Published in final edited form as:

Med Hypotheses. 2009 May ; 72(5): 596–602. doi:10.1016/j.mehy.2008.09.057.

Exploiting Cellular-Developmental Evolution as the Scientific Basis for Preventive Medicine

J.S. Torday, PhD^{1,2,*} and V.K. Rehan, MD¹

¹ Department of Pediatrics, David Geffen School of Medicine, Los Angeles, California; Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California

² Department of Obstetrics and Gynecology, David Geffen School of Medicine, Los Angeles, California; Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California

Abstract

In the post-genomic era, we must make maximal use of this technological advancement to broaden our perspective on biology and medicine. Our understanding of the evolutionary process is undermined by looking at it retrospectively, perpetuating a descriptive rather than a mechanistic approach. The reintroduction of developmental biologic principles into evolutionary studies, or evo-devo, allows us to apply embryologic cell-molecular biologic principles to the mechanisms of phylogeny, obviating the artificial space and time barriers between ontogeny and phylogeny. This perspective allows us to consider the continuum between the proximate and ultimate causes of speciation, which was unthinkable when looked at from the descriptive perspective. Using a cell-cell interactive ‘middle-out’ approach, we have gained insight to the evolution of the lung from the swim bladder of fish based on gene regulatory networks that generate both lung ontogeny and phylogeny, i.e. decreased alveolar size, decreased alveolar wall thickness, and increased alveolar wall strength. Vertical integration of cell-cell interactions predicts the adaptivity and maladaptivity of the lung, leading to novel insights for chronic lung disease. Since we have employed principles involved in all of development, this approach is amenable to all biologic structures, functions, adaptations, maladaptations, and diseases, providing an operational basis for preventive medicine.

Introduction

In the post-genomic era, we must broaden our perspective on biology and medicine to make maximal use of this technological advancement. Since evolution is all of biology (1), such an approach would accommodate our needs. How can we effectively apply evolutionary thinking to medicine? Like Darwin and Wallace, our understanding of Natural Selection continues to be based on retrospective, descriptive analysis, reasoning after the fact-if we think of the chronology of life as an “arrow of time” traveling from left to right, we must begin thinking about evolutionary complexity from its unicellular origins, progressing in time and space from left to right, rather than being seen in the conventional reverse direction from right to left. At best, Darwinian theory retrodicts phylogeny, but how do we move to evolution in the forward direction, reflecting how life evolved from unicellular organisms to metazoans? This paradox is a vestige of the view that the only evidence we have for evolution as scientists is the fossil

*Author Responsible for Correspondence: J.S. Torday, MSc, PhD, E-mail: jtorday@labiomed.org, Phone: 310-222-8186, FAX: 310-222-3887. E-mail: vrehan@labiomed.org, Phone: 310-222-1965, FAX: 310-222-3887.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

record. But that has changed with the advent of evolutionary-developmental biology and the effective application of molecular biology to decipher both phylogeny and ontogeny.

Cellular-Development and Evolution

We know a lot about the cell-molecular mechanisms of embryogenesis-how genes determine structure and function-which can be used as an experimental platform for testing Natural Selection for gene regulatory networks (GRNs) that determine phylogenetic phenotypes. Exploitation of developmental models to experimentally test phylogenetic hypotheses would allow us to challenge evolutionary theory in Real Time, experimentally. Unicellular organisms dominated the earth for the first 4.5 billion years-it is only during the last 500 million years that multicellular organisms have emerged (2). The prevailing theory as to why this occurred is that unicellular organisms began experimenting with metabolic cooperativity (3), a process that is mediated by cell-cell signaling. That process resulted in a selection advantage, initially because the bigger the organism became, the less likely it was to be eaten. But beyond this simple explanation for increased size, as pro- and eukaryotes continued to compete, the development of progressively more complex systems conferred a further selection advantage through division of labor. D'Arcy Thompson had pointed out that the variability in animal size is not a function of cell size, which is fairly constant, but in cell morphologies, which are highly variable (4).

