A Cell–Molecular Approach Predicts Vertebrate Evolution

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Abstract

In contrast to the conventional use of genes to determine the evolution of phenotypes, we have functionally integrated epithelial–mesenchymal interactions that have facilitated lung phylogeny and ontogeny in response to major geologic epochs. As such, this model reveals the underlying principles of lung physiology based on the evolutionary interactions between internal and external selection pressures, providing a novel understanding of lung biology. As a result, it predicts how cell–molecular changes in this process can cause disease and offers counterintuitive insights to diagnosis and treatment based on evolutionary principles.

Key words: lung, phylogeny, ontogeny, ecology, mesenchymal–epithelial interaction, selection pressure.

Introduction

In contemporary biology and medicine, we are faced with the challenge of merging phenotypic patterns with the genomic and molecular biologic data that generated them. These interrelationships are most apparent during development because the genetic changes that determine morphogenesis are causal (Warburton et al 2010). Developmental homeostatic interrelationships can be exploited to deconvolute the evolution of the lung and other organs (Torday and Rehan 2007, 2009) but only as a model for the molecular evolution of physiologic principles because we will never know what actually transpired. That relationship is depicted in figure 1. By focusing on the role of lung surfactant as the driving force behind the progressive increase in the gas-exchange surface area during lung development and phylogeny (fig. 1, left-hand panel) (Torday et al. 2010), we have envisioned how lung cell–cell interactions might have facilitated the metabolic demand for oxygen in preparation for birth developmentally; we have speculated that these same principles facilitated the evolution of the lung from the fish swim bladder because the same cell–cell interactions that determine lung surfactant production and function in reducing the alveolar diameter (and hence the efficiency of gas exchange) are involved in lung phylogeny. The phylogenetic increase in surfactant production, and the thinning of the alveolar wall, in combination with stretch regulation of ventilation–perfusion matching, have facilitated the decrease in alveolar size. That process has allowed for the increase in the gas-exchange surface area-to-blood volume ratio, optimizing gas transfer across the alveolar wall (Torday and Rehan 2004).

Mechanistically, this process was mediated by parathyroid hormone-related protein (PTHrP) (fig. 1, right-hand panel), which is synthesized and secreted by the alveolar epithelial type II cell. In the absence of PTHrP, the alveoli do not form (Rubin et al. 2004). PTHrP stimulates adepithelial interstitial fibroblast differentiation into lipofibroblasts, culminating in their production of leptin (Torday and Rehan 2002). This results in efficient alveolar gas exchange in adaptation to the metabolic demands of the organism, as determined by the stretching of the alveolar wall, coordinately stimulating PTHrP and leptin secretion, and their cognate cell surface receptors on the lipofibroblast and type II cell, respectively. These cell-mediated interactions between PTHrP and leptin coordinate the "on-demand" production of surfactant (Torday and Rehan 2002) with alveolar capillary perfusion (Gao and Raj 2005) or ventilation–perfusion matching, the functional basis for alveolar physiology. This mechanism has emerged over the history of vertebrate evolution, or phylogeny, and is recapitulated during embryonic lung development. The premise that all of biology is an evolutionary continuum (Riedl 1975; Torday and Rehan 2007) offers the opportunity to exploit this observation in order to determine where it began, and how it was perpetuated through Darwinian selection pressure.

Evolution occurs as a result of genetic adaption to changes in the environment under selection pressure. Conventionally, based on natural selection and genetics, macro- and microevolutionary adaptations are viewed as being independent processes of genetic mutation and selection, yet the patterns of diversity would suggest that there is an integrated continuum between the proximate and ultimate causes of evolution. To explore this idea, the schematic shown in figure 2 was generated by regressing the sequence of microevolutionary cell–molecular paracrine mechanisms of vertebrate lung development against the sequence of major macroevolutionary environmental changes that occurred during epochs relevant to vertebrate lung evolution. Using a "case study" approach, we will demonstrate how environmental factors have affected cell–cell signaling in a sequence consistent with the ontogeny, phylogeny, and evolution of the lung. Moreover, these selection pressures did not induce new gene expression but instead facilitated the cis regulation of housekeeping genes, which is what Jacob described as "tinkering" (Jacob 1977) and may be why humans have far fewer genes than were originally predicted by the Human Genome Project.

