylogeny and ontogeny: (1) AMPs; (2) VDR; (3) type IV collagen; (4) GR; (5) 11β HSD; (6) βAR; (7) ADRP; (8) leptin; (9) leptin receptor; (10) PTHrP; and (11) SP-B. Steps 12–17 represent the pleiotropic effects of leptin on the EGF in oval signaling pathways integrating steps 1–6, 10, and 11. Steps 18–20 are major geologic epochs that have "driven" intrinsic lung evolution [3].

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four compass points, not unlike da Vinci's idealized portrayal of Vitruvian Man. The ultimate size of these entities was pragmatically determined by need, constrained by capacity, and formulated by unicell using the Base 10 to emulate the number of fingers and toes – this is rapidly changing with our efforts to translate everything into the Base 2. Here we show how to merge the binary system with a new understanding of physiology.

We have always considered our own physiology from its ends instead of its means. The conventional view has examined the physiology of complex organisms as an association of parts, and generally linked steps, when in fact physiology is a highly integrated process that has evolved intact from our unicellular origins, beginning some 500 million years ago. Physiology only

seems to be complex because we have been reasoning after the fact within a conceptually limiting teleological frame of reference. In reality, physiology is quite simple if examined from its first principles, moving forward, having been established by relatively simple unicellular eukaryotic organisms. From this point of origin, physiology is properly assessed as the culmination of metabolic adaptations to the environment in support of epigenetic inheritance.

Unicellular eukaryotes, defined as those organisms with a nuclear envelope, evolved from bacteria, or prokaryotes, some two billion years ago. With the advent of cholesterol synthesis, and its incorporation into the eukaryotic cell membrane, eukaryotes were able to efficiently perform the three key functional tasks that characterize vertebrate physiology – locomotion, endocytosis/exocytosis, and respiration. Each of these traits evolved as a direct result of the physicochemical thinning of the cell membrane caused by the insertion of cholesterol.

Prokaryotes and eukaryotes continually compete with one another. Prokaryotes evolved the capacities for forming biofilm, and for quorum sensing, which are pseudo-multicellular properties. But eukaryotes actually evolved the capacity to form truly multicellular organisms as a result of competition with prokaryotes over the course of the last billion years.

Physiologic stress was a major driver for vertebrate evolution, epitomized by the water–land transition (W-L-T). As a result of the epic ecologic selection pressure brought on by the biota, increasing the amount of carbon dioxide in the atmosphere, causing lakes and rivers to evaporate (Romer Hypothesis), there were three known gene duplications that facilitated specific land-adaptive traits as a result of their amplification. These duplications supported many disparate processes or their functioning organs: skeletal structure in adaptation to increased gravitational force on land, lung physiology for air breathing, the kidneys for water and electrolyte regulation, the skin for barrier function, and the brain to integrate all of this newly acquired complex physiology. The three gene duplications – parathyroid hormone-related protein (*PTHrP*) receptor (*PTHrPR*), the β adrenergic receptor (*βAR*), and the glucocorticoid receptor (*GR*) – were all instrumental in facilitating the evolution of all these traits.

There were at least five known attempts by vertebrates to breach land during the W-L-T based on the fossil record that involved crucial skeletal and concomitant visceral organ adaptations based on these duplications. The *PTHrPR*, which is essential for bone remodeling, is also necessary for the development of the lung and skin and is indirectly involved in the development of the kidney. Physiologic stress would have caused shearing of the microvasculature, particularly in key tissues and organs necessary for adaptation to land (skeleton, lung, skin, kidney), consequently generating radical oxygen species known to cause gene duplications. The over-expression of the *βAR* gene due to duplication overcame the constraint caused by the shared regulation of blood pressure in both the lung alveoli and the peripheral circulation; and in turn, glucocorticoid signaling in response to physiologic stress would have facilitated *βAR* overexpression. These physiologic adaptations may all ultimately have been facilitated by the evolution of the mammalian lung, during which intermittent phases of hypoxia would have stimulated the pituitary–adrenal axis. This would have increased the production of adrenaline by the adrenal medulla, culminating in relief of the hypoxic constraint by increasing surfactant secretion into the alveoli, making the alveoli more distensible due to reduced surface tension on the alveolar wall. As a consequence, there would have been increased production of PTHrP, fostering alveolarization and vascularization of newly formed alveoli. Such positive selection for PTHrP signaling may have fostered the aforementioned expression of PTHrP in both the pituitary and adrenal cortex, further amplifying the stimulation of adrenaline production. This stress-mediated mechanism thus enhanced both alveolarization and caused release of free fatty acids from peripheral fat cells. This would have resulted in increased overall metabolism, body temperature, and surfactant bioactivity, since the latter is 300% more active at 37 °C than at 25 °C. Thus, endothermy evolved as a result of these positive adaptations, mediated by the genes known to have been duplicated during the W-L-T in complicated physiologically directed linkages.

As added evidence of this evolutionary mechanism for the adaptation of vertebrate visceral organs during the W-L-T, the adrenal medulla of mammals formed vascular arcades during

this period. These further amplified the production of adrenaline due to the increased microvascular surface area. These vascular arcades may have been generated by *PTHrP* secreted by the adrenal cortex since it is angiogenic. In fish, the adrenal cortex and medulla are separated, lacking this amplification mechanism. However, under such functionally mediated positive selection pressure, the adrenal cortex and medulla evolved into one integrated structure sharing a common vasculature. That positive selection pressure for adrenaline amplification may have been a balancing selection for the hypoxic stress due to pulmonary insufficiency. This might explain why land-dwelling vertebrates have glomeruli, globular capillary complexes, which make fluid and electrolyte regulation more efficient for land habitation. PTHrP expression within the renal artery may have fostered the evolution of the glomerulus from the glomus, a much simpler vascular kidney invagination, since PTHrP is expressed in the podocytes lining the glomeruli, signaling to the mesangium for regulation of fluid and electrolytes. Therefore, the internal and external selection pressures for skeletal remodeling, air breathing, neuroendocrine stimulation, and kidney evolution were all positively benefited by the evolution of PTHrP signaling from fish to man due to the *PTHrPR* gene duplication.

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Critically, these particular gene duplications for vertebrate land adaptation are the very same genetic adaptations involved in the evolution of unicellular eukaryotes. Facilitated by cholesterol, linked physiologic drivers from the unicellular state yield metabolic complexity, locomotion, and respiration. So positive selection for these attributes should not come as a surprise, given the deep phylogenetic "history" of these biologic traits, referring all the way back to the Big Bang of the Cosmos [1]. All of these were part of the continuing attempt every organism must exert to maintain its homeostatic equipoise.

