What We Talk About When We Talk About Evolution

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ABSTRACT: Currently, the biologic sciences are a Tower of Babel, having become so highly specialized that one discipline cannot effectively communicate with another. A mechanism for evolution that integrates development and physiologic homeostasis phylogenetically has been identified—cell-cell interactions. By reducing this process to ligand-receptor interactions and their intermediate down-stream signaling partners, it is possible, for example, to envision the functional homologies between such seemingly disparate structures and functions as the lung alveolus and kidney glomerulus, the skin and brain, or the skin and lung. For example, by showing the continuum of the lung phenotype for gas exchange at the cell-molecular level, being selected for increased surface area by augmenting lung surfactant production and function in lowering surface tension, we have determined an unprecedented structural-functional continuum from proximate to ultimate causation in evolution. It is maintained that tracing the changes in structure and function that have occurred over both the short-term history of the organism (as ontogeny), and the long-term history of the organism (as phylogeny), and how the mechanisms shared in common can account for both biologic stability and novelty, will provide the key to understanding the mechanisms of evolution. We need to better understand evolution from its unicellular origins as the Big Bang of biology.

KEYWORDS: evolution, paracrine growth factor, parathyroid hormone-related protein, endothermy, pituitary adrenal axis

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> "A hen is only an egg's way of making another egg"
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> —Samuel Butler

Introduction

The current state of the biologic sciences is reminiscent of the Tower of Babel; they have become so highly specialized that one discipline cannot effectively communicate with the other due to the hermeneutic, self-serving languages we all employ. In contrast to that, there is a strong sense that biology is the product of the process of evolution and that there is an underlying driving mechanism, which we have not quite been able to figure out yet, sometimes referred to as teleonomy. That sense was perhaps best and most famously expressed by Dobzhansky's dictum that "Nothing in biology makes sense except as evolution".[1] He set the bar, and we have been trying to hurdle it ever since.

The fact that metazoans begin life as single-celled zygotes and reproduce from that single cell is indisputable. The mechanism involved in generating a whole organism from a fertilized egg involves cell-cell interactions mediated by soluble growth factors and their receptors, which mediate cell signaling through pathways that determine morphogenesis.[2] More recently, experimental evidence has been put forth to indicate that single-celled organisms possess the complete genomic toolkit for multicellular organisms, documenting that single-celled organisms are the basis for the process of evolution.[3] That provides the empiric rationale for examining evolution from its unicellular origin.

Statement of the Problem

When we think of evolution in terms of contemporary biologic phenotypes, we make the systematic error of reasoning backwards from the present to the past. Yet reasoning after the fact, by definition, is illogical. All of biology is formed from and by cells, which emerged from the primordium 3–4 billion years ago, likely as primitive micelles formed from lipids.[4] Such structures are semipermeable, generating intracellular chemical gradients, a process referred to as chemiosmosis,[5] ultimately allowing for the reduction of entropy within the cell, transiently circumventing the Second Law of Thermodynamics.[6,7] It was under these conditions that life began on Earth, initiated by prokaryotes[8] and perpetuated by the perennial competition between prokaryotes and eukaryotes, a battle which rages on to this day.

It is because of the emergent and contingent nature of life that Polanyi failed to reduce it to physical principles,[9] and Prigogene similarly failed.[10] Back in the 1980s, a group of physiologists—ER Weibel, CR Taylor and H Hoppeler[11]—attempted to determine if physiologic mechanisms were consistent with physical principles. They referred to their hypothesis as Symmorphosis. In the end, they concluded that physiology could not be predicted by the laws of Physics.

So by default, life merely imitates the physical world, sparked by the reduction in entropy within unicellular organisms.

The Solution to the Problem

A mechanism that integrates development and physiologic homeostasis phylogenetically has been identified—cell-cell interactions.[12–15] Why not apply that mechanism to Evolutionary Biology as the long-term basis for phylogenetic change? Using that approach at the cell-molecular level offers the opportunity to determine how cellular composition has accommodated adaptation. In a recently published book, entitled *Evolutionary Biology, Cell-Cell Communication and Complex Disease*,[16] we exploited this approach to understand how the lung evolved to accommodate metabolic drive, based on the role of surfactant in facilitating both the developmental and phylogenetic increases in lung alveolar surface area for gas exchange. By reducing this process to ligand-receptor interactions and their intermediate downstream signaling partners, we were able, for example, to envision the functional homologies between such seemingly disparate structures and functions as the lung alveolus and kidney glomerulus, the skin and brain, and the skin and lung.

Using such a reductionist approach to functional genomics has led to a mechanistic understanding for how internal selection pressure, brought on by physiologic stress within Claude Bernard's *milieu interieur*,[17] may have given rise to such lung diseases as Goodpasture Syndrome[16] and asthma.[16] By linking together the cell-molecular pathways for basic physiologic mechanisms independently of their overt structural and functional appearances, particularly as they relate to extrinsic ecologic selection pressures,[14,15] one can discern the "how and why" of evolution. By starting from the "middle" of the mechanism,[18] tracing the signaling pathways linking genes to phenotypes, one can see how such pathways evolved across the space and time of biology as ontogeny and phylogeny.

The classic dissociation of proximate and ultimate causation in biology was elaborated by Ernst Mayr.[19] Proximate causes deal with the mechanisms responsible for the makeup and functioning of the individual phenotype. Ultimate causes refer to the past conditions having led to the information encoded in DNA. According to Mayr,[19] proximate causation takes place once the encoded genetic program is actualized in the individual, whereas ultimate causation determines the shaping of the program itself. This dichotomous scheme may be viewed as a logical consequence of the Weismannian[20] separation of the soma from the germ line. It assumes that we need different means to understand the phenotype and the genotype. Biologists studying proximate causes ask "how" questions about mechanisms, whereas those studying ultimate causes ask "why" questions about evolutionary epistemology. The phenomena involved at these different levels of causation occur on different time scales and are referred to as diachronic. For example, by showing the continuum of the lung phenotype for gas exchange at the cell-molecular level, being selected for increased surface area by augmenting lung surfactant production and function in lowering surface tension, we have determined an unprecedented structural-functional continuum from proximate to ultimate causation in evolution.[12–15] Beginning with cholesterol facilitating gas exchange through the unicellular plasma lemma,[21] this process culminated in the alveoli of the mammalian lung by tracing the cell-cell interactions that have facilitated surfactant production both ontogenetically and phylogenetically.[12–15] By analogy, we can do the same using the example Mayr himself used to dissociate proximate and ultimate causation- that of migratory birds.[19] At the time, without being able to reduce physiology to the cellular-molecular level, it was impossible to discern such a continuum for this complex physiologic trait, yet nowadays that behavior can be broken down to seasonal changes in the wavelength of ambient light, its effect on the pineal gland in controlling neuroendocrine hormones, which ultimately determine the feeding patterns and reproductive strategies for bird migratory habits.[22] By reducing this complex process to its cellular-molecular constituents, its causal nature can be hypothesized and experimentally tested, obviating the artificial siloing of biology as proximate and ultimate.

One fundamental insight from such molecular analyses is that the time dimension for evolutionary processes is a quantitative artifact of Descriptive Biology; once the underlying mechanisms are identified, the time dimension falls out of the analysis, other than to provide the sequence of events. Once achieved, the vertical integration literally and figuratively eliminates time. And the space occupied by the myriad forms of multicellular organisms is also eliminated once it is acknowledged that multicellular organisms evolved from unicellular organisms.