Letter

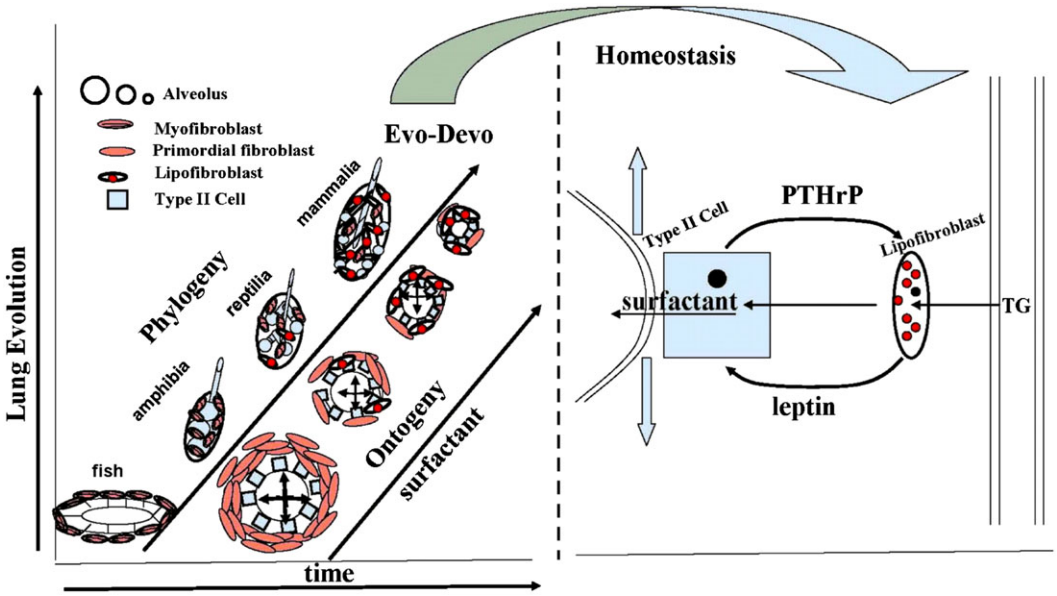

Fig. 1. The continuum from lung phylogeny and ontogeny to alveolar homeostasis. The left-hand panel depicts the cellular changes that occurred during the evolution of the lung from the fish swim bladder phylogenetically with mammalian lung development ontogenetically. Note the transition from myofibroblasts to lipofibroblasts during both processes, which facilitated surfactant production, allowing for the decrease in alveolar diameter that was necessary for the reduction in the alveolar surface area-to-blood volume ratio, increasing the efficiency of gas exchange across the alveolar wall. The right-hand panel depicts the mechanism of alveolar homeostasis mediated by PTHrP production by the alveolar type II cell, stimulating both lipofibroblast production of leptin and alveolar capillary perfusion. Leptin, in turn, stimulates type II cell surfactant production. The combined effects of PTHrP and leptin on surfactant and blood flow results in ventilation–perfusion matching, the physiologic mechanism of alveolar homeostasis.

A Working Model for Cell–Molecular Evolution: Integration of Ontogeny, Phylogeny, and Ecology

The causal interrelationships highlighted in the model depicted in figure 2 are supported by their well-recognized relevance to lung developmental mechanisms—namely the selection for host defense (step 1), antimicrobial peptides (AMPs), and the counterbalancing effects of vitamin D; (step 2), the vitamin D receptor (VDR); (step 3), type IV collagen (type IVcol), which acts as a molecular barrier, the glucocorticoid receptor (GR); (step 4), which determines the timing of lung maturation; (step 5), 11 beta hydroxydehydrogenase (11β HSD), which locally regulates the activation or inactivation of glucocorticoids; (step 6), the beta adrenergic receptor (βAR), which allows for local regulation of lung vascular blood pressure; (step 7), Adipocyte differentiation-related protein (ADRP), which mediates lipid trafficking for surfactant synthesis; (step 8), leptin, which stimulates surfactant synthesis; (step 9), the leptin receptor (LR), which mediates the effect of leptin on surfactant synthesis; (step 10), PTHrP, which coordinately regulates the synthesis of surfactant lipid and Surfactant protein-B (SP-B); (step 11), with alveolar capillary perfusion in the process of lung homeostasis; and (steps 12–17) represent the evolution of the lipofibroblast, and the consequent pleiotropic effects of its secretory product leptin on (steps 1–6, 10 and 11), which are all stimulated through the epidermal growth factor (EGF) pathway, highlighted by the oval. The inference of the schematic (fig. 2) is that the ontogeny and phylogeny of the lung have been determined by the iterative impact of extrinsic environmental factors (lying along the *x*-axis) on the selection for intrinsic cell–molecular mechanisms (steps 1–11) of lung development and phylogeny, interacting to select for lung structure and function.

This process resonates all the way back to the origins of life as micelles forming in response to environmental factors (Hanczyc and Szostak 2004). All the "molecular steps" in lung development employed in the model have been shown to be causally related experimentally (Warburton et al. 2010). And in some cases, the phenomena can be mechanistically explained based on the underlying nature of the mediators. For example, salinity inhibits host defense in fish, an effect that is compensated for by vitamin D hydroxylation products that stimulate local immunity in target tissues (Sundh et al 2007), acting as balancing selection to facilitate evolutionary adaptation; the selection pressure for type IV collagen synthesis for structural support of the alveolus; the effect of glycerrhetinic acid, the product of rancidification of land vegetation, on the specialization of the mineralocorticoid and glucocorticoid receptors, that is, pentacyclic triterpenoids inhibiting 11β HSD1, causing increased blood pressure, resulting in balancing selection for both the GR and 11β HSD1 local activation of glucocorticoids within cells and tissues; or the impact of fluctuating oxygen tension in the environment over the past 500 My (Berner 1999) on the differentiation of the lipofibroblast (Csete et al. 2001) to protect the alveolar wall against oxidant injury (Torday et al. 2001). It would be expected