How might such complex, interrelated fundamental physiological mechanisms and evolutionary strategies bear on social systems? Even more importantly, what might be gleaned from these deeply rooted physiologic pathways that could productively relate to our all too "human" interactions? Obviously, civilizations have developed to support our physiologic needs for water, food, shelter, and mobility as organisms. These physiologic adaptations evolved in support of homeostasis as the prevailing

mechanism of evolution by such deeply linked mechanisms as have just been illustrated earlier. In contrast to the ad hoc nature of human cohabitation in large groups – village, city, state, country, nation state, or world community – social systems might in future be designed to effectively support human homeostasis in ways that would optimize physiology simultaneously on multiple scales, thereby maximizing our human potential. Through contemporary technological tools such as social networking, people would be enabled to provide biofeedback that would be used to fine-tune and "servo-regulate" such social systems in real time. Data strings could be used to both monitor and modify the social system in order to maintain societal equipoise as a thriving construct, avoiding "clogged arteries" and social decay; perhaps even more insidious, it is known that physiologic stress can directly give rise to psychological depression, having the opposite effect on society for multiple generations.

Such an organic construct would synergize human activity, empowering individuals to grow and flourish within their environments in concert with their own genetic makeup, referring all the way back to their unicellular origins. The critical point is that our physiologic mechanisms are profoundly interlocking and constantly monitored and assessed within us as biologic organisms, yet our human responses to our physiologic stresses are never systemically assayed in real time. Might that not benefit society if we had the ready means? Why would we leave such useful reciprocal feedback mechanisms to stimuli to the whim of such top-down entities as advertisers and governments? Or to myth and custom, perpetuating racial, gender, and ageist biases.

Witness Mark Twain's Huckleberry Finn, which is about social pathology. In Nafisi's *The Republic of Imagination* [5] she states that "…everything that is accepted as the norm, as respectable, is in essence not normal or respectable. It is a book in which 'educated' people are the most ignorant, stealing is 'borrowing,' people with 'upbringings' are scoundrels, goodness is heartless, respectability stands for cruelty, and danger lurks, most especially at home." Twain wrote the book as a way of making us aware of the pathology. Elsewhere, Nafisi states that "Ignorance of the heart, in this book, is the greatest sin." Entraining such metaphoric physiology in all of society is what we are inferring herein.

Any model such as this could be designed to effectively determine how the by-products of our living interactions might effectively be incorporated into our social structure, or discouraged in order to maximally benefit its inhabitants. It is clear that there are agencies within the environment responsible for disease and pathology. For example, smoking directly afflicts the smoker, but also causes deposition of nicotine in the environment, affecting newborns and toddlers by causing asthma. At all stages of the life cycle, deleterious agents may epigenetically affect any individual. Conversely, there are organic substances in the environment that are known to be beneficial, or might be shown to be so. These might be productively identified and husbanded for our benefit. With an appropriate feedback system, deliberate systematic inclusion and exclusion of a variety of substances could initially be based on experimental evidence, but could also be monitored on an on-going biofeedback-based mechanism, since there may be subtle effects not predicted by the model.

Importantly, the Physiologic First Principles model allows for monitoring of biologic systems based on homeostatic principles, instead of "input/output" metrics. It can be imagined just as one might consider a patient in the intensive care unit (ICU) recovering from a heart attack. The physician measures fluid and electrolytes in urine to try and bring the patient back into homeostatic balance. Because the heart is in failure, the lungs are filling with fluid, and the kidneys have shut down due to shock. The hope is that the patient will reset his homeostatic mechanisms by normalizing outputs downstream of the regulatory mechanisms. Care is concentrated on assessing fluid inputs and outputs that are only indirect biomarkers of renal, cardiac, and ultimately lung function. Yet, both the alveolus and glomerulus are "pressure transducers" which utilize endodermal PTHrP to regulate physiology by signaling to specialized fibroblasts in both structures. When these signaling mechanisms fail, the fibroblasts in both conditions default to the molecular Wingless/int (Wnt) pathway. In response, peroxisome proliferator activated receptor gamma agonists inhibit Wnt, attempting to normalize the homeostatic pathways of both the lung and kidney. This is the means by which physiology actually facilitates recovery of homeostasis. Certainly, a deeper level of understanding offers unique opportunities to intervene at an effective and

direct level as compared to any indirect assessment means. Such a true mechanistic understanding of physiology allows for higher-order regulation and correction based on fundamental operating principles.

The philosopher W.O.V. Quine [6] and his predecessor Duhem [7] had expressed concern that science was "underdetermined," sensing a lack of competency, leading to subjective conclusions instead of deterministic results. Some of this lack of clarity may have stemmed from misunderstanding our own physiologic makeup, leading instead to default decisions based on custom or subjective opinion rather than data-driven principles. Just like the patient in the ICU whose care could be rewardingly directed by a deep understanding of physiology, an alternative social system based on physiological realities using contemporary feedback tools could empower a society that is both empathetic and genuinely enlightened. Instead of the artificial mind–body duality of Descartes, we would have a totally integrated model of physiology on which to build social systems directly reflective of Man himself, not of his environment. What then are such deeply rooted, cell-based physiologic principles? They are the means by which cells govern themselves and have evolved: close collaboration, partnership, and reciprocality, as well as competition. A better human society, which systematically avoids stigma and suffering, has to be based on enacting and amplifying these cell-based physiologic principles. The means to do so by creatively utilizing modern feedback systems is a method that is finally within our grasp, though not yet in hand.

AQ3 ## References

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15

Evolution, Deception, and Public Health

Summary

The aggregate of ultimate and proximate causal relationships in evolution can be envisioned by reducing developmental and phylogenetic processes to their cellular–molecular elements, since both comply with developmental mechanisms, but in different time-frames. They are ultimately driven by large-scale environmental changes, particularly when the mechanisms of homeostasis and dyshomeostasis (pathology) are superimposed. Viewing descriptive biology in the forward direction from unicells onward, physiology can be understood logically, rather than dogmatically. By understanding what makes us "tick" at this fundamental level, we can better realize how we fit into the great scheme of things personally, societally, and as a species among species. Acknowledging that we exist through ambiguity, using deception to cope with this natural "sleight of hand" would help us stop deceiving ourselves, progressing as a species as a result.