This ultrareductionist point of view yields a very different perspective on life being simple, rather than complex. Moreover, it begs the question as to whether metazoans are merely a further extrapolation of such prokaryotic pseudometazoan traits as lateral inheritance, biofilm, and quorum sensing. Perhaps protozoans evolved such metazoan phenotypes as a way of monitoring the environment over multiple time-frames. After all, H.G. Wells wanted to teach us humility by having bacteria save mankind in *War of the Worlds*.[23]

Putting Humpty Dumpty Back Together Again Based on Epigenetic Principles

Darwin initiated the search for the origin of species in 1859,[24] using the metaphor of Natural Selection for its mechanistic basis. Ever since, those interested in pursuing the evolutionary process have been prone to using metaphors instead of mechanisms that could elucidate how and why evolution has occurred. Ironically, biology had a 50-year head start on cosmology in its reductionist approach, yet the physicists have long since determined how the universe "evolved",[25] having determined that quantum mechanics and $E = MC^2$ enable us to see how the cosmos was generated by the Big Bang. In contrast to that, biology lacks a central dogma to unify it. In any endeavor to formalize knowledge, the first phase involves collecting,

describing, and organizing the information. Eventually, the scientific method is applied to the data to determine causation. Evolutionary Biology has been in the descriptive mode for more than 150 years, whereas in the interim, physicists have been able to devise theories and methods to determine the origins and composition of the universe.[26]

A systematic error in the reductionist approach to Evolutionary Biology is our failure to recognize that it is a mechanism, not a "thing", namely DNA. In order to understand how and why evolution works, one must first reduce it to its smallest functional unit of activity—the cell. In contrast, evolutionists describe the process dichotomously at the genetic and population biologic levels, neither of which is the smallest functional unit. Perhaps that is why Cell Biology is not part of the conventional analysis—it is not considered to be necessary,[27] yet it is the fundamental mechanism of ontogeny—it is only in the recent past that we have been able to determine the mechanisms underlying morphogenesis based on cell-specific production of soluble growth factors and their cognate receptor signaling partners on the surfaces of neighboring cell types. These developmental mechanisms culminate in homeostatic control, providing a unified functional basis for physiology, repair, and regeneration. And since such processes are amenable to modification under selection pressure, they are also the mechanisms for phylogeny. Such cellular signaling mechanisms common to both ontogeny and phylogeny provide insights to the mechanisms of evolution, complying with the "emergent and contingent" nature of the evolutionary process.

It is high time that evolution moved on to the mechanistic phase. In order to do so, it must re-embrace Cell Biology, from which it isolated itself back at the turn of the 20th century because embryologists such as Ernst Haeckel[28] and Hans Spemann[29] were unable to provide experimental evidence for Ontogeny Recapitulating Phylogeny (the Biogenetic Law) or identify the Organizing Principle, respectively. Instead, the evolutionists turned their attention to the burgeoning field of genetics, concluding that mutation (as variation) and Natural Selection were the only mechanisms necessary for Descent with Modification. As a consequence, evolutionists merely show associations between randomly occurring gene mutations and phenotypes, rather than how genes determine evolving phenotypes. On the other hand, Cell Biology functionally integrates genes and phenotypes, and nowhere else is that more evident than in the case of Developmental Biology, particularly as it relates to physiology.

We humans have succeeded as a species because of our highly evolved brains. We have an obligation to both our ancestors and offspring to use our minds effectively so that we do not destroy ourselves, the biota, and the planet in the process.[30] If we understood where we evolved from, and therefore where we are evolving to as a species, perhaps we would act in more socially responsible and humane ways.[30] The key is to deconvolute Evolutionary Biology, which has become so complicated as to be useless in utilizing the Human Genome for the prediction and prevention of disease.[31]

The solution to the puzzle of evolution is right under our noses, but instead we generate more and more neologisms and metaphors that allow us to circumlocute and evade the solution.[32]

Conrad Hal Waddington actually foresaw that Cell Biology would reveal the workings of evolution, as expressed in his book *The Strategy of the Genes*,[33] in which he stated that "somewhere hidden among the deepest secrets of the physiology of the cell, there must be the process by which the hereditary factors undergo those sudden mutations which are the basis for the long time-scale evolution." Those secrets were first revealed in the late 1970s, when it was discovered that cells secreted soluble growth factors which bound to their cognate receptors on nearby target cells, communicating to determine their mutual growth and differentiation during embryogenesis.[34] We have used this approach to deconvolute the evolutionary process,[12–15] which Waddington described as three time scales: evolution, development, and physiology.[33] He contrasted biology with physics:

> "Perhaps the main respect in which the biological picture is more complex than the physical one, is the way in which time is involved in it. In the Newtonian system, time was one of the elements in the physical world, quite separate from any of the others; a material body given mass just existed, unchanging and, indeed, quite indifferent to the passage of time. But time and change is part of the essence of life. Not only so; to provide anything like an adequate picture of a living thing, one has to consider it as affected by at least three different types of temporal change, all going on simultaneously and continuously.
>
> These three time elements in the biological picture differ in scale. On the largest scale is evolution; any living thing must be thought of as the product of a long line of ancestors and itself the potential ancestor of a line of descendants. On the medium scale, an animal or plant must be thought of as something which has a life history. It is not enough to see the horse pulling a cart past the window as the good working horse it is today; the picture must also include the minute fertilized egg, the embryo in its mother's womb, and the broken-down old nag it will eventually become. Finally, on the shortest time-scale, a living thing keeps itself going only by a rapid turnover of energy or chemical change; it takes in and digests food, it breathes, and so on".[33]

He recommended that this was the way to think of the process of evolution, but cautioned that it was still difficult to envision. Indeed, it would be another 20 years before growth factor signaling for embryogenesis would be discovered,[34] providing the wherewithal to do as Waddington had suggested.

And with all due respect to Waddington, he was misguided by the seeming complexity of life, when in fact it may be the opposite. As discussed below, if you start from the premise that it is the unicellular state that is actually being selected for, then time and space can be factored out of the analysis.[12–16]

Paracrine Growth Factors—From Morphogenesis to Homeostasis

Up until the late 1970s, there was no known mechanism that explained how genes generated phenotypes. Up until then, biology was solely descriptive, attracting those who were skillful at limning biologic phenomena, like Goldschmidt,[35] Waddington[33] and Gould.[36] Then the soluble growth factors that mediate morphogenesis during development were discovered, beginning with experiments performed by Clifford Grobstein,[37] who demonstrated that organs could autonomously develop in a totally defined culture medium in tissue culture and that if he separated the endodermal and mesodermal layers of the developing kidney or lung, the isolated tissues would ball up and fail to develop. But if the tissues were recombined in culture, with a semipermeable membrane interposed between the tissue layers, they would inexplicably continue to grow and differentiate.

In 1967, Taderera[38] subsequently showed that low-molecular-weight developmental "principles" produced by the mesenchyme could be transmitted across a semipermeable membrane, thereby implicating soluble molecules in the mesenchymal regulation of organ development. The later discovery that specific growth factor receptors and their downstream second messenger signaling cascades determine form and function developmentally opened up the field of cellular-molecular embryology.[39] This fundamental mechanistic insight to well-defined spatio-temporal relationships in biology has been totally ignored by the evolutionists, who are satisfied with merely characterizing the superficial genetic or phenotypic changes that occur over the course of ontogeny or phylogeny, reflecting their descriptionist heritage, which is why such individuals have self-selected to enter the field of biology. Alternatively, there are theoretical biologists who derive information for the sake of mathematically modeling the process of evolution. And there are pure philosophers who try to devise scenarios for the Darwinian "tangled bank" de novo. Here, it is maintained that these descriptive activities are all the direct consequence of a culture that has rejected Cell Biology for historic reasons.

Contemporary molecular embryology is based on growth factors signaling via their cognate receptors, depending upon spatio-temporal relationships that determine morphogenetic patterns. As such, these mechanisms provide a predictive magnitude and direction for the formation of structure and function. In this sense, it is no different from what we expect of a mechanistic basis for Evolutionary Biology, which is also trying to comprehend the magnitude and direction of biologic change, though the time scales are (seemingly) very different. But perhaps that's just an artifact of the descriptive modality. Once we transition to a mechanistic approach, such time and space considerations are independent of the mechanisms of interest, other than providing the nominal sequence of events.