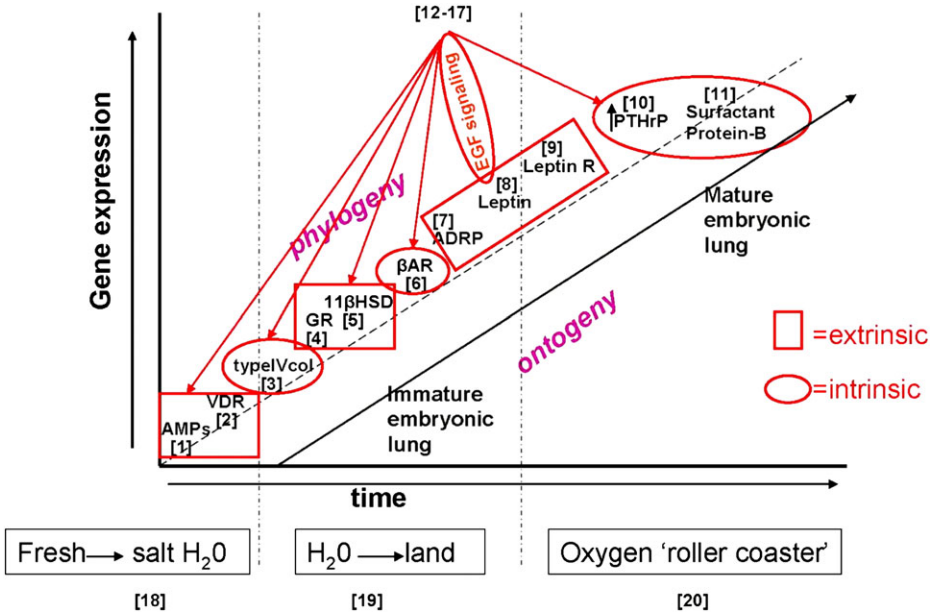

Fig. 2. Alternating extrinsic and intrinsic selection pressures for the genes of lung phylogeny and ontogeny. The effects of the extrinsic factors (salinity, land nutrients, and oxygen on the *x*-axis) on genes that determine the phylogeny and ontogeny of the mammalian lung alternate sequentially with 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.

that if this were "positive selection" that the emerging adaptations would positively interact with the earlier adaptations or exaptations, that is, glucocorticoids having a positive effect on the VDR; oxygen positively affecting glucocorticoid signaling and the VDR—perhaps this is seen best as the effect of oxygen on the emergence of the lipofibroblast (fig. 2, [steps 7–9]), producing leptin, which then pleiotropically stimulates AMPs, the VDR, type IV collagen, 11β HSD, βAR, and the surfactant via EGF signaling (fig. 2, [steps 12–17]), thereby acting to facilitate the expansion of the blood–gas barrier.

Lung Evolution by the Numbers

Salinity and the VDR

The speculation that external selection pressure has primarily been on the lung innate host defense system is reinforced by the first example of an external selection pressure affecting molecular lung development and evolution, as follows: the increase in the salt content of the oceans (see fig. 2, [step 18]), possibly a consequence of the drying up of water environments thought to have driven vertebrates onto land (Romer 1967), is depicted in the schematic on the *x*-axis as a geochemical selection pressure for lung evolution. The hypothesized mechanism of action of salinity resulted from its inhibition of antimicrobial peptide bioactivity (fig. 2, [step 1]), which was counterbalanced by increased vitamin D hydroxylation (fig. 2, [step 2]). Vitamin D independently stimulates tissue-specific innate host defense because fish experimentally exposed to elevated salinity show increased vitamin D hydroxylation (Sundh et al. 2007). This selection pressure for host defense may, in turn, refer back to the anatomy of the physostomous fish swim bladder, which is connected to the gut tube by the pneumatic duct, allowing the fish to inflate its swim bladder by rising to the surface and "inhaling" air shortly after hatching. This phenomenon may have been the antecedent to the transition by ancestors of this class of fish from water to land. It may also have created the selection pressure for host defense because bacteria could have entered the swim bladder via the pneumatic duct. In this context, it is interesting to note that leptin, the endogenous cytokine produced by the lipofibroblast, and lipopolysaccharide (LPS), which composes the gram-negative bacterial coat, both have the same effect on lung epithelial cell differentiation. One interpretation of this phenomenon is that the LPS effect was the atavistic mechanism for innate host defense by the gut, and that the lung epithelial lining, which evolved from the gut, generated the intrinsic leptin signaling mechanism (Torday and Rehan 2002). Such a scenario would provide an evolutionary, mechanistic explanation for the effects of leptin on frog lung development (Torday et al. 2009), which is the same as that on mammalian lung, namely the thinning of the blood–gas barrier and the stimulation of surfactant production. These effects on the frog lung are paradoxical because it does not require such mammalian lung properties for gas exchange, suggesting that the selection pressure for leptin's effects on the lung are primarily related to the innate host defense provided by the defensins surfactant proteins A and D as well as the AMP produced by the epithelial barrier.