Part I. Deception Is Deceiving: The Exception that Proves the Rule

> *We all know that Art is not truth. Art is a lie that makes us realize truth at least the truth that is given us to understand. The artist must know the manner whereby to convince others of the truthfulness of his lies.*
>
> *–Picasso.*

Evidence-Based Evolutionary Medicine, First Edition. John S. Torday, Neil Blackstone, and Virender Kumar Rehan

Introduction

Did you ever wonder why Man seems to be intimately connected with Nature, the planets, stars, and the Cosmos? Recognition of the First Principles of Physiology (FPP) as an integrating process for biology and physics offers the opportunity to understand how and why we have derived from the environment. By reducing developmental and phylogenetic processes to their cellular–molecular elements ultimately driven by large-scale environmental changes, the aggregate ultimate and proximate causal relationships can be envisioned, particularly when the mechanisms of homeostasis and dyshomeostasis (pathology) are superimposed. Viewing descriptive biology in the forward direction from unicells, physiology can be understood logically rather than dogmatically. By understanding what makes us "tick" at this fundamental level, we can better realize how we fit in to the great scheme of things – personally, societally, and as a species among species.

Having made these observations regarding the integration of the animate and inanimate, why is life full of deceptions, obfuscations, dualities, dialectics, cheating? There is no question that this is the case, as chronicled by Robert Trivers [1] in his landmark book *The Folly of Fools: The Logic of Deceit and Self-Deception in Human Life*. Perhaps in this case, the exception proves the rule? We would like to make the case for deception being innate to our origins, so naturally it would pervade our existence.

In the Beginning

One theory of the origin of life on Earth emphasizes the formation of the oceans, generated from snowball-like asteroids striking the planet's surface. Those asteroids also contained polycyclic hydrocarbons, which became suspended in the bodies of water. As the Sun warmed the waters during the day the lipids liquefied, expressing their hysteretic property, which is a physical form of "memory," deforming and reforming, ultimately generating protocells with semipermeable membranes. Within these structures endomembranes partitioned ions into positively and negatively charged species, creating bioenergetic flow. This electrical

potential fostered negentropy within the cell, circumventing the Second Law of Thermodynamics, regulated by homeostasis. In the aggregate, this configuration of negentropy, chemiosmosis, and homeostasis constitutes the FPP, and the first niche construction.

Epigenetics and Niche Construction

Possibly, as life thrived on Earth, it generated carbon dioxide, causing a "greenhouse effect" that warmed the atmosphere and dried out bodies of water on the Earth's surface. This caused some of the waterborne organisms to transition onto land, adapting to terrestrial life over eons. Two major characteristics that land life acquired, epigenetic inheritance and niche construction, were critically important for the successful adaptation to land. Epigenetic inheritance is the ability of the organism to acquire information directly from the environment; niche construction is the organism's ability to modify its immediate surrounding environment. When these two properties merge, it generates a dynamic capacity for the organism to adapt to its environment, maximizing its likelihood of survival and ongoing evolution. And when niches impinge on one another and/or coalesce, they form networks for ever-expanding niches, ultimately covering the surface of the Earth. In the aggregate, this is the mechanism underlying the Gaia Theory described by James Lovelock [2].

The Deception Proves the Rule

Robert Triver's book *The Folly of Fools* documents the foibles of Nature as deception. Cheating seems to be pervasive in Nature, yet biology is founded on the principles of cooperativity, so how can life constitute both of these characteristics? This seeming paradox is testament to the great "prank" that life has foisted on its physical environs, defying a fundamental law of Nature (see above), which behooves us to acknowledge this inherent slight of hand in order to be true to ourselves. There are so many dualities, dialectics, paradoxes, and counterintuitives encountered in human experience that could be resolved by acknowledging the inherent fallacy engendered in transitioning from the physical to the biologic.

The Quantum Physicist David Bohm [3] said that we misperceive our physical reality in his book *Wholeness and the Implicate Order* because we experience our physical surroundings through our subjectively evolved senses. The recognition that biology is pseudo-physics is of equal if not greater importance, disabusing ourselves of the pervasive notion, for example, that we are not machines. We are merely a mechanism for converting the physical into the animate, monitoring our ever-changing environment in order to survive, thrive, and communicate knowledge from one generation to the next effectively.

Armed with this more informed perspective, many otherwise dogmatic aspects of our being could be realized as a continuum from our origins forward. In a series of articles we have redefined many terms in biology as mechanisms in service to biology as communication – natural selection, the cell, homeostasis, pleiotropy, heterochrony, and the life cycle. With these insights, we are enabled to see how and why we have evolved as an integrated whole, as an agent for collecting information from the environment rather than as the result of random mutations, seemingly without rhyme or reason – no wonder people default to belief rather than science. Importantly, this holistic vision offers the opportunity to fully appreciate our ecology, ourselves, and all organisms as one grand scheme, as referred to in the opening paragraph of this chapter.

Our Own Personal Heliocentrism

We could even formulate a periodic table of biology, integrating all of the natural sciences into one functionally predictive database. A similar realization that the Earth is the center of the Solar System fundamentally changed human thought and action – likewise, a firm understanding of where we came from (ontology), and how (epistemology), would have equal if not greater impact on human thought. Prior to the recalibration of the Earth as one of the planets circling the Sun, autocrats and soothsayers had control of humanity, striking fear in their hearts and minds through ignorance. But then came technological breakthroughs like the telescope and microscope, offering knowledge of our insides and outsides that raised our sights

and curiosity. And with the advent of the scientific method, we were enabled to "know what we do not know."

But the stigma of deception remains as a barrier to our fullest knowledge of who and what we are as a species. Like our instrumentation, there is a "signature" in our perception of Nature that distorts our ability to fully realize our own potential. Purging our outlook of that signature would finally allow us to understand who and what we are, unencumbered by the baggage of our origins in deceit. And perhaps, for the first time in human history, ethics would precede technology because we will have figured out the "rules" and used them as guidelines for space exploration, human genetic engineering, artificial intelligence, artificial reproduction, genetically modified foods, and human discourse.

Deception and Social Pathology

Deception arose from the very origins of life itself, "cheating" Mother Nature by circumventing the Second Law of Thermodynamics. By utilizing the FPP as a means of instituting self-organization and self-reference, life has been able to generate a mechanism distinctly different from the physical laws of the Universe. Bohm has stated that the end result has been two different realms, the explicate and implicate. The explicate realm is the one we think of as reality, when in fact it is one of our own making, distorted by our subjective, evolved senses. The true reality, which Bohm refers to as the implicate realm, exists on another perceptual plane. This duality is what has led to the deceptions we are familiar with in the explicate realm, offering the opportunity to cope with the inherent paradoxes we encounter daily. Our own physiology has equipped us with the ability to endure such duplicity, but the consequence of that is "stress" – the stimulation of the hypothalamic–pituitary–adrenal (HPA) axis. In its optimal state, the stress reaction facilitates learning, offering the opportunity to dominate the circumstances and evolve novel structures and functions that mitigate and can even eliminate the source of the stress – evolving means internalizing otherwise-toxic substances in the environment, metabolic cooperativity/multicellularity, endothermy/homeothermy – or what we think of as physiologic evolution. Ultimately, such adaptive

strategies, in combination with niche construction and epigenetic inheritance, can lead to homeostatic balance, both physically and physiologically, at least for the moment. However, there are conditions that are not conducive to such harmonious outcomes. In human evolution, there are social constructs that are not conducive to homeostatic balance because they are predicated on false principles – autocracies, communism, and oligarchies – what Jared Diamond [4] discusses in his book *Collapse* as the inability of social systems to integrate with their environmental surroundings. Such conditions perpetuate stress, resulting in elevated levels of cortisol and adrenocorticotrophic hormone (ACTH), causing physiologic wasting in the host and transgenerational depression in the off-spring.