More recent experiments have further demonstrated that paracrine growth factors such as Sonic Hedgehog (Shh), Wingless/int (Wnt) proteins, Bone Morphogenetic Protein 4 (Bmp4), Scatter Factor, and Fibroblast Growth Factor 10 (Fgf10) all play important roles in the lateral branching of the mouse lung bud. Genes encoding Bmp4, Wnt2, and Shh are expressed at high levels in the bud-forming distal epithelium, while genes encoding Fgf10 and the Shh receptor Patched (Ptc) are expressed in the distal mesenchyme.[40–42]

In the embryonic mouse lung, Fgf10 determines the position and expansion of the lung bud.[43] Mice homozygous for loss-of-function mutations of Fgf10 lack limbs and lungs, while endodermal expression of a dominant negative for the Fgf receptor Fgfr2IIIb causes mice to lack terminal buds in their lungs.[42] Moreover, the addition of Fgf10 to 11.5-day old embryonic mouse lung rudiments cultured in Matrigel™ causes extensive budding.[44] Fgf10 is seen in the mesenchyme around both the terminal and lateral branches.[40–42]

The regulation of Fgf10 appears to be controlled, at least in part, by Sonic Hedgehog and Bmp4. Shh is expressed throughout the respiratory epithelium, with the highest expression occurring within the terminal buds. In lung rudiments where Shh is overexpressed, Fgf10 transcription is reduced significantly. During normal mouse lung development, the lateral buds become surrounded with Shh-expressing mesenchyme after they form. During bud outgrowth, Shh and Wnt7b from the epithelium induce FGF10 and cell proliferation of both the epithelium and mesenchyme cells. As outgrowth progresses, the levels of Bmp rise in the distal tip, and it reaches a level where it can inhibit Fgf10. Fgf10 expression then appears more laterally, where it initiates the formation of new buds. At the most distal region, a cleft appears, and extracellular matrix molecules stabilize this cleft.[40–42]

During the fetal period of lung development, immature mesodermal cells are dominated by the Wnt/catenin pathway, which confers the myogenic fibroblast phenotype.[45] The developing epithelium expresses Shh, which stimulates mesodermal Wnt/βcatenin through its receptor-mediated downstream interactions with Ptc and Gli, actively promoting the myogenic fibroblast phenotype.[46] Descriptively, as the endoderm and mesoderm of the alveolar interstitium mature, endodermal Shh signaling through the mesodermal Wnt/_catenin pathway decreases as endodermal parathyroid hormone-related protein (PTHrP) signaling to the mesodermal PTHrP receptor signaling pathway is concomitantly upregulated.[47] We have exploited the stretch regulation of PTHrP to test the hypothesis that fetal lung fluid stretches the alveolar interstitium and stimulates PTHrP signaling, which downregulates the mesodermal Wnt/βcatenin pathway through cAMP-dependent PKA inhibition of Gli, upregulating the PTHrP signaling pathway, inducing the lipofibroblast phenotype (Fig. 1).[48] The mature lipofibroblast produces leptin,[49] which induces endodermal type II cell differentiation.[49] The downregulation of endodermal Shh expression by the mature epithelial type II cell ensures constitutive downregulation of the Shh/Wnt/βcatenin GRN, molecularly stabilizing these key alveolar interstitial phenotypes.

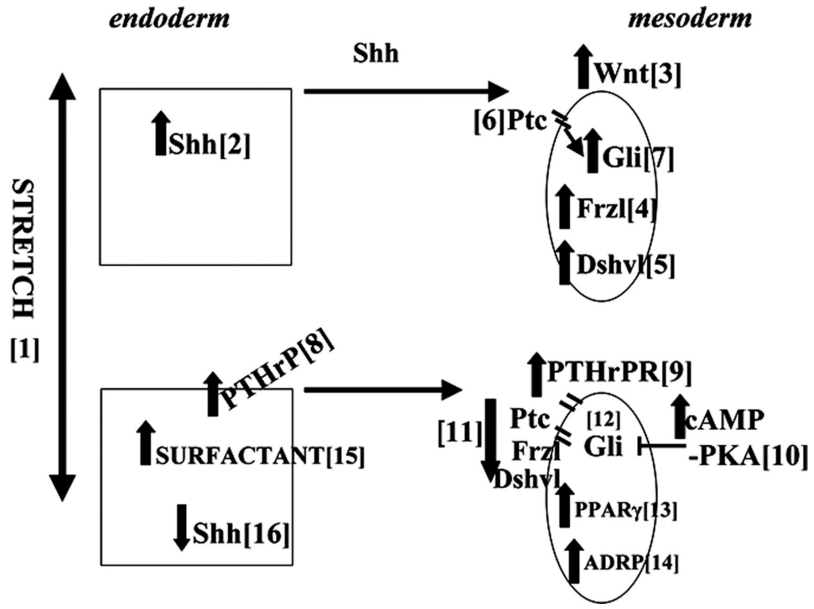

Figure 1. Schematic representation of maturation of the alveolar acinus.
Notes: During early embryonic lung development (*upper panel*) endodermal Shh [**2**] signals to the mesodermal Wnt/Ptc/Gli pathway [**3**–**7**]. Maturation of the interstitium is driven by alveolar fluid distension [**1**], which upregulates the PTHrP signaling pathway between the endoderm and mesoderm [**8**–**16**], down-regulating the Wnt pathway by inhibiting Gli [**12**] and upregulating PPARγ [**13**] and ADRP [**14**]. Differentiation of the lipofibroblast stimulates differentiation of alveolar type II cell surfactant synthesis [**15**] and inhibition of Shh [**16**] expression.

The Bottom Line

So what is the value added in using a cell-molecular mechanistic approach? Using such an approach, we have been able to envision this continuum and how it has fostered the evolution of the lung, for example. Based on our working knowledge of how paracrine growth factor-receptor interactions have mediated the development of the mammalian lung, we considered the overall ontogeny and phylogeny of the lung phenotype, that is, its evolution, as an overall selection pressure for increased surface area, from fish to man in service to the metabolic drive underpinning the water-to-land transition. This has been realized by a progressive decrease in the size of the gas-exchange units, which increase the gas-exchange surface area to blood volume ratio over phylogenetic and ontogenetic space time (Fig. 2).[12–16] This process could not have occurred without an increase in the net production of lung surfactant, which must physicochemically compensate for the increased surface tension resulting from the decrease in alveolar diameter (by the Law of Laplace that the surface tension is inversely related to the diameter of a sphere). The cellular regulation of surfactant production, in turn, is orchestrated by interactions between the alveolar epithelial lung cells that synthesize the surfactant, known as alveolar type II cells, and the adepithelial connective tissue fibroblasts that underlie them within the alveolar wall. The cell-cell interactions that regulate surfactant production have evolved from the secretion of cholesterol, the simplest form of surfactant, into the lumen of the swim bladder of fish to prevent the walls from adhering to one another,[50] to a progressively more efficient means of synthesizing and secreting a more complex biochemical surfactant mix of lipids and proteins in order to accommodate the increase in surface area as the lung has evolved phylogenetically.[51] Along with the decrease in the diameter of the alveoli, the alveolar walls also became progressively thinner,[52] further facilitating the gas exchange between the alveolar space and the lung microcirculation. The "invention" of tubular myelin,[53] an extracellular latticework of surfactant proteins and phospholipids generated from the lamellar bodies secreted by the alveolar type II cell, provides an extracellular homolog of the lipid barrier formed by the stratum corneum of the skin, including both the lipids and the antimicrobial peptides packaged within the lamellar bodies.[54]

It is maintained that tracing the changes in structure and function that have occurred over both the short-term history of the organism (as ontogeny) and the long-term history of the organism (as phylogeny), and how the mechanisms shared in common can account for both biologic stability and novelty, will provide the key to understanding the mechanisms of evolution.[12–16] Like solving a mathematical fraction problem, the cellular-molecular approach determines the "least common denominator" for both ontogeny and phylogeny, eliminating the artifactual temporal-spatial differences between these processes.