Type IV collagen and Increased Surface Area of the Lung

The next major environmental selection pressure occurred when vertebrates moved out of water onto land (fig. 2, [step 19]) some 300 Ma, causing selection pressure for type IV collagen (fig. 2, [step 3]), which nominally acts to physically support the walls of the lung air sacs or alveoli. We know from studies of Goodpasture's syndrome (MacDonald et al. 2006) that the three alpha isoform of type IV collagen evolved sometime between fish and amphibians. This resulted from selection pressure for specific amino acid substitutions that rendered this protein more hydrophobic and negatively charged, preventing the exudation of water and proteins from the microcirculation into the alveolar space and glomerulus.

Goodpasture's syndrome is an autoimmune disease caused by simultaneous kidney and lung epithelial barrier failure. This catastrophic failure is due to pathogenic circulating autoantibodies targeted to a set of discontinuous epitope sequences within the noncollagenous domain-1 (NC1) of the α3 chain of type IV collagen (α3(IV)NC1), referred to as the Goodpasture autoantigen. Basement membrane extracted NC1 domain preparations from *Caenorhabditis elegans* (worm), *Drosophila melanogaster* (fly), and *Danio rerio* (fish) do not bind Goodpasture autoantibodies, whereas frog, chicken, mouse, and human α3(IV)NC1 domains bind autoantibodies. The α3(IV) chain of type IV collagen is not present in worms (*C. elegans*) or flies (*D. melanogaster*), and is first detected in frogs. Three-dimensional molecular modeling of the human NC1 domain suggests that evolutionary alteration of electrostatic charge and polarity due to the emergence of critical serine, aspartic acid, and lysine amino acid residues, accompanied by the loss of asparagine and glutamine, contributes to the emergence of the two major Goodpasture epitopes on the human α3(IV)NC1 domain as it evolved from fish over 450 My. The evolved α3(IV)NC1 domain forms a natural physicochemical "barrier" against the exudation of serum and proteins from the circulation into the alveoli and glomeruli due to its hydrophobic and electrostatic properties, respectively. These modifications of type IV collagen were more than likely the molecular selection pressure for the evolution of this protein, given the oncotic and physical pressure on the evolving barriers of both the alveolus and the glomerulus.

Differentiation of the GR from the Mineralocorticoid Receptor

Another recently discovered vertebrate physiologic trait that evolved during the transition from water to land was the specialization of the steroid hormone receptor into the mineralocorticid receptor and GRs (fig. 2, [step 4]). The primary selection pressure may have been due to the rise in ocean salinity, which was accommodated structurally by type IV collagen, combined with sodium regulation by the steroid hormone receptor mineralocorticoid activity. But the subsequent counterbalancing selection pressure may have been caused by the ingestion of pentacyclic triterpenoids by land vertebrates. Such compounds are produced by the rancidification of carbohydrates, a process that is unique to land vegetation. Pentacyclic triterpenoids such as glycerrhetinic acid inhibit glucocorticoid inactivation by 11β HSD2 (fig. 2, [step 5]), causing glucocorticoid stimulation of mineralocorticoid receptors, and the resultant elevated blood pressure. This would have created positive selection for both 11β HSD1, which inactivates glucocorticoids, counterbalancing the blood pressure elevating effect of the mineralocorticoids (fig. 2, [step 5]) and the specialization of the mineralorticid receptor and GR through two amino acid substitutions in the steroid binding site of the GR in tetrapods (Bridgham et al. 2006).

The Role of βARs in Increased Lung Surface Area

The combined positive selection pressures for 11β HSD1 due to the salt counterbalancing selection for the VDR (see fig. 2, [step 2]), and by the pentacyclic triterpenoid inhibition of 11 β HSD2 (see fig. 2, [step 5]) may have led to the subsequent positive selection pressure for lung βARs (fig. 2, [step 6]) independently regulating pulmonary and systemic blood pressure. Lipofibroblasts subsequently appeared in response to rising atmospheric oxygen tension (fig. 2, [steps 7–9]): lipofibroblast 11β HSD1 may have been constitutively up-regulated by vitamin D_3 produced by lung epithelial cells in response to salt, leading to constitutive lipofibroblast leptin expression (fig. 2, [step 8]). Leptin would have acted to further stimulate AMPs (fig. 2, [step 1]), type IV collagen (fig. 2, [step 3]), and surfactant production (fig. 2, [step 10]). Such synergy would have increased barrier function at multiple levels, providing a stable platform for further increases in lung surface area and vertebrate evolution on land.