Conversely, if we were able to recognize the systematic problem in perpetuating societal deception, perhaps we could live in a more harmonious environment. Peter Whybrow addresses this in his book *American Mania*, seeing the pathology from the point of view of a social scientist. And this problem is becoming endemic and pervasive with the advent of computer technology because it feeds into narcissistic behavior that resulted from the deceptions in the first place. Dacher Keltner [5] has pointed out that we humans are naturally cooperative in his book *Born to be Good*, which is based on experimental evidence.

Physiologic Stress

Hans Selye [6] coined the term stress in *The Stress of Life (1956)*. It describes the physiology of the "fight or flight" mechanism. Stimulation of the HPA axis under duress is critical for survival, fostering learning under optimal conditions, but when overstimulated it can also cause disease.

The evolution of this integrated mechanism is most apparent during vertebrate adaptation to land, when the adrenal cortex and medulla evolved into one structural–functional unit. Prior to that, these two elements of the adrenal gland were physically separate structures. The merging of these two components of the adrenal gland constituted more than just a physical change; it had a profound effect on physiologic adaptation since the microvasculature of the corticoid-producing cortex was continuous with that of the catecholamine-producing medulla.

Under stress conditions, increased production of ACTH by the anterior pituitary stimulates corticosteroid production by the adrenal cortex; the corticoids produced by the cortex pass through the adrenal medulla, stimulating the rate-limiting step in catecholamine production, phenylethanolamine-*O*-methyltransferase (PNMT). Consequently, catecholamine production is increased, augmenting many tissues and organs necessary for adaptation to physiologic stress – vasodilation, increased lung function, and glycogenolysis/gluconeogenesis.

In a recent article, the evolution of endothermy/homeothermy in mammals and birds was attributed to this mechanism [7]. Briefly, the lung evolved in a stepwise manner mediated by cell–cell interactions during the water–land transition in response to the increasing demand for metabolic drive. Periodically, the evolving lung would be inefficient for gas-exchange as evidenced by the fossil evidence for at least five independent attempts to breech land, suggesting a salutatory process of trial and error that would also have affected visceral organ development. That speculation is supported by the fact that when parathyroid hormone-related protein (*PTHrP*) gene is deleted in the developing mouse embryo, it results in the failure to alveolarize the lung, calcify bone, and fully develop skin barrier function. The PTHrP signaling mechanism was amplified during the water–land transition due to the duplication of the PTHrP 1 receptor (PTH1R), likely due to the internal selection pressure for these specific tissues and organs generated by microvascular shear stress in adaptation to land.

In tandem with the above-mentioned stresses, the stimulation of the HPA axis would have increased the production of catecholamines, alleviating the stress on the lung by stimulating lung-surfactant production by the alveoli, acutely increasing the alveolar surface area for gas-exchange. Ultimately, this way of alleviating the constraint on lung gas exchange would have increased the gas-exchange surface area constitutively since the increased distension of the lung alveoli would have stimulated PTHrP production by the alveolar type II cells, fostering more the formation of additional alveoli.

In parallel with their effect on the evolution of the lung, catecholamines would also have stimulated the secretion of fatty acids from fat cells in the periphery, increasing body

temperature due to increased metabolism. This acute increase in body temperature would have been positively selected for since warm-blooded organisms require only one enzyme isomer per metabolic function, whereas cold-blooded organisms require several isozymes in order to accommodate their ambient environmental temperature efficiently. The former is much more energy efficient than the latter, favoring endothermy/homeothermy. This is consistent with the huge decrease in the genome of vertebrates in the post-Cambrian Burst era.

Elsewhere, we have speculated that the evolution of endothermy in mammals and birds may have fostered bipedalism (both humans and birds are two-legged) since it takes more energy to walk on two legs than on four. The freeing of the forelimbs for specialized functions like flight and tool making would have offered positive selection for this cascade, putatively culminating in the higher consciousness seen in humans and birds.

In support of this hypothetical mechanism for the evolution of endothermy/homeothermy, the PTHrP signaling mechanism appeared in the pituitary and adrenal cortex in association with expansion of the microcirculation in the adrenal medulla. These novel mechanisms were likely due to the duplication of the *PTHrPR* gene, amplifying the stress reaction by increasing the production of ACTH and corticosteroids. The increased microvasculature in the adrenal medulla may have been a consequence of the increased PTHrP production by the adrenal cortex since PTHrP is angiogenic. This expansion of the adrenal medullary microcirculation would have further amplified the production of catecholamines by increasing the surface area for the corticoid stimulation of PNMT. Why there was such positive selection for the fight-or-flight mechanism in mammals may have been because it was advantageous to be "nimble" in evading predators, particularly in the case of hominids, who evolved from small rodent-like creatures.

Therefore, stress had a positive effect on vertebrate evolution. Yet, too much of a good thing may lead to the law of unintended consequences. For example, we know that excessive myelination of neurons may lead to neurodegenerative diseases. And there may be long-term consequences of physiologic stress, causing transgenerational depression.