It is important to bear in mind that there are certain gene-phenotype homologous relationships that are fairly readily apparent because of their position as "barriers" at the interface between the environment and the organism, such as the lung, skin, and gut, likely having originated from the cell membrane in unicellular organisms as their "common denominator".[12–16] And then there are other homologies that

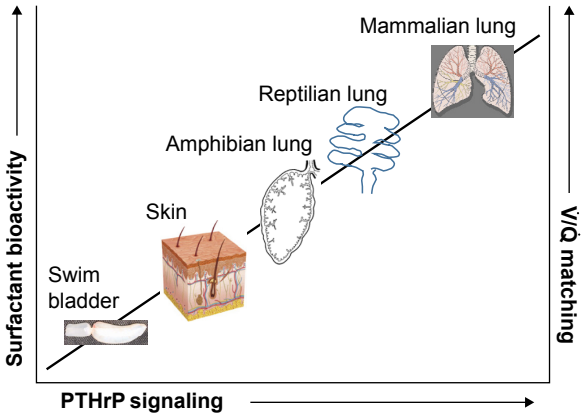

Figure 2. Structural evolution of the organ of gas exchange.
Notes: During phylogeny from fish to mammals, the organ of gas exchange becomes more and more complex, starting with the swim bladder of fish, the skin of amphibians, and the lung, increasing in surface area to accommodate the metabolic demand for oxygen. This is particularly true of the arboreal conducting airways and clustering of alveoli in the mammalian lung. Cellular changes in the interstitium of the lung from amphibians to reptiles and mammals are characterized by a decrease in myofibroblasts and an increase in lipofibroblasts. There is a concomitant decrease in the diameter of the alveoli. We hypothesize that the structural changes are due to the progressive increase in the PTHrP/PTHrP receptor amplification signaling (*x* axis), which enhances surfactant production and V̇/Q̇matching (*y* axes).

are "derived" from those more readily apparent properties that must be deciphered based on their short- and long-term histories, particularly as they derive from those primary mechanisms.[16] Instead of taking a "top-down" or "bottom-up" approach to understand physiologic evolution based on superficial appearances, we have advocated for a "middle-out" approach based on the underlying cell-cell communication to determine the evolutionary origins of cell-molecular traits.[55]

We have demonstrated the utility of a cell-molecular developmental physiologic approach in deconvoluting lung evolution, providing a mechanistic continuum from development to physiologic homeostasis and regeneration.[12–16] Moreover, this tack allows for understanding the interrelationships between tissues and organs at a fundamental cell physiologic level, independent of their contemporary appearances and functions, effectively replacing the need for illogically reasoning after the fact. This approach has provided novel insights to the mechanisms of evolution for the more directly evolved structures/functions of the lung, namely skin and bone, as well as for the deeper homologies of the kidney and brain, based on cell-cell signaling as the integrative mechanism, for the first time.[56]

We have learned from cell culture experiments that normal metazoan cells are not structurally or functionally autonomous; over time, differentiated cell types lose their phenotypes.[57,58] They exist within microenvironments created during development by cell-cell interactions between cells derived from different cell lines.[59,60] The underlying mechanisms of development, physiologic homeostasis, and regeneration are mediated by soluble growth factors and their cognate receptors, which signal through second messengers to determine the metabolic and proliferative status of their surroundings.[61] We maintain that these mechanisms are the basis for the evolution of complex biologic traits and that by systematically analyzing these diachronic signaling mechanisms over time within and between species, the mechanistic basis for evolution can be discerned.[12–16]

A Mechanistic Evolutionary Riddle: When is an Alveolus Like a Glomerulus?

As a prototypical working example of how to understand the evolution of a derivative structure, the lung and kidney appear to be distinctly different based on their overt structures and functions dedicated to gas—versus fluid/electrolyte—exchange, respectively. However, by starting with the developmental and physiologic commonalities between the alveolus and glomerulus as the functional units of the lung and kidney, one can find cell-molecular evolutionary homologies by ignoring the superficial differences. Both organs function to produce amniotic fluid during mammalian gestation,[62] demonstrating developmental functional homology. But more importantly, these two seemingly disparate structures have common physiologic roots since both act as "professional" pressure transducers. Alveolar distension mediates gas exchange between the internal and external environments, whereas the glomerulus mediates fluid and electrolyte balance to regulate the internal physiologic water and electrolyte milieus. Despite such functional differences, the physiologic distension of either the alveolus or the glomerulus is transduced by the same communicating cell types (Fig. 3): in the case of the alveolus (Fig. 3, left-hand side of schematic), the distension of the alveolar wall stimulates the cross-talk between the alveolar epithelial type II cell and the interstitial lung fibroblast, causing coordinately increased production of PTHrP by the alveolar type II cell, increased production of leptin by the lipofibroblast, and increased prostaglandin E_2 production by the alveolar type II cell.[63] As a result of the integrated upregulation of these molecules and their cognate receptors on their complementary epithelial and mesodermal cell types, more surfactant is produced in response to the increase in alveolar surface area, maintaining reduced alveolar surface tension; alveolar capillary perfusion is also coordinately increased, since PTHrP is a potent vasodilator;[64] calcium in the alveolar hypophase is regulated, since PTHrP is calciotropic,[65] maximizing surface tension-reducing activity, allowing for efficient gas exchange in response to the expansion of the lung.

In the case of the kidney (Fig. 3, right-hand side of schematic), the podocytes that line the glomerulus also produce PTHrP,[66] signaling to PTHrP receptors located on the surface of the mesangial fibroblasts;[67] the mesangium monitors and controls fluid and electrolyte flux within the kidney in determining urinary output.[68] The functional relevance of these evolved mechanisms is reflected by the fact that in the case of both the alveolus and glomerulus, failure of the PTHrP

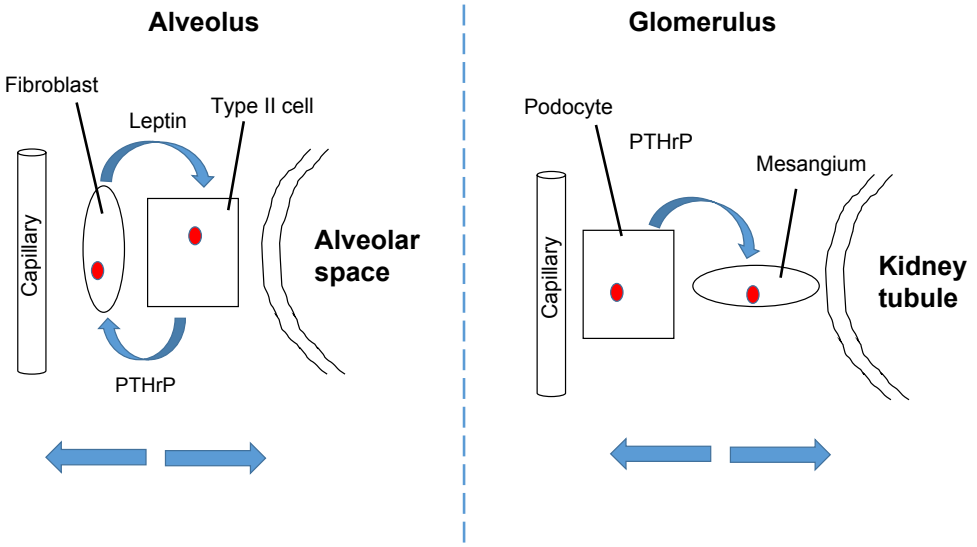

Figure 3. When is an alveolus like a glomerulus? The alveolus and glomerulus are stretch sensors.
Notes: In the lung (left panel), the alveolar epithelium (square) and fibroblast (oval) respond to the stretching of the alveolar wall by increasing surfactant production. In the kidney (right panel), the mesangium (oval) senses fluid pressure and regulates blood flow in the glomeruli.

homeostatic signaling mechanisms described above, due to a wide variety of insults (barotrauma, oxotrauma, infection, and xenobiotics), causes increased Wnt signaling in the fibroblasts of both organs (Fig. 3),[69,70] resulting in either lung[71] or kidney[72] fibrosis and scarring due to the transdifferentiation of the resident homeostatic fibroblasts to myofibroblasts.[73,74] This process of injury repair compromises both lung and kidney functions, yet it sustains organ function in an evolutionarily advantageous, quasi-homeostatic state, allowing the organism to survive and reproduce, passing its genetically adaptive cellular-molecular motifs on to its offspring. The ability to accommodate such vital injuries is a mechanistic expression of Survival of the Fittest.