Atmospheric Oxygen, Adipocytes, Surfactant, Body Temperature, and Lung Evolution

Over the course of vertebrate evolution during the Phanerozoic period (the last 500 My), the amount of oxygen in the atmosphere has increased to its current level of 21%. However, it did not increase linearly; instead, it increased and decreased several times, reaching concentrations as high as 35%, falling to as low as 15% over this time period (Berner et al. 2007). The increased oxygen tension may have caused the differentiation of muscle cells into lipofibroblasts in the lung, as the first anatomic site where the increased atmospheric oxygen would have affected selection pressure for evolutionary change. Experimentally, muscle stem cells will spontaneously differentiate into adipocytes in 21% oxygen (room air) but not in 6% oxygen (Cseste et al. 2001), suggesting that as the atmospheric oxygen tension increased over evolutionary time, lipofibroblasts could have formed spontaneously. Consistent with this hypothesis, we have previously shown that the lipids stored in alveolar lipofibroblasts protect the lung against oxygen injury

(Torday et al. 2001). In turn, the leptin secreted by the lipofibroblasts binds to its receptor on the alveolar epithelial cells lining the alveoli, stimulating surfactant synthesis (Torday and Rehan 2002). The increased production of surfactant would have reduced the alveolar surface tension, resulting in a more deformable gas-exchange surface. Such positive selection pressure could ultimately have selected for the stretch-regulated PTHrP coregulation of surfactant and microvascular perfusion (Gao and Raj 2005). This mechanism could ultimately have given rise to the mammalian lung alveolus, with maximal gas exchange resulting from coordinate stretch-regulated surfactant production and alveolar capillary perfusion, thinner alveolar walls due to PTHrP's apoptotic or programmed cell death effect on fibroblasts, and a blood–gas barrier reinforced by type IV collagen (West and Mathieu-Costello 1992). We speculate that this last feature may have contributed generally to the molecular bauplan for the peripheral microvasculature of evolving vertebrates, given its effect on angiogenesis (Sierra-Honigmann et al. 1998).

As a result of the increased oxygen exposure, the lipofibroblasts of the lung alveoli were able to accumulate lipids, mediated by ADRP (fig. 2, [step 7]), which would have protected the evolving lung against oxygen toxicity. These lipofibroblasts produce leptin (fig. 2, [step 8]), which stimulates surfactant production by neighboring epithelial cells (fig. 2, [step 11]), making the alveolar sacs more compliant, or "stretchy," permitting more efficient oxygen exchange between the air and systemic circulation, further increasing tissue oxygenation for metabolic drive. One consequence of this may have been the induction of fat cells in the peripheral circulation, which led to endothermy or warm bloodedness (Mezentseva et al. 2008). The increase in body temperature synergized increased lung oxygenation because lung surfactant is 300% more active at 37 °C than at ambient atmospheric temperature (body temperature for cold-blooded organisms). These major physiologic changes in response to the increase in atmospheric oxygen would have been severely challenged by subsequent episodes of hypoxic conditions (Berner et al. 2007), perhaps being accommodated in the survivors of such mass extinctions by increased βAR production, bearing in mind that hypoxia is the most potent physiologic stress stimulus for adrenal epinephrine production. This, in turn, would have facilitated the coevolution of the pulmonary and adrenocortical systems. We speculate that this may also have led to selection pressure for the on-demand alveolar homeostasis in reciprocating breathers, that is, stretch regulation of barrier function (surfactant production, AMPs, and type IV collagen) in combination with alveolar capillary perfusion through the coordinate up-regulation of PTHrP and leptin. The preadaptations to salt water (fig. 2, [steps 1 and 2]), and to the water-to-land transition (fig. 2, [steps 3–5]) provided the lung with the genetic and epigenetic means for organisms to survive and adapt to the oxygen fluctuations (fig. 2, [steps 6–9]) that have occurred over the last 500 My.

Predation, PTHrP, Leptin, and Stretch-Regulated Alveolar Homeostasis

As a result of the evolution of increased oxygenation, the transitional tetrapods (amphibians and reptiles) would have been more metabolically active, putting further selection pressure on the lung to evolve efficient gas exchange. This was achieved by the "invention" of the stretch-regulated surfactant system, that is, the stretching of the alveolar wall increased PTHrP production by the epithelial cells (fig. 2, [step 10]), stimulating leptin production (fig. 2, [step 8]) by the lipofibroblasts, causing more surfactant production and further increased stretching of the alveoli, creating further selection pressure for stretch regulation of the leptin receptor (fig. 2, [step 9]). This PTHrP–leptin stretch-regulated mechanism was reinforced by PTHrP stimulation of blood flow through the alveolus because PTHrP is a potent vasodilator (Gao and Raj 2005), acting to further facilitate gas exchange in synchrony with increased surfactant facilitation of alveolar wall stretching, driving positive selection pressure. Endogenous leptin production by lipofibroblasts further reinforced all these evolutionary steps (fig. 2, [steps 12–17]) by coordinately stimulating the formation of AMPs (fig. 2, [step 1]), protecting the increased surface area of the lung against infectious agents and coordinately increasing type IV collagen (fig. 2, [step 3]) and surfactant production (fig. 2, [step 10]). That adaptation which would have prevented the rupturing of the alveolar blood–gas barrier under physiologic stress as vertebrates evolved from cold- to warm-blooded animals and from more prey-like to more predator-like animals ("fight or flight" mechanism).