Ambiguities in Biology

- **The cell** – We conventionally think of the cell as the smallest functional unit of life. But when it is seen from the perspective of evolution, it constitutes the FPP. Life was constrained by the Second Law of Thermodynamics, but the cell solved that problem by generating negentropy through chemiosmosis, regulated by homeostasis. It is those foundational principles that allowed for both sustaining and changing the phenotype when necessary.
- **Homeostasis** – Homeostasis is conventionally thought of merely as a synchronic (same time) servo-mechanism that maintains the status quo for organismal physiology. However, when seen from the perspective of developmental physiology, homeostasis is a robust, dynamic, intergenerational, diachronic (across-time) mechanism for the maintenance, perpetuation, and modification of physiologic structure and function. The integral relationships generated by cell–cell signaling for the mechanisms of embryogenesis, physiology, and repair provide the needed insight into the scale-free universality of the homeostatic principle, offering a novel opportunity for a systems approach to biology. Starting with the inception of life itself, with the advent of reproduction during meiosis and mitosis, moving forward both ontogenetically and phylogenetically through the evolutionary steps involved in adaptation to an ever-changing environment, biology and evolution theory need no longer default to teleology.
- **Aging** – Organisms have survived because they have devised adaptive genomes that allow them to change in response to the ever-changing nature of Earth's environments. This has come in the form of their reproductive strategy, which is optimized to generate the largest number of offspring suited for the environment into which they are born. This comes at a cost, because the energy of reproduction is selected to optimize the organism's internal physiologic milieu. But that energy debt must somehow be repaid because the Second Law of Thermodynamics cannot be violated – the first and second laws of thermodynamics state that the total energy content of the universe is constant, and that total entropy is continually increasing.

This assumes that there is a finite amount of energy during the lifecycle. Hayflick has unequivocally stated that longevity is genetically determined, whereas aging is epigenetic. Therefore, by definition, there must be a finite amount of energy generated during the lifecycle of any organism, which is then distributed throughout the period between birth and death in response to selection pressure for reproductive success. As a result, the bioenergetics are optimized during the reproductive phase, followed by a progressive loss of energy during the post-reproductive phase of life, leading to the breakdown in cell–cell communication, aging, and ultimately death, as a result of the progressive increase in entropy. This mechanistic explanation for the process of aging is consistent with descriptive theories of aging such as the mutation theory, antagonistic pleiotropy, and the disposable soma.

- **Pleiotropy** – In contrast to the probabilistic way of thinking about pleiotropy as the random expression of a single gene that generates two or more distinct phenotypic traits, it is actually a deterministic consequence of the evolution of complex physiology from the unicellular state. Pleiotropic novelties emerge through recombinations and permutations of cell–cell signaling exercised during reproduction based on both past and present physical and physiologic conditions, in service to the future needs of the organism for its continued survival. Functional homologies ranging from the lung to the kidney, skin, brain, thyroid, and pituitary exemplify the evolutionary mechanistic strategy of pleiotropy. The power of this perspective is exemplified by the resolution of evolutionary gradualism and punctuated equilibrium in much the same way that Niels Bohr resolved the paradoxical duality of light as complementarity.
- **Life cycle** – Based upon observation, the life cycle describes the milestones of an organism, starting with birth, infancy, childhood, adolescence, teenage, adult, senescence, and death. Yet, we know that there is a great deal of variability in these stages of life, both within and between species. Hominids have a protracted infancy and childhood, which is usually attributed to the amount of time required to form our oversized brains; neoteny is the process by which an organism retains its juvenile phenotype; longevity is highly variable, as

exemplified by the May Fly, which only lives for a day, and the Giant Sequoia, which lives for thousands of years. What should we make of this variability? Elsewhere we have laid claim to the idea that since the epigenetic marks acquired during the life cycle are not expunged during meiosis, that their incorporation into the developing conceptus during embryogenesis is similarly a means of determining the "fit" of those epigenetic marks based on homeostatic principles. Based on that idea, why should we assume that the influence of epigenetic inheritance stops at the time of birth? Perhaps the phases of the life cycle are also a way of utilizing epigenetic inheritance.

Since the stages of the life cycle are determined by the endocrine system, that would be a place to look for the influence of epigenetics. As it turns out, epigenetics does affect the endocrine system, substantiating the fact that epigenetics affects the organism at all stages of the life cycle.

- **Phenotype** – The conventional understanding of phenotype is as a derivative of descent with modification through Darwinian random mutation and natural selection. Recent research has revealed Lamarckian inheritance as a major transgenerational mechanism for environmental action on genomes whose extent is determined, in significant part, by germ line cells during meiosis and subsequent stages of embryological development. In consequence, the role of phenotype can productively be reconsidered. The possibility that phenotype is directed toward the effective acquisition of epigenetic marks in consistent reciprocation with the environment during the life cycle of an organism is explored. It is proposed that phenotype is an active agent in niche construction for the active acquisition of epigenetic markers as a dominant evolutionary mechanism rather than a consequence of Darwinian selection toward reproductive success. The reproductive phase of the life cycle can then be appraised as a robust framework in which epigenetic inheritance is entrained to affect growth and development in continued reciprocal responsiveness to environmental stresses. Furthermore, as FPP determine the limits of epigenetic inheritance, a coherent justification can thereby be provided for the obligate return of all multicellular eukaryotes to the unicellular state.

- **Economics** – Whybrow P. *American Mania* [8], "Hence, in simple terms, it is the dynamic tension between innate desire and social learning that determines individual behavior and underpins the extraordinary complexity of the myths and social agreements that we call human culture. And because of this tension we rarely exercise the basic instincts of self-preservation as solitary animals but rather do so in competitive collaboration with others. The give-and-take of a market economy may be understood within such a conceptual framework as a natural by-product of human social evolution, one where competitive collaboration is exploited as a collective benefit. Thus, with the adoption of a few rules – such as honesty in competition, respect for private property, and the ability to exchange goods for money – a market culture is essentially an ordering of human instinct and competition by those traditional cooperative, sharing practices that our forebears found to be fruitful and successful. Through the give-and-take of social interaction, and through internalization of the conventions and customs it promotes, instinctual self-interest is liberated and molded to the common good. The capitalist enterprise is founded on this dynamic principle."

Part II. Resolution of the Ambiguities by Assimilating the Deception

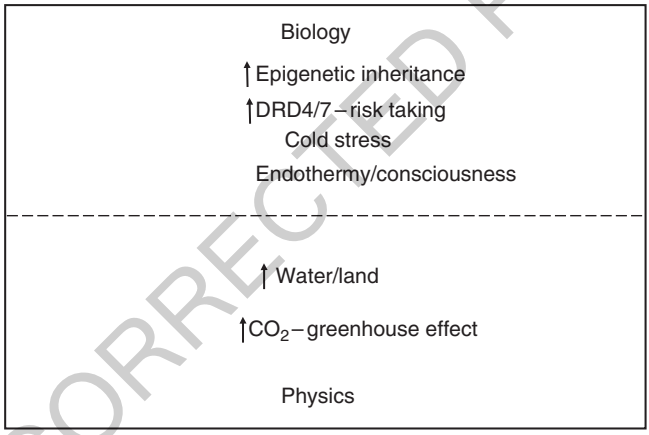
Introduction

Part I explained the origin of the ambiguities in biology resulting from failing to acknowledge the deception of the Second Law of Thermodynamics. Once that is achieved we can resolve many of the misunderstandings that have been become dogma in biology, as follows:

The Cell as the First Niche Construction – Self-Organization Overcomes the Ambiguity

Niche construction nominally describes how organisms can form their own environments, increasing their capacity to adapt to their surroundings. It is hypothesized that the formation of

the first cell as "internal" niche construction was the foundation for life, and that subsequent niche constructions were iterative exaptations of that event. The first instantation of niche construction has been faithfully adhered to by returning to the unicellular state, suggesting that the life cycle is zygote to zygote, not adult to adult as is commonly held. The consequent interactions between niche construction and epigenetic inheritance provide a highly robust, interactive, and mechanistic way of thinking about evolution being determined by initial conditions rather than merely by chance mutation and selection. This novel perspective offers an opportunity to reappraise the processes involved in evolution mechanistically, allowing for scientifically testable hypotheses rather than relying on metaphors, dogmas, teleology, and tautology.