This counterintuitive, middle-out approach to understand the cell-molecular origins of physiologic homologies is in contrast to the efforts of others to understand kidney evolution by a more superficial top-down molecular approach, as described by Raff and Kaufman in *Embryos, Genes and Evolution*.[75] Focusing on how the kidney handles nitrogen waste in the form of ammonia or urea, on the one hand, or hemoglobin synthesis on the other, does not recapitulate phylogeny; it is a "snapshot" of the consequences of the evolutionary mechanisms that have occurred over the course of the history of the organism. And to emphasize the difference between the top-down and middle-out approaches, unlike the evolutionary accommodation of gas or water through pressure transduction, there is no need to modify structure, so there is no demonstrable structural change. It's the determination of the "historic" functional cell/molecular homologies that reveal the evolutionary selection pressure and genotypic-phenotypic result.[12–16] In the case of ammonia, urea, or hemoglobin, the level of selection pressure is perceived to only be molecular, hence the lack of an integrated, structurally evolved trait. Another way to think about this is that biology cannot accommodate gas exchange by modifying oxygen; instead, it accommodates it by increasing the surface areas of the lung and kidney for exchange of gases, liquid, and electrolytes. Seen in this light, the kidney may have been exapted from those members of the species best able to upregulate PTHrP signaling for lung evolution, now facilitating kidney function during one of the reiterative water-land transitions in order to prevent desiccation.

The Water-Land Transition, PTHrP Amplification, and the Adaptation to Land

The evolution of PTHrP signaling, known to have occurred during the water-land transition,[76] would provide a mechanistic explanation for the morphing of fish into land vertebrates, like Neil Shubin's Tiktaalik, the fossil remains of the transitional tetrapod discovered in 2006.[77] All of the essential water-land adaptations—lung, skin, kidney, gut, and brain—would have been facilitated. At first glance, this event may seem like a "just-so story" for vertebrate adaptation to land, yet we know that there were at least five separate attempts by vertebrates to breach land based on skeletal fossilized remains;[78] this could not have occurred independently of the evolution of the visceral organs, particularly because many of the same genetic mechanisms are common to both bone and visceral organ development (PTHrP, Wnt/βcatenin, TGFβ, PKA, PKC, and Shh), so these events should also be viewed in the context of hypothetical internal selection mechanisms for cellular adaptation.

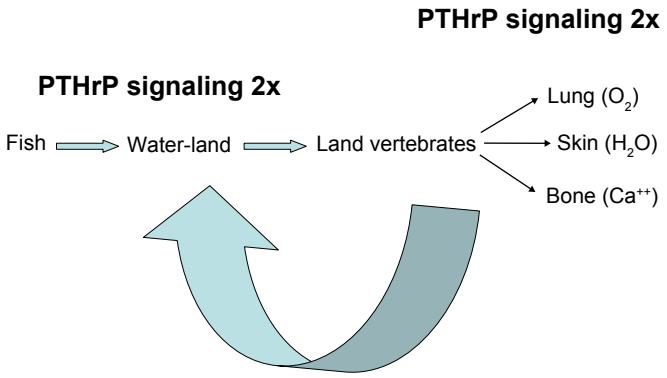

Figure 4. Role of PTHrP amplification in the water-land transition.
Notes: Duplication of the PTHrP receptor occurred during the water-land transition, "amplifying" the PTHrP-PTHrP signaling pathway, fostering key adaptations for life on land. The lung, skin, and bone are all dependent on PTHrP signaling for their development; since development is the mechanism of phylogenetic change, PTHrP signaling may also have facilitated the evolution of these structures.

As mentioned above, mechanistically (Fig. 4), the PTHrP receptor gene is known to have duplicated during the water-land transition,[76] amplifying the PTHrP signaling pathways for the adaptive morphing of the lung, skin, and bone—all of these organs are dependent on the PTHrP signaling pathway for their development and homeostasis. Though the literature describes this as though it occurred by chance, it could well have happened as a direct consequence of the generation of excess oxygen radicals and lipid peroxides due to vascular shear stress within the microcirculations of these very same tissues.[79] On the one hand, these tissues and organs would have constrained land adaptation, but on the other, increased PTHrP signaling would have been advantaged by such gene duplication events. This process is formally known as the Baldwin Effect.[80]

In fact, if adaptation is thought of in the context of internal selection caused by vascular shear stress, the concept of plasticity becomes much more relevant, not to mention being experimentally testable; constitutive genes are the ones that were most vulnerable to mutation, since they were the genes being targeted by such selection mechanisms. And perhaps such unconventional internal selection was followed by classic Darwinian population selection for those members of the species that were best fit to regulate those constitutive genes to survive, rendering the newly evolved homeostatic mechanisms regulatable. Theoretically, this may have been due to the fact that regulated mechanisms would be more resilient and therefore less likely to generate mutagens than nonregulated constitutive genes. And this may also explain why humans have fewer than the predicted number of genes based on descriptive instead of mechanistic biology.

There have been numerous attempts to reconstruct biology from its component parts. Darwinian thought fostered the works of Haeckel, Waddington, Riel, Seilacher, and Gould, to name only a few of those who have attempted to further our insights to evolution. And more recently, Morowitz,[81] and West and colleagues[82] have gained much notoriety by formulating comprehensive analyses of physiology, but the problem with their approaches is that they reason backwards from existing structures and functions. They do not predict the changes that have occurred over the course of evolution, even given all the moving parts, and they thus leave biology as a loosely linked series of anecdotes and medicine as virtually nonpredictive and ultimately incomplete in its philosophic and functional scope.

Those members of the species best able to upregulate their PTHrP signaling in support of any one or all of the land adaptive traits—bone, skin, and lung—would have had a higher likelihood of surviving on land. In turn, the other tissues and organs would also have been positively selected for their amplified PTHrP signaling capacity, making them more likely to survive. This is particularly relevant to the glomeruli of fish kidneys, which range from large (salt water) to small (fresh water) to being absent in some species[83] but are ubiquitous in land vertebrates. Shear stress within the renal vasculature could have given rise to PTHrP signaling for glomerular function—PTHrP-mesangium signaling for water and electrolyte flux.[67] Similarly, PTHrP is expressed in the pituitary[84] and adrenal cortex[85] of land vertebrates, making for a more robust physiologic stress "fight or flight" mechanism since the corticoids stimulate epinephrine secretion as they course their way from the adrenal cortex through the adrenal medulla.[86] But this amplified epinephrine response to stress is only applicable to amphibians and beyond phylogenetically since fish have an independent adrenal cortex and medulla.[87] Such an evolved stress mechanism would have been advantageous for various physiologic adaptations to land, not the least of which would have been the positive selection for brain evolution—epinephrine inhibits flow through the blood–brain barrier, generating more neuronal interconnections within the central nervous system due to increased epinephrine and norepinephrine production within the brain.[88]

Again, this is not merely a tautologic rationalization of the data. Developmentally, if you experimentally delete the PTHrP gene in the embryonic mouse, the bone, skin, and lung fail to develop the self-same characteristics for land adaptation.[89] Phylogenetically, the PTHrP signaling pathway has been amplified through gene duplication, fostering stronger skeletal support, skin barrier function, and lung gas exchange.