The inference of this model of lung evolution driven by the interactions between extrinsic and intrinsic factors is that the external selection pressures caused the internal physiologic adaptations in response to these interactions. The genetically regulated mechanisms originate as housekeeping genes and evolve into regulated paracrine mechanisms in response to deep homologic selection pressures linked together through selection pressure for increased alveolar surface area. By inference, these cell–molecular mechanisms facilitated the evolution of the lung phenotype in those organisms that were able to molecularly adapt, whereas those organisms that could not adapt became extinct and therefore do not appear in this model. By ignoring the time scales over which these genes have evolved (development, homeostasis/regeneration, reproduction, and aging), we have been able to delineate their putative roles in the evolution of the blood–gas barrier. This application of parsimony for deriving the interrelationships between genes and phenotypes as evolutionary processes is applicable to all tissues and organs.

Not only does this model of lung evolution functionally integrate the genetic mechanisms of ontogeny and phylogeny with ecology, but it also lends itself to a mechanistic way of merging together such seemingly disparate processes as gradualism and punctuated equilibrium as a continuum. Under what might be thought of as normal

variation in environmental selection pressure, there may have been gradual changes in genetic expression of housekeeping genes in response to natural selection, not unlike those described for eco-devo mechanisms (Gilbert and Epel 2009). However, under conditions of severe global selection pressures that would have threatened the species with extinction, like those that are depicted in the schematic (fig. 2), for which there were no other options for finding environmental niches, major adaptive strategies would have constituted the emergence of paracrine signaling mechanisms derived from preadapted, gradualist genetic amplifications—evolve or become extinct!

As experimental evidence for the above, Thornton et al. (Bridgham et al. 2006) have shown that the GR evolved from the mineralocorticoid receptor coincident with vertebrates emerging from water onto land, seemingly by chance. However, this is a Just So Story that does not address what the selection pressure on the hormone receptor and 11β HSD mechanisms was, and how it facilitated physiologic adaptation.

The cascade described in figure 2 starts with balancing selection for host defense, inhibited by salinity, counterbalanced by increased vitamin D hydroxylation to stimulate antimicrobial activity locally in specific tissues and organs. As a result, the biochemical activation of vitamin D would have facilitated the positive selection for other "housekeeping" genes, such as 11β HSD, the leptin receptor, PTHrP, and lung surfactant. This would have provided the exaptations for the subsequent effects, starting with the adaptation to land, and then for adaptation to the fluctuations in atmospheric oxygen, generating the lipofibroblast, its production of leptin, stimulating lung surfactant production, increased distensibility, positive selection for stretch regulation of PTHrP, culminating in stretch-integrated surfactant production and ventilation–perfusion matching—all of which are emergent and contingent, which would provide a mechanistic basis for this well-recognized description of the evolutionary process for the first time.

The Model is Predictive and Refutable

The scientific power of this model is that it is empirically testable and refutable in compliance with Popper's criteria for a scientific approach (Popper 1963). More importantly, it provides a novel way of thinking about positive selection pressure, given the common cell–molecular pathway from the fish swim bladder to the evolution of the mammalian lung, predicting that the genes in this paracrine pathway will be highly polymorphic, which they are. The strength of the model provides the plasticity for physiologic evolution, on the one hand, and a basis for understanding genetic lung disease, on the other.

Evolutionary biology is a paradoxical reconciliation of the need for both constancy and novelty in homeostatically regulated mechanisms. Historically, Severtsov had coined the term aromorphosis as a descriptor for this "black box" mechanism (Severtsov 1939). And E. D. Cope had generated the neologism physiogenesis to describe the physicochemical changes caused by the environment, and the individual's ability to respond effectively to those changes, a complex process which he also referred to as accommodation. Jantsch (1980) approaches a mechanistic explanation by stating that the system is a self-organizing, autonomic, autocatalytic process that is self-referential with respect to its own evolution. This is the result of self-generating disequilibrium, as Prigogene had described for nonliving dynamic systems in Order Out of Chaos (Prigogene and Stengers 1984) in which nonequilibrium can be the source of order, or organization, becoming the basis for nonlinear thermodynamics of irreversible processes that generate spontaneous structuration. Jantsch then allows for mechanisms of coevolution and ecological niche complexification. He emphasizes the behavior of the organism in relation to these factors, allowing it to "determine within relatively wide boundaries to which natural selection it subjects itself" but also to participate in further evolutionary progress by responding to new "stresses" that arise from its new activities. This sounds like a functional definition of evolvability (Kirschner and Gerhart 1998), and for the first, and to our knowledge only time, suggests the possibility of cell–molecular interactions between the organism and the environment that drive evolution.