The Evolution of Endothermy as Internal Niche Construction; or, Self-Organization Overcomes Biologic Ambiguities

Only mammals and birds are warm-blooded, or endothermic. How this trait evolved has never been explained based on an integrated physiologic mechanisms emanating from the ontogeny and phylogeny of visceral organs. A recent paper on the role of physiologic stress in the evolution of endothermy based on the appearance of specific physiologic traits in birds and mammals has provided such an explanation for the first time, as follows [7]:

Conditional endothermy – It has been hypothesized that endothermy evolved as a direct consequence of intermittent hypoxia during the water-to-land transition. Briefly, vertebrates breeched land several times based on fossilized skeletal evidence in order to avoid the extinction of drying up bodies of water. Since our overarching hypothesis is that visceral organs evolved through cell–cell interactions, as the lung evolved from the swim bladder of fish there would have been stages at which the lung was inefficient, resulting in hypoxia; hypoxia is the most potent of all physiologic agonists, causing stress, stimulating the HPA. The net result would have been increased catecholamine production, which would have alleviated the constraint of the inefficient lung by stimulating surfactant production, increasing the distensibility of the alveoli and thus their surface area, increasing oxygenation acutely. Over time, this ad hoc response to hypoxia evolved into increased numbers of alveoli since stretching of the lung stimulates PTHrP, which promotes alveolarization of the lung. As evidence for this mechanism, PTHrP appears in the pituitary of mammals and birds, where it augments ACTH production. PTHrP also appears in the adrenal cortex of mammals and birds, where it augments the effect of ACTH on corticosteroid. Corticosteroids produced in the adrenal cortex of mammals and birds stimulate catecholamine-*O*-methyltransferase activity in the adrenal medulla, amplifying epinephrine production. As a note added in proof of the evolutionary amplification of the HPA by PTHrP, Wurtman has shown that the microvasculature of the adrenal medulla is augmented in rats, increasing the surface area of the capillaries for corticosteroid amplification of the epinephrine production.

In tandem with the facilitating effect of catecholamines on air breathing, it also stimulates free fatty acid secretion by fat cells in the periphery, providing substrate for enhanced metabolism, increasing body temperature. Ultimately, the increase in endotherm body temperature would have been selected for since warm-blooded metabolism is much more efficient than cold-blooded. In order to metabolize efficiently, a warm-blooded organism requires several forms of the same enzyme to accommodate metabolism at different environmental temperatures, whereas endotherms/homeotherms only require one. This increased metabolic efficiency is evolutionarily advantageous in being much more functionally efficient.

The causal nature of the interrelationship between physiologic stress, catecholamines, and endothermy/homeothermy is validated by the reverse effects of hibernation or torpor on lung-surfactant lipid composition and cell membrane fatty acid composition. Under such conditionally low stress conditions, decreased catecholamine production results in both increased surfactant cholesterol, rendering lung surfactant less surface active, and decreased unsaturated fatty acid content of cell membranes, adaptively reducing oxygen uptake. And there are commonalities between stress, endothermy/homeothermy, hibernation, and meditation, leading to thoughts about the role of this mechanism in fostering higher consciousness (see below).

Constitutive endothermy – Ultimately, the endothermic phenotype became functionally integral to the organism. Recently, it was discovered that deletion of the oxytocin gene in mice inhibited their ability to thermoregulate, indicating that this hormone is central to endothermy.

Stress-Induced Evolution of Endothermy by Stepwise Changes in Physiology Predicts Bipedalism, Evolution of the Avian and Hominid Forelimbs, and Higher Consciousness

It is noteworthy in the context of metabolic evolution that both birds and humans are bipedal, which may have been a consequence of their both being endotherms. Being upright is metabolically costly, but by increasing their body temperatures in adaptation to land, both birds and humans have become much more metabolically efficient; cold-blooded organisms require multiple isoforms of the same metabolic enzyme to survive at ambient temperatures, whereas endotherms usually have only one isoform. Bipedalism may have resulted, freeing the forelegs to evolve into wings and hands with prehensile thumbs through common genetic motifs.

Hobson and Friston [9] have hypothesized that the brain must actively dissipate heat in order to process information. This physiologic trait is functionally homologous with the first instantiation of life formed by lipids suspended in water-forming micelles allowing the reduction in entropy (heat dissipation). This circumvents the Second Law of Thermodynamics permitting the transfer of information between living entities, enabling

them to perpetually glean information from the environment, that is felt by many to correspond to evolution per se. The next evolutionary milestone was the advent of cholesterol, embedded in the cell membranes of primordial eukaryotes, facilitating metabolism, oxygenation, and locomotion, the triadic basis for vertebrate evolution. Lipids were key to homeostatic regulation of calcium, forming calcium channels. Cell membrane cholesterol also fostered metazoan evolution by forming lipid rafts for receptor-mediated cell–cell signaling, the origin of the endocrine system. The eukaryotic cell membrane exapted to all complex physiologic traits, including the lung and brain, which are molecularly homologous through the function of neuregulin, mediating both lung development and myelinization of neurons. That co-option later exapted as endothermy during the water–land transition, perhaps being the functional homolog for brain heat dissipation and conscious/mindful information processing. The skin and brain similarly share molecular homologies through the "skin–brain" hypothesis, giving insight into the cellular–molecular "arc" of consciousness from its unicellular origins to integrated physiology. This perspective on the evolution of the central nervous system clarifies self-organization, reconciling thermodynamic and informational definitions of the underlying biophysical mechanisms, thereby elucidating relations between the predictive capabilities of the brain and self-organizational processes.

Cold Stress and DRD4–7: Did Risk-Taking Drive Us Out of Africa?