In further support of the causal relationship between the water-land transition and the evolution of specific physiologic traits that actively accommodated the adaptation to life on land, there were two other gene duplications that occurred during the water-land transition: the β-adrenergic receptor (βAR)[90] and the glucocorticoid receptor.[91] The evolution of the βARs was necessitated by the demand for independent regulation of the systemic and pulmonary blood pressures to accommodate the expanding surface area of the

evolving lung.[92] The evolution of the glucocorticoid receptor from the mineralocorticoid receptor was necessitated by the increase in blood pressure due to the increased effect of gravity on land, causing increased blood pressure,[93] generating further selection pressure for the βAR mechanism in alleviating the constraint on the expansion of the lung surface area; the effective stimulation of the βARs by glucocorticoids[94] caused further positive selection pressure for the co-evolution of both genes. Again, as in the case of the duplication of the PTHrP receptor, the specific effects of the physiologic stress due to land adaptation on shear stress in the lung and kidney may have specifically precipitated gene duplications in these capillary beds, functionally alleviating the physiologic constraints on these tissues and organs through internal selection, further fostering these physiologic adaptations through external selection. For example (Fig. 5), the episodic bouts with hypoxia due to the unmet physiologic needs of the organism as it attempted to adapt to land would have caused physiologic stress since hypoxia is the most potent physiologic stressor known, stimulating the pituitary-adrenal axis (PAA), pituitary ACTH stimulating glucocorticoid (GC) production by the adrenal cortex, and subsequently amplifying epinephrine production by the adrenal medulla as the GC passes through it, stimulating phenylethanolamine-N-methyltransferase, the rate-limiting step in epinephrine production; acutely, epinephrine would have alleviated the hypoxic stress by stimulating surfactant secretion by the evolving alveoli,[95] and the GCs would have increased βAR density, acting synergistically with epinephrine. As a result, the increased distension of the alveoli would have stimulated PTHrP production by the alveolar type II cells,[96] promoting further alveolarization, alveolar capillary perfusion, and angiogenesis of both the capillaries and lymphatic vessels; those organisms that were most fit to upregulate this cascade would have been more likely to survive, providing a mechanism for its Natural Selection. Taken together, the evolution of alveolar PTHrP signaling coordinates the secretion and homeostasis of surfactant with gas exchange across the microvasculature at both the macrolevel, and at the microlevel, since it functionally coregulates calcium in the alveolar fluid hypophase with the regulation of surfactant removal from the alveolus via the lymphatic drainage. In the aggregate, this adaptive integration of the PAA and the pulmonary system would have fostered the phylogenetic adaptation of land vertebrates. And this cascade of physiologic adaptations may explain the evolution of PTHrP signaling for pituitary ACTH and adrenocortical GC, since it would have further facilitated the positive selection for land adaptation by PTHrP receptor gene duplication.

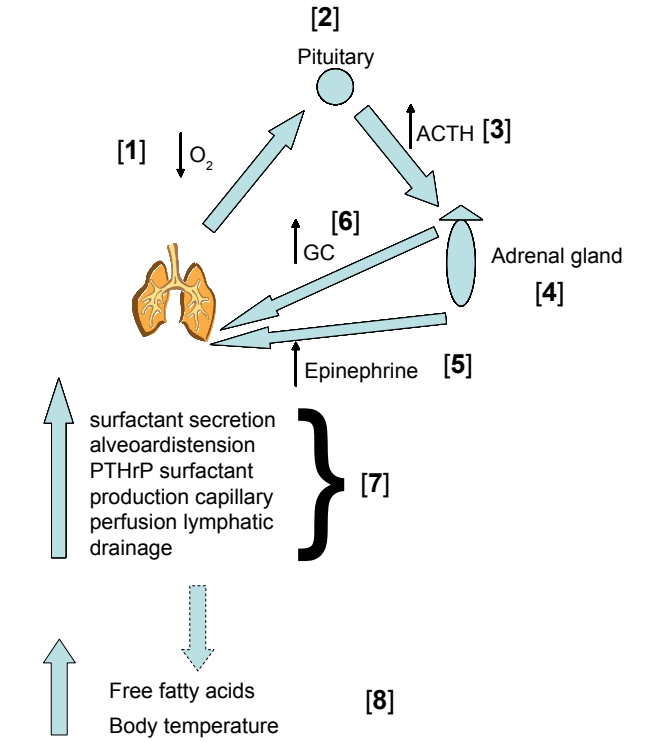

Figure 5. Parathyroid hormone-related protein (PTHrP) evolution.
Notes: Periodic hypoxia [**1**] during evolutionary adaptation to land stimulated pituitary PTHrP [**2**], amplifying ACTH [**3**], stimulating adrenocortical PTHrP [**4**], amplifying epinephrine [**5**] via glucocorticoid (GC) production [**6**]. GC enhances epinephrine activity in the lung, amplifying epinephrine-stimulated lung surfactant secretion [**7**], alveolar distension, increased PTHrP, increasing surfactant production, alveolar capillary perfusion, and lymphatic drainage. Epinephrine also causes free fatty acid secretion from peripheral fat cells [**8**], further increasing metabolism, generating "body heat", increasing lung surfactant activity, further increasing oxygenation.

Bear in mind that these events did not occur all at once; it took place over eons of land vertebrate evolution, both within and between species. Consistent with this scenario, elsewhere we have shown that in the course of lung evolution, there were alternating intrinsic and extrinsic selection pressures for the genes that facilitated the increased surface area of the lung.[96] This pattern may atavistically reflect the original mechanism by which the cell membrane of unicellular organisms facilitated the adaptation of the cell to the environment.

The Cellular-Molecular Approach to Evolution is Predictive

The predictive power of this cellular-molecular approach for understanding the evolution of complex physiology is underscored by the synergistic evolution of the lung and endothermy. There are a number of theories for the evolution of endothermy,[97–99] but there are none that integrates it in a functionally relevant way to the ontogeny and phylogeny of vertebrates. In contrast to that, the following hypothesis for the origin of endothermy is based on the physiologic interactions between the respiratory, neuroendocrine, and metabolic systems that would have occurred under the episodic hypoxic conditions encountered during the water-land transition and the subsequent fluctuations in ambient oxygen levels theorized by Berner.[100] The epinephrine effect on surfactant secretion in response to hypoxia alluded to above would have stimulated the secretion of fatty

acids from peripheral fat cells,[101] providing substrate for the tandem increases in respiration, metabolism, and the consequent increase in body temperature. This would have caused further positive selection for lung evolution since surfactant phospholipid is 300% more active at 37°C than at 25°C,[102] thus providing additional oxygen for metabolic drive. The consequent stress-induced increases in GCs would have further enhanced the epinephrine effect by amplifying βAR activity in fat cells. At the cellular level, these effects of epinephrine and GCs are consistent with their mechanism of action on phospholipid composition in both the lung surfactant and in somatic cell membranes; during the processes of ontogeny and phylogeny, there is an increase in saturated phosphatidylcholine in lung surfactant, caused by the effect of glucocorticoids on its synthesis in the alveolar type II epithelial cell.[103] In the periphery, epinephrine has been found to increase the unsaturated phosphatidylcholine content of the cell membrane,[104] similarly amplified by the effect of glucocorticoids on βARs. In the lung, the increased production of surfactant saturated phosphatidylcholine is physiologically advantageous because its phase transition temperature (the temperature at which it fluidizes) renders it more surface active in reducing surface tension at higher body temperatures;[105] in the periphery, the opposite occurs, since unsaturated phosphatidlycholine renders the cell membrane more fluid at lower body temperatures due to its lower phase transition temperature, making it more permeable to oxygen.[106] Hence, the same epinephrine mechanism that facilitated lung evolution also facilitated gas exchange in the periphery—a synergistic "win–win" that put mammals at advantage in adapting to land life.