The causal nature of all the adaptive changes of the lung cited above are documented by experimental evidence for these structural and functional traits. Moreover, there is evidence that the more distal changes complement and reinforce the more proximal changes—recognized by evolutionists as the principle of terminal addition (Hughes and Jacobs 2005)—suggesting that the origins of the selection pressure for the lung may derive from deep homologies. The overt selection pressure appears to be for the increased efficiency of the lung surfactant system, allowing for the progressive decrease in alveolar diameter, resulting in the increased area of the gas-exchange surface area-to-blood volume ratio (Torday and Rehan 2007). Yet, we have made the paradoxical observation that leptin stimulates this process in the frog tadpole lung in the same manner that it does in the mammalian lung (Torday et al. 2009), increasing the surface area of the lung in association with stimulation of the surfactant system. The frog lung does not require the same gas-exchange efficiency as the mammalian lung because the gas-exchange surface is composed of faveoli, which are air sacs that are so large in diameter that the reduction in surface tension at the faveolar surface is superfluous. These data more likely point to the primary selection pressure on increased expression of innate host defense mechanisms, such as the antimicrobial surfactant proteins A and D. This insight may also explain why bacterial infection stimulates lung development, another counterintuitive phenomenon requiring an evolutionary perspective.

The self-reinforcing effect of leptin for alveolar homeostasis represents epistasis because each of these physiologic traits—barrier function, host defense, and alveolar compliance—refers to ancestral traits expressed all the way back to unicellular organisms. The earliest mechanism by which eukaryotes evolved from prokaryotes was through