Morphogenesis is determined by cell-cell communication, which is the driving force behind vertebrate evolution. As such, it provides insights to the macro- and microevolutionary strategies that have succeeded over biologic time. Gene duplication has been a key genetic adaptational mechanism (5), but this is an obvious strategy, like increasing body size- more is better at a very basic level in all systems. And increasing size and gene duplication describe processes without providing underlying mechanisms. As long as we continue to tell 'Just So Stories' (6), we legitimize Intelligent Design and fail to exploit genomics to advance our knowledge of biology and medicine (7). One set of molecular mechanisms common to both development and phylogeny are the ligand-receptor interactions that mediate growth, differentiation, homeostasis and aging (8). Sydney Brenner has referred to this as the 'Middle-Out' approach (9), though he didn't suggest exploiting the ligand-receptor relationship as a way of deconstructing the evolved pathways. These mechanisms are plastic, and represent mechanisms that mediate the on-going interactions between the organism and its environment that are at the core of the evolutionary process (9,10). Such a mechanistic strategy is superior to descriptive 'top-down' approaches like Natural Selection (11) or The Great Chain of Being, or 'bottom-up' approaches like those of West (12,13) and Morowitz (14) based on hierarchical metabolic pathways. For example, the Creationist Michael Behe has suggested that for evolution to have generated novel protein features through point mutations it would have required a minimum of 10^9 individuals (15). But the middle-out mechanism would require much smaller numbers since it is based on active selection for traits. The model also provides the basis for evolutionary experimentation. By examining GRNs that determine development of structure and function, one can identify other functionally related GRNs within that organism, and in homologous tissues in the same and phylogenetically-related organisms (16). Such an analysis, based on adaptational strategies, is far more likely to provide useful information about evolution and physiology than the stochastic approach currently being used to elucidate Systems Biology (17). For example, as depicted in the Schematic (Fig. 1) above, a GRN common to the phenotypes for development, homeostasis, repair and aging of a given structure/function (lung, kidney, liver, brain, etc.) can be depicted as changing over chronologic time (x axis) as a family of idealized parallel lines. Such a set of simultaneous equations can then be solved for these GRN/phenotype interrelationships in biologic time, or evolution, independent of chronologic time, i.e. all of the biological processes are now relative to one another, independent of chronologic time. Such a self-referential property of evolved structure

and function reflects the modular nature of the cell-cell interaction principle. But that primary process of evolution is complicated by the fact that selection is for genes in specific cell populations as they relate to specific physiologic functions, such as breathing, locomotion, digestion, micturation, cognition, etc., etc. But those same genes are expressed in all of the cells in the population, both for the primary structure/function site, and for all of the other tissues and organs where that cell population is present. The descriptive term for this phenomenon is exaptation, as coined by Gould and Vrba (18). What they had not considered were the developmental implications of such a process. Such a mechanism would create scenarios in which cells of differing germline origins would be forced into spatiotemporal juxtapositions based on developmental principles, whereas the formation of novel gene regulatory networks would either create novel structures and/or functions, or not, depending upon whether they were compatible with viability limited/constrained by the reproductive process.

Such a seemingly haphazard mechanism could explain why the fish swim bladder evolved into the vertebrate lung, for example, as follows: the swim bladder is a gas-filled out-pouching of the gastrointestinal tract in physostomous fish (19). It has allowed fish to adapt to the force of gravity, maintaining equilibrium in order to forage efficiently at various levels in the water, rather than having to expend additional energy by constantly swimming, and to sleep at the bottom at night by emptying the bladder. The 'invention' of the surfactant, a lipid complex, further facilitated this mechanism by making the bladder more compliant (20)- selection pressure for the transition of vertebrates from water to land may have been facilitated by the overlapping of the processes of gas exchange and metabolic activity (feeding) through the production of surfactant, selecting for progressively greater surfactant production efficiency to increase the surface-to-volume ratio of the gas exchange organ (21).

Cell-Cell Signaling, Evolution and the Development of Physiologic Novelties

It has been challenging to understand why evolution takes 'big leaps' from time to time (22). Our prediction is that because the cell-molecular model for evolutionary novelty selects for genes within specific cell populations, the resultant genetic 'legacy' acquired by all cells in that population (e.g., endoderm, mesoderm, ectoderm), not just those of the structure/function being selected for, i.e. genetic adaptation spilling over into other structures/functions, creates opportunities for novelty by virtue of the fact that the genetic trait may become useful, i.e. adaptive, under emergent conditions. This process may explain why, for example, respiration and metabolism (23), photoreception and circadian rhythms (24), cerebation and radical oxygen species signaling (25), renal function and erythropoiesis (26), or the formation of eyes and ears (27) have co-evolved. Ideally, such counter-intuitive logic should emerge from a paradigm shift in our thinking about evolutionary mechanisms in cell-molecular terms from the "middle out". Ultimately, such a fundamental change in the way we think about the processes of evolution will provide the mechanistic answer to such perennial questions as the nature of canalization, gene sharing, reproductive isolation and evolutionary novelty.