Such mutual positive selection for both lung gas exchange and increased body temperature is consistent with the evolution of endothermy. And these interrelationships may have been exapted since both the lung lipofibroblast and the peripheral fat cell produce leptin;[107] in the lung, leptin promotes surfactant synthesis,[108] whereas in the periphery, leptin increases body temperature;[109] among its many physiologic effects, it has been shown to increase body temperature, perhaps due to its inflammatory interleukin homology. Interleukins have been implicated in the evolution of endothermy as a mechanism in support of host defense.[109] Experimentally, treating ectothermic Fence Lizards with leptin increases their basal metabolic rate and body temperature.[110] Thus, the integration of pulmonary physiology and host defense may have led to selection pressure for endothermy.

As a note added in proof, in hibernating animals, hypoxia is associated with decreased unsaturated cell membrane phospholipids, rendering the cell less permeable to oxygen at low temperature.[111] This metabolic adaption in heterothermic animals is a "reverse-evolutionary" strategy for conserving oxygen under hypoxic conditions.

Dinosaurs and birds are also warm-blooded, but this mechanism does not apply because their lungs are affixed to the thorax,[112] so the above-cited adrenalin effect on surfactant does not apply. This may be why the bird adrenal is not compartmentalized into cortex and medulla, instead is being composed of randomly associated corticoid and chromaffin cells that would not have amplified adrenalin production as in the case of mammals.[113]

This is not surprising since there is a functional homology between host defense and the surfactant systems. There are four surfactant apoproteins: A, B, C, and D. A and D are collectins, which are members of the host defense system. In experiments designed to determine the role of leptin in Xenopus's lung development, we treated frog tadpole lung tissue with leptin and found that it had the same effect on alveolar development that it does in mammals—increased surfactant synthesis in combination with the thinning of the gas exchange surface.[114] Yet this was counterintuitive since frogs are buccal breathers—they actively force air into muscle-lined faveoli, which are gas exchange spaces 1,000 times larger than an alveolus, unaffected by surface tension, obviating the need for surfactant to prevent atelectasis. However, in retrospect, the stimulation of surfactant proteins necessary for host defense makes sense since the lung evolved as an expansion of the foregut,[115] creating a potential site for infection. Therefore, the impetus for surfactant production by the evolving lung may have been predicated on increased antimicrobial peptides, followed by surfactant phospholipids, known to be produced by the gut. Thus, the forward-directed approach to evolution provides a causal chain of events rather than a series of loose associations, at best.

The functional interrelationship between the neuroendocrine and respiratory systems and endothermy is an exaptation that refers all the way back to the origins of eukaryotic life itself. The advent of cholesterol fluidized the cell membranes of unicellular eukaryotes, facilitating gas exchange, metabolism, and locomotion, the three major traits in vertebrate evolution.[116] This may have been the molecular evolutionary prototype for the coevolution of the neuroendocrine and surfactant systems that fostered endothermy. As a note added in support of this hypothesis, the EGF signal mediator neuregulin is fundamental to both lung development[117] and myelinization,[118] for example.

Moreover, the mutual positive selection for endothermy and gas-exchange efficiency was driven by an ever-more robust neuroendocrine system, marked by the progressive physical integration of the adrenal cortex and medulla during the water-land transition. The latter must have been due to Darwinian selection.

As added evidence for the interrelationship between key gene duplications that occurred during the water-land transition and physiologic stress causing internal selection, type IV collagen also evolved novel polymorphisms in the basement membranes of the lung and kidney phylogenetically from fish to humans during this period.[119] The NC1 domain of type IV collagen forms a natural physicochemical barrier against fluid exudation from both the lung and kidney due to its molecular electrostatic and polar properties, preventing the loss of fluid

across the alveolus and glomerulus that would otherwise have occurred due to the increased physiologic demand on these structures during the water-land transition.[120]

Moreover, pathophysiologically, loss of any of these evolutionarily adaptive properties causes cellular-molecular malfunctions consistent with "reverse evolution". For example, loss of PTHrP expression by alveolar epithelial type II cells due to over-distension, infection, or oxidant injury causes transdifferentiation of lipofibroblasts to myofibroblasts, causing increased alveolar diameter, reverting to earlier phylogenetic forms of the lung seen in reptiles and amphibians.[120] Compromised βAR function similarly leads to chronic lung disease,[121] and functional GC deficiency leads to bronchopulmonary dysplasia in the developing lung.[122] And the abnormal molecular composition of the NC1 domain of type IV collagen in Goodpasture syndrome can cause physiologic failure of both the lung and kidney.[123]

Contrasting Evolutionary and Developmental Biology as Descriptive Versus Mechanistic

If the "key" to understanding evolution is as a mechanism for spatial-temporal relationships of genes as determinants of phenotypes, and these relationships are mediated by soluble growth factors and their cognate receptors, then by following the latter, we can understand the former. After all, how can you generate an "arrow of time" without a mechanism for the magnitude and direction of its trajectory? Ironically, the Evolutionary Biology literature has virtually no orientation to growth factors as the mediators of evolution, or their signaling to their cognate growth factor receptors, which are the determinants of the "arrow of time" described by evolutionists.[124] As a result, Evolutionary Biology is purely descriptive, offering no biologic mechanism to explain Natural Selection.

On the other hand, as mentioned earlier, contemporary Developmental Biology is predicated on the functions of growth factors and their receptors as the determinants of morphogenesis. The big breakthrough in molecular embryology occurred in the late 1970s with the discovery that soluble growth factors and their receptors underlie and mediate the patterns of development. And developmental physiology as the outcome of embryonic development acknowledged that the denouement of development is integrated homeostasis. Recognition of such developmental and homeostatic mechanisms as a continuum provides deep insight into the mechanisms of evolution. By superimposing cell-cell signaling on conventional ways of thinking about descriptive evolution, one can begin to understand such otherwise nebulous terms and concepts as Survival of the Fittest, Descent with Modification, Natural Selection, the Biogenetic Law, Spemann Organizers, Canalization, Genetic Assimilation, Exaptation, Modularity, Evolvability, Systems Biology, Developmental Systems Theory, Pleiotropy, etc.

Conrad Waddington invoked Canalization, aka homeostasis, in the context of evolution.[33] When a cell biologist looks at Waddington's adaptive landscapes, which resemble tents, supporting poles, and all, they want to look under the canvas and see what has caused those hills and valleys. In so doing, they have been able to determine the cellular/molecular basis for morphogenesis, which is where evolutionists began in the 19th century, but were unable to provide the mechanistic basis for Haeckel's Biogenetic Law[28] or Spemann's Organizer.[29] So the geneticists wrested the subsequent inquiry into evolution from the embryologists and have been reducing Evolutionary Biology to mutation and selection ever since. Cell Biology has literally been eliminated from Evolution Theory for these historic reasons, yet it has revealed how single cells can create whole organisms, much the same as evolution has. And suffice it to say that evolutionists are not trained in cell biologic methods. Therefore, it would seem productive to let the cell biologists back into the tent. How would this advance our understanding of the mechanisms of evolution? Perhaps by addressing some of the major concepts in Evolution Theory in cellular terms (see above), we may see how Developmental Biology would facilitate our thinking in this field, which has the potential for being the basis for a unifying theory of biology in practice, as well as in principle "science is deductive, not inductive". We suffer from too many metaphors and too few experimentally refutable hypotheses.

As mentioned above, Ernst Mayr[19] artificially (and in the present day and age, artifactually) separated Evolutionary Biology into proximate and ultimate causation in an effort to protect biology against the onslaught by reductionist physicists back in the 1950s and 1960s. The advent of genomics has yet again threatened to reduce Evolutionary Biology to Systems Biology, but the reasons for the breakdown between these subdisciplines have been resolved, potentially creating a rapprochement between the "biologies".