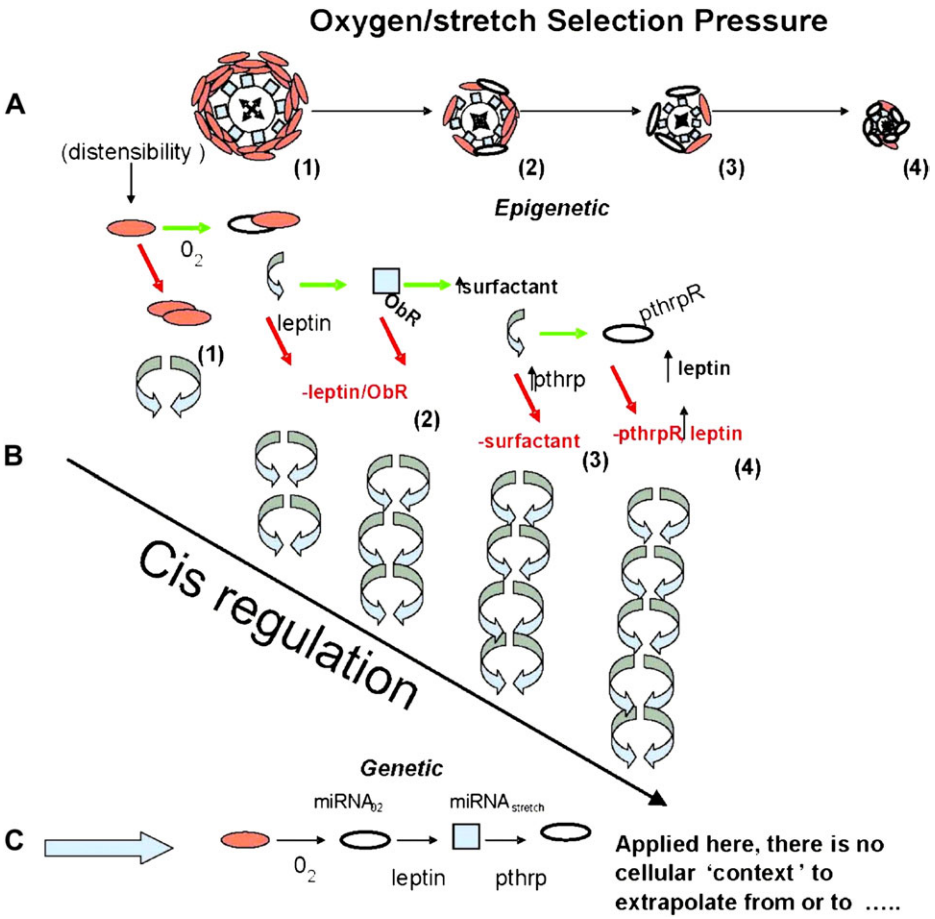

Fig. 3. Evolutionary developmental origins of the lung. The top panel (*A*) depicts the paracrine cellular mechanism of alveolar development, phylogeny, and evolution. The middle panel (*B*) depicts the cell–cell signaling mechanisms that have facilitated alveolar evolution, mediated by soluble factors such as leptin and PTHrP, and the cis regulatory elements that evolved to facilitate the signaling mechanisms, depicted as curved arrows pointing to one another, increasing in number from left to right to symbolize their increasing complexity. The cascade shows how oxygen and stretch may have "driven" a series of cellular interactions for lung evolution, (1) from the advent of the lipofibroblast to leptin production, causing increased surfactant (2), increased alveolar distensibility, placing positive selection pressure on stretch regulation of PTHrP signaling (3) for homeostatic surfactant synthesis (4). This process facilitated the progressive decrease in alveolar diameter, increasing the gas-exchange surface area-to-blood volume ratio for increased oxygenation. The bottom panel (*C*) is a traditionally descriptive genetic pathway for the same processes shown in *A* and *B*.

the formation of a nuclear envelope as a barrier to protect its DNA, or host defense. And we have previously shown that the stretch signaling by PTHrP is an adaptation to gravitational force (Torday and Rehan 2003), which is among the most ancient effectors of biologic adaptation, present in the environment before the oceans, salinity, or oxygen. In fact, we have shown that this is a property of single cells experimentally (Torday 2003). When lung and bone cells are placed in a microgravitational environment, the PTHrP messenger RNA level decreased; when these cells were put back in unit gravity, the PTHrP messenger RNA level returned to normal. And to demonstrate the biologic significance of this gravitational effect on PTHrP expression, we further showed that PTHrP messenger RNA is decreased in the bones of rats that had been in deep space for 2 weeks (Torday 2003). This finding was significant because microgravity causes osteoporosis in astronauts, potentially due to decreased PTHrP in bone. This effect of gravity on PTHrP may also be the underlying cause for the lack of normal alveolar gas distribution in the lungs of astronauts (Prisk 2000), given that PTHrP coordinates lung alveolar ventilation–perfusion matching.

Chronic Lung Disease as "Evolution in Reverse"

A central tenet of this model is that the evolutionarily conserved cell–cell interactions that facilitate adaptation underlie the fundamental mechanisms of vertebrate evolution. Based on that precept, it is conceivable that the inhibition of such mechanisms, particularly using specific agents that mimic the primordial factors that generated the evolutionary phenotypes would either block and/or reverse these putative evolutionary traits. It is reaffirming, therefore, that such agents as oxygen, pressure, infection, and nicotine all act to simplify lung structure in a reverse evolutionary direction (Cerny et al. 2008). This suggests that both the forward and the reverse directions of lung

development and injury represent evolution in both the positive and the negative direction. Limitations on the reversal of the evolved trait may reflect the original constraint, and even the functional basis for the extinction of transitional organisms. These observations are perhaps all the more significant because they provide a novel and counterintuitive way of thinking about the treatment of chronic lung disease (Torday and Rehan 2009).

Evolution of Cis Regulatory Mechanisms

One of the hallmarks of the evolution of complex physiology is the transition from housekeeping genes to cis regulatory genes through cell–cell communication, as depicted in figure 3. For example, the epithelial lining cells of the fish swim bladder express surfactant protein and phospholipid in an unregulated housekeeping fashion. In contrast to this, the amphibian lung epithelium expression of surfactant is regulated by leptin produced by lipofibroblasts (Torday et al. 2009), which are the key to mammalian lung evolution—the cellular paracrine interactions between cells that mediated lung evolution (depicted in fig. 3*A*, 1–4) are underpinned by the evolution of cell–cell signaling through leptin and PTHrP, soluble factors that bind to their cell surface receptors, triggering a downstream cascade of cis regulatory mechanisms (fig. 3*B*, 1–4). Figure 3*B*, step 1, for example, depicts the evolutionary effect of increasing atmospheric oxygen on muscle fibroblast differentiation into lipofibroblasts, which store lipid substrate for surfactant and produce leptin; in figure 3*B*, step 2, leptin binds to its receptor (ObR) on the alveolar type II cell, stimulating surfactant synthesis; in figure 3*B*, step 3, surfactant makes the alveoli more distensible, generating positive selection pressure for the stretch-regulated gene PTHrP, subsequently generating positive selection pressure for the emergence of the stretch-regulated PTHrP receptor on lipofibroblasts in figure 3*B*, step 4. This completes the stretch-regulated paracrine regulation of surfactant synthesis. These cell surface receptor-mediated signaling mechanisms act through second messengers, which interact with DNA, requiring progressively more complex cis regulatory complexes, symbolized by the increasing stacks of curved arrows pointing toward one another under each step. This progression from housekeeping genes to cell–cell communications as the basis for evolution is distinctly different from the conventional descriptive genetic perspective, which is depicted in fig. 3*C*.

Therefore, in contrast to the conventional genetic analyses of evolved phenotypes, which are out of context with their cell biologic function, by systematically analyzing the ontogenetic and phylogenetic progression of cis regulation, we can deconvolute the mechanisms of vertebrate evolution and ultimately determine the first principles of physiology and medicine.

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