Peter Whybrow's book *American Mania: When More Is Not Enough* [8] makes the case for the Dopamine Receptor DRD4–7 being the cause for primates migrating out of Africa, since it is associated with risk taking. He also mentions that at the time of the migration out of Africa that the world was a lot colder than it is now … based on geological evidence until about thirteen thousand years ago when the world began warming up, glaciers covered North America and arctic conditions came and went with the seasons. Land masses were interconnected by ice bridges, facilitating human dispersal both north and east.

Migrant behavior is of considerable biological importance because it leads to "gene dispersal" and reproductive advantage. "Out-migration," or dispersion as the primatologists call it, is dangerous (risky) but it opens up new opportunities. In most primate species, some animals will ultimately leave the group of their birth and seek another habitat. Commonly it is the males, but for some – in chimpanzees, gorillas, and spider monkeys for example – it is the females. Most out-migration occurs in adolescence, when risk taking increases. "It is important to understand that in most monkey groups the adolescents leave because they want to, not because they are driven out." There is a second factor that interacts with risk-taking predisposition of those who migrate, the competition for scarce resources. This is where social rank becomes important in determining which animals leave the troop. In bad times, when there is not enough food to go around, the high-ranking animals usually stay in place and the aggressive lower-ranking animals are those most likely to leave. Such dispersion does not happen regularly or in every generation but when it does occur it has a major impact on future generations by weeding out the parent troop and potentially seeding new ones.

Four adult male rhesus monkeys formed a new social group with 13 adult females. The male who became dominant (alpha) showed a progressive increase in plasma testosterone. The male who became subordinate to the other three males showed an 80% fall in testosterone from baseline levels. After 7 weeks, this group was introduced to a well-established breeding group, and all four males became subordinate to all members of the breeding group. All four males evidenced a fall in testosterone during the first week after introduction, and within 6 weeks their levels were approximately 80% of pre-introduction values. The alpha male of the breeding group showed a large increase in testosterone (238%) 24 h after he successfully defended his group and became the dominant animal of the larger, newly formed group. Thus, plasma testosterone levels appear to be significantly influenced by the outcome of conflict attendant to alterations in status of rhesus monkeys living in social groups.

Numerous studies of migrant populations all over the world support Fairbanks's conjectures…. Optimism, self-interest, curiosity (often described as restlessness or novelty seeking),

and a vigorous ambition are the best predictors of emigres' adjustment to their new environment. Studies show that ambition and optimism are more commonly expressed in the men than in the women who migrate.

During the Miocene, 20 million years ago, a global cooling began, and it was under these challenging circumstances, as the food supply dwindled and competition for survival increased, that our direct forebears emerged. We know from the fossil record and genetic studies that humans, gorillas, and chimpanzees all descended from common ancestors – small ape-like creatures, called hominids, that were distinguished by walking upright – who lived late in the Miocene period, some five to seven million years ago.

Novelty seeking, curiosity, and impulsive behavior are interrelated. Fairbanks has found that the most impulsive and risk-taking males in her colony are those who have the lowest levels of the serotonin breakdown product 5-hydroxyindolacetic acid (5-HIAA) in their cerebrospinal fluid (CSF) (serotonin modulates behavior, opposing the curiosity provoking dopamine superhighway and the alerting drive of norepinephrine). In some individuals or subspecies, serotonin only weakly opposes the dopamine drive, so they may not be genetically "programmed" for migratory behavior.

Jay Kaplan [10] has found that those rhesus males that remain within a troop beyond puberty have higher levels of 5-HIAA in their CSF. In baboons in the Rift Valley, in whom dispersal occurs around puberty, there is an inverse relationship between serotonin levels and dispersal, again suggesting a strong role of dopamine drive in migratory behavior.

How Androgens Act to Reduce Ambiguities of Life

The sex ratio is defined as the number of males to females. At the time of conception, the sex ratio is 4 : 1, whereas at birth it is 1 : 1, which raises the question as to why three out of four males die during development. There are two peaks of fetal demise during pregnancy, the first occurring at 16–18 weeks gestation, the second during the peripartum period. The cause of excess male deaths during the peripartum period is largely due to the relative immaturity of the male lung, caused by the

production of androgens in the male conceptus delaying lung development. The earlier demise at 16–18 weeks is the much larger population of spontaneous abortions, which is also due to the production of androgens by the fetus, as follows. At this stage of development the maternal ovary produces progesterone that maintains the pregnancy. The progesterone in turn stimulates human chorionic gonadotropin (HCG), which is produced by the placenta and stimulates development of the fetal gonads. The fetal testis and ovary synthesize androgens in response to HCG, which pass from the fetus to the mother via the placenta. Androgens can inhibit progesterone synthesis if they are produced in too large an amount, causing the abortion of the fetus. This mechanism selects out such "super males," culling such fetuses that produce large amounts of androgen, causing fetal overgrowth, endangering the life of both the conceptus and the mother at birth because the fetus cannot pass through the birth canal. So we see here an example of how the sex steroids are being exploited as a fail-safe mechanism for reproductive health.

How Art Seemingly Resolves the Deception of Life

When we view works of art, we often find solace or escape from "reality" in the content because it provides reassuring evidence that there is logic or truth in Nature … this is because the artist is providing a way of seeing reality in ways that are self-organizing and self-referential, much like our biologic origins. The artist who painted the first cave paintings in Lascaux, France, was probably telling a story about the hunt, providing a rationale for life. The use of techniques in painting that encourage the viewer's eye to come full circle in appreciating the content of the work, for example, giving one the sense of an integral whole. So art encourages us to think that there is harmony in the Universe, if only we could see it.

How Music Resolves the Deception of Life

Music similarly teaches us that there is harmony in the Universe, likes Holst's "Music of the Spheres." Again, we find refuge here but fail to find resolution outside of the musical construct. Instead, at least for us, it was encouragement to think that

perhaps science could resolve this ambiguity. My realization that biology is a deception, cheating Nature by circumventing the Second Law of Thermodynamics, providing deep insight into the fundament of life as a pseudo-physical construct. Many physicists, such as Prigogine, Polanyi, and LL Whyte, have tried to understand this interrelationship but have failed. Prigogine assesses life's irreducible complexity in his book *Order Out of Chaos*, in which he concludes that biology is too complicated to define. In contrast to such attempts to understand biology by analyzing it in its present synchronic form, we have approached the question of the mechanism underlying evolution by starting from its cellular origins, moving forward in biologic time diachronically, eliminating time and space to reveal the absolute nature of the process. This is analogous to the physicists viewing the Universe as having originated from the Big Bang, and understanding such phenomena as the patterned distribution of the elements and the cosmic microwave background, with the formation of black holes and supernovas as a result.