Raff's recounting of this era[75] makes it clear that "a boundary discipline exists, and its investigations can yield important complementary insights not possible in either discipline alone", namely Evolutionary Developmental Biology. The reintegration of Developmental Biology and Evolutionary Biology was a major step in advancing our understanding of both disciplines. But there is still a huge gap in this effort due to the strong presence of Cell Biology in Developmental Biology and its virtual absence from Evolutionary Biology. The gap appears to be due to the long-standing rift between these two disciplines, yet Walter Garstang[125] observed that because the morphology of animals arises anew in each generation, evolution of new animal forms had to be viewed as a problem in the evolution of development. In reformulating the Modern Synthesis, those advocating for the reintroduction of Developmental Biology into Evolutionary Biology failed to challenge the evolutionary community to use contemporary methods of cell-molecular embryology, which is dependent on the mediation of gene products by soluble growth factors and their receptors expressed on different cell types that participate in morphogenesis. One can speculate as to why this

lapse occurred, but for whatever reason, it seems to have left Evolutionary Biology without a way of integrating genes and phenotypes in the same way that Developmental Biology does. This is ironic, since these principles have resolved the problem of the Spemann-Mangold "organizer"[29] by demonstrating how soluble growth factors and their cognate receptors mediate spatio-temporal signaling to generate form and function, providing the basis for developmental physiology. By determining the molecular basis for the development of physiologic principles, we now have a working model for a mechanistic continuum from development to homeostasis, repair, and aging. By focusing on the serial mechanisms that generate phenotypic change in adaptation to the environment, we eliminate the need for "time", other than as the sequence of events. And "space" is also eliminated, if indeed we are all derivatives of unicellular organisms. Therefore, the "evolution" of such biologic mechanisms should obviate the need for the artificial dissociation between the proximate and ultimate mechanisms of evolution, yet such precepts persist, impeding the functional integration of genomics into Evolution Theory.

For example, Bonner had introduced the concept of "modularity" into Evolutionary Biology,[126] which was seen as a breakthrough idea that would advance thinking in the discipline. Had the evolutionists embraced Cell Biology, they would have avoided the need to introduce yet another metaphoric circumlocution into the discipline, alleviating the need to devise experiments to determine how developmental motifs form the basis for evolution at the cell-molecular level.

It is universally held that genes determine biologic structure and function. However, genes do not directly interact with other genes, and therefore, they must be considered within their cellular contexts. Nowhere is this more apparent than in the process of development, in which genes determine morphogenesis by spatio-temporally regulating soluble growth factors and their receptors, dictating the growth and differentiation of other cells within their niche. This phenomenon was first described by Driesch[127] and later refined by Spemann and Mangold[29] as morphogenetic fields, but without having knowledge of ligand-receptor interactions; that mechanism only emerged in the late 1970s. Similarly, it is acknowledged that development mediates evolutionary change, yet evolutionists rarely, if ever, reduce the analysis to cells and their products. The reason for this is somewhat obscure; in her book *Unifying Biology: The Evolutionary Synthesis and Evolutionary Biology*, Smocovitis[128] has attributed the absence of Cell Biology from Evolution Theory to the rift between evolutionists and embryologists in the late 19th century. It is unfortunate that those who have been advocating for the rapprochement between Evolutionary Biology and Developmental Biology, or Evo-Devo, have overarched cell biology yet again in favor of random mutation and selection—biology is not stochastic, it is pragmatic and existential in nature.

One often reads of molecular biologists alluding to the highly conserved nature of genes of interest as validation for their relevance to some biologic process or structure, but what does that mean functionally? That it is expressed far back in the history of the organism, inferring that it has been present through much of the evolution of the species. But rarely if ever is this pursued mechanistically in order to determine how and why such a conserved gene was involved in the evolutionary mechanism. Other than the process of development, there is no system in which to test such mechanisms.

Although this is a simple concept, there was considerable difficulty in actually executing studies based on the idea. Development and evolution certainly offer a facile sort of analogy to each other: both are processes of change. Although this analogy was compelling during the 19th century, it was sterile until the developmentalists discovered soluble growth factors and their cognate receptors, which were able to mediate the spatiotemporal aspects of the developmental process. Development is a programmed and reproducible process. If we accept Darwinian mutation and selection, evolution can be neither. Evolution can consist of internal and external selection, with internal stability being homeostasis, which can exhibit "reaction norms" that are heritable based on the Baldwin effect. The process of evolution is described as "emergent and contingent". Canalization can be seen in the context of homeostatic regulation, which, when it fails, can generate cryptic genes that represent the history of the organism, now reprised to provide a physiologic "safety net" that allows for the healing to occur; as such, it allows for reproduction even in the face of illness. The apparent inevitability of development was daunting. To connect it effectively with evolution, two major ideas had to be accepted. The first, pointed out by Garstang,[125] is that the larval stages also face the rigors of life (reminiscent of the Barker Hypothesis, that adult diseases originate in utero). Mendelian genetics allows new traits to appear at any developmental stage, and Natural Selection potentially operates upon them as it does upon traits expressed in adults. The second major point is that although ontogeny appears inevitable and inextricably orchestrated in its flow, it is not a single process. There are a large number of processes at work, some more or less coupled to others. It was Joseph Needham who, in 1933,[129] using an engineering metaphor of shafts, gears, and wheels, suggested the idea of dissociability of elements of the developmental machinery. He pointed out that it is possible to experimentally separate differentiation from growth or cell division, biochemical differentiation from morphogenesis, and some aspects of morphogenesis from one another. The implication of this idea is enormous: developmental processes could be dissociated in evolution to produce novel ontogenies out of existing processes, as long as an integrated developmental program and organismal function could be maintained.

Epistemology—Maybe We Got it Backwards?

The integrated mechanism for physiology has long been accepted to be a fait accompli, yet we know that there are

processes of development, evolution, and regeneration-repair that comply with some unknown, underlying bauplan. The recent experimental evidence for the complete metazoan toolkit being present in the unicellular state of sponges provides the rationale for such an integration of structure and function, by definition. Mechanistically, the insertion of cholesterol in the plasma membrane of eukaryotes facilitated endocytosis, locomotion, and respiration, providing the impetus for their evolution. Moreover, it is striking that the cytoskeleton collectively mediates homoestasis, mitosis, and meiosis alike, suggesting the phenotypic autonomy of these unicellular organisms. The significance of this is evinced by subjecting yeast, the simplest eukaryotes, to microgravity, causing both loss of polarity and failure to bud. Without polarity, there is no calcium flux or reason to locomote—where is up, down, sideways? And budding is the reproductive strategy of yeast. Loss of these fundamental traits by "disorienting" the cytoskeleton underscores the adaptation to the one element in the environment that is omnipresent, is unidirectional, and was there from the inception of the planet. So perhaps multicellularity was merely the eukaryotic ploy used to combat lateral inheritance, biofilm, and quorum sensing in our age-old competitors, prokaryotes.

Conclusion

The multicellular form may merely be a derivative of the unicellular state, acting as a matrix for it to monitor the oncoming environment so that the gene pool knows what epigenetic marks acquired during the multicellular phase of the life cycle to include or exclude in the next generation. That perspective would herald a sea change in our perspective on the life cycle, re-centered on the unicellular state instead of the adult phenotype. For example, Dictyostelium exists in two forms, a free-swimming amoeboid form and a colonial fruiting body. Under conditions of abundant nutrients, the slime mold remains in its free-swimming amoeboid form; under low food abundance conditions, the amoeboid free-swimming phenotype forms colonies. Logic would dictate that this organism evolved under high nutrient abundance conditions, and therefore, its unicellular form is the primary phenotype, the colonial form being derivative.

We need to better understand evolution from its unicellular origins as the Big Bang of biology.

Author Contributions

Conceived the concepts: JST. Wrote the first draft of the manuscript: JST. Developed the structure and arguments for the paper: JST. Made critical revisions: JST. The author reviewed and approved of the final manuscript.

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