Evo-Devo as a Scientific Basis for Preventive Medicine

The use of a developmentally mechanistic approach to chronic lung disease provides a perspective on genes of interest in pathologic processes as a subset of maladaptation. Not only does a developmental approach identify the acute, proximate genes involved, but it also reveals the mechanistically linked genes that are up-stream, and pre-clinical, leading to novel diagnostic genetic markers for Chronic Lung Disease and molecular targets for the prevention of such diseases. By broadening our perspective to the adaptive and maladaptive evolutionary origins of the lung (initial conditions, cell-cell cooperativity) we effectively eliminate the constraint of chronologic time incurred by a retrospective view by demonstrating the

commonality of all lung biologic processes (28) - development, homeostasis, repair, aging- demonstrating how all lung biologic processes can be expressed as a family of parallel lines, or simultaneous equations, representing the Gene Regulatory Networks held in common. By this approach we have effectively eliminated the artifactual precept that there are distinct proximate and ultimate causes of evolution, as first suggested by Mayr in 1961 (29). Therefore, by breaking down the barriers between the various aspects of lung biology we can now freely examine the specific interconnected gene regulatory networks involved, in both the forward (phylogeny, ontogeny, homeostasis) and backward (chronic disease, aging) direction, molecularly and biologically.

Moreover, this is a robust model for all complex diseases, since it is based on the normal developmental mechanisms for all tissues and organs. This model contrasts with gene maps, interactomes and other Systems Biologic approaches that identify genes outside of their biologic context, i.e. abiotically.

A Paradigm Shift for Medicine from Osler to Darwin

For centuries we have used disease to leverage our knowledge and understanding of health, and visa versa, since all we had available were descriptive phenotypes. However, with the advent of genomics, we can now address the questions of health and disease as a continuum, based on genetic mechanisms as they apply to the relevant phenotypes. Furthermore, because of the recognition of the commonalities between phyla, and the sequencing of fish, amphibian and avian genomes, we can now let evolutionary-developmental biology act as the Rosetta Stone for deciphering the nature of disease as maladaptation, rather than defining health as the absence of disease. Since biomedical research is in a crisis with regard to translating genes into phenotypes (12), this transition constitutes what Thomas Kuhn referred to as a paradigm shift in "The Structure of Scientific Revolutions" (30).

Evolutionary biology is a catch phrase for all of biology (1), and so by plugging such genes of interest into a developmental model that approximates evolution (28), it offers an opportunity to decipher the disease process based on a robust integration of genes and phenotypes independently selected for by evolutionary pressures.

A Practical Guide to Evolution, from Start to Finish

Volumes have been written on the subject of evolution, yet we still haven't solved the paradox of biologic stability with change (31), or the origins of novelty (32). The answer to this puzzle may lie in the origins of metazoa as unicellular organisms and how single cells have evolved to form the structures and functions of multicellular organisms. King et al (33) have made a seminal observation in this regard- that choanoflagellates, the free-living form of sponges, already had evolved all of the 'genetic tool kit' to generate multicellular organisms, i.e. adhesion molecules, growth factors and their receptors, long before metazoans evolved, indicating that in generating colonial organisms, all of the building blocks were already present. And as to why unicellular organisms evolved into multicellular organisms, it has been conjectured that rising oxygen tension in the atmosphere forced the existing organisms to evolve or become extinct. Metabolic Cooperativity among such primordial organisms was an effective strategy for survival, characterized by the generation of soluble factors that would facilitate communication between individuals to promote efficient use of nutrients and oxygen. As these early metazoans increased in size- an acknowledged strategy for survival since the bigger they were, the less likely they would be to be eaten- there was selection pressure to specialize cell-types to accommodate the increased need for metabolic efficiency. Therefore, the selection pressure was for cells with specific phenogenetic traits, keeping in mind that the selection was for cell populations. This