Literature (Deceptively) Resolves the Ambiguities of Life

Liturgy Resolves the Ambiguities of Life: Back to the Garden?

Genesis teaches us that we originated in the Garden of Eden, an ideal world that was "lost" through knowledge. We would submit that that "knowledge" failed to recognize the ambiguity, instead reinforcing it through the teachings of religion, institutionalizing our ignorance of our true biologic origins. Once this paradox is realized, we as a species can rid ourselves of the falsities imbedded in our mores and reasoning, much like the frame-shift caused by the realization that the Sun is the center of the Solar System, which gave rise to the Age of Enlightenment.

Part III. Deception and Public Health

Assuming that deceit can lead to stress, the magnitude of the deception would correlate with the incidence of disease, given that physiologic stress inhibits the immune system, promulgating infectious disease, chronic disease, and cancer. Chronic stress

also leads to depression, both within and between generations, attributed to chronically elevated cortisol levels. For these reasons, it is important to recognize the existence of deception both in society and within ourselves; it is when we begin to believe in the deceptions that we are most affected by them. Witness the effect of the lending fraud that caused the collapse of the world financial system in 2008; or the Tulip "bubble" in the sixteenth century. And more recently, it has been acknowledged that Americans have not received a pay raise in three or four decades, associated with increased incidence of premature death among white males, and the escalating use of opiates. There is experimental evidence in rodents, for example, that if the mother is stressed, her offspring will suffer from physiologic depression, causing intergenerational malaise, as was seen in the Soviet Union. Or the attitude of the people in the Hundred Years War – in Berthold Brecht's Mother Courage – when told that peace had been declared, Mother Courage says "Oh no, now what shall we do."

Cognitive Dissonance: Scientific Principles, Disease, and Health

Most of hominid history has been dominated by myth making. It is only in the last 500 years that we have begun to emancipate ourselves intellectually using the scientific method as a way of "knowing what we do not know." The use of science to leverage truth is a powerful weapon against the deception built into our DNA. The mere fact that Creationism has held sway over evolution theory speaks to the fact that there is currently no scientific evidence for the latter, so the debacle comes down to one belief system versus another.

We must be able to address evolution theory using scientifically testable and refutable methods. It has been proposed that a cellular–molecular approach be used for scientifically determining the evolution of vertebrate physiology based on cell–cell communication [11]. Thus far, this approach has been used to redefine a series of otherwise dogmatic concepts in biology – natural selection, the cell, homeostasis, heterochrony, pleiotropy, phenotype, life – successfully showing the value added in understanding these processes mechanistically rather than

descriptively. Moreover, experimental data have demonstrated developmental and phylogenetic properties common to amphibian and mammalian lung, hypothesizing that leptin evolved as a cytoprotective mechanism against oxidant injury. Since evolution is a structurally–functionally linked series of exaptations, it was predicted that leptin would have the same effect on the amphibian lung as it does on the mammalian lung. Elsewhere, it has been argued that the use of cell–cell communication will reveal the same evolutionary mechanisms for all of physiology [11], given that it can be traced back to the unicellular eukaryotic state using cholesterol-related traits as the common denominator to vertically integrate physiology.

Part IV. Prediction: Bioethics Based on First Principles of Physiology

The origin of life on Earth began with the formation of the first cell, composed of the polycyclic hydrocarbons that were transported on the asteroids that formed the oceans, or composed of mineral pores in deep-sea alkaline hydrothermal vents. Partitioning the cellular internal milieu and the external environment, chemiosmosis fostering negentropy regulated by homeostasis were the FPP. In turn, this construct paradoxically fostered both Free Will, but it was determined by such FPP. The Earth was populated by unicellular life for the first four billion years of its existence; it is only in the last 500 million years that complex multicellular organisms have existed. In all likelihood, this transition occurred because of competition with prokaryotes that have the capacity to act as pseudo-multicellular organisms through the formation of biofilm and quorum sensing. Eukaryotes cooperate through cell–cell signaling mediated by soluble growth factors and their cognate receptors. Such cooperativity was important because the rising levels of oxygen in the atmosphere created positive selection pressure for cells to utilize environmental nutrients through oxidative metabolism.

Darwinian evolutionists would have us think that this process was dictated by survival of the fittest, which implies competition rather than cooperation. The British Philosopher Deryk Parfit pondered the paradox of cooperativity and competition in a

biography entitled "How to be Good" published in *The New Yorker* magazine in 2011. In the article he openly questioned the dichotomy of these two processes. Based on the first 14 chapters of this book, internal cellular functions have evolved through cooperation. It is only when we turn to the overt behavior of animals that we witness the "tooth by jowl" combat that Darwin thought was the genesis of evolution. But such activity cannot generate phenotypic novelty. It is more likely that such behavior increases adaptation to a niche environment, fostering the acquisition of epigenetic marks from the environment. If such a process occurs recurrently, it could foster novel traits.

Darwin's contribution to humanity was extricating us from The Great Chain of Being. The metaphors of his theory of evolution ("survival of the fittest" and "natural selection"), however, are not conducive to scientific testing and as a result there is no experimental evidence for these processes of evolution. The process of evolution is instead based on epigenetic inheritance, which stems directly from the environment – and not just an arbitrary environment, but the niches we personally construct, like beavers building dams, worms conditioning the soil around them to accommodate their water-adapted kidneys, or humans building cities and cultural environments, referred to as Niche Construction Theory [12]. It is within those ecologic niches that environmental change is monitored over the course of the organism's life cycle, incorporated into germ cells as epigenetic marks, either maintaining equipoise or "evolving" accordingly due to sorting of the epigenetic marks during meiosis, embryogenesis, and over the course of the life cycle [12]. That process refers all the way back to the first eukaryotic cell, based on the FPP. The construction of that protocell, distinguishing external from internal (Claude Bernard) niche, conferred both free will and determinism on life as the origin of morality [12].

In an earlier essay published in the Humans & Nature *Minding Nature* journal, entitled "Man is Integral with Nature," [13] William Miller and I made the case for the intimate relationship between physics and biology, annealed by the formation of the first cell from the lipids delivered by snowball-like asteroids during the early history of the planet. Briefly, lipids will spontaneously form primitive "cells," or micelles, in water. Such semipermeable membrane-bound spheres can generate bioenergy

and reduce the entropy, or order, within them, referred to as negentropy [14], controlled by homeostasis. That founding relationship, based on the FPP allows biology to circumvent the Second Law of Thermodynamics. That Faustian pact affords us the free will to test those constraints, but the aforementioned physiologic principles of life are determined. We live between those two boundary conditions, which we refer to as morality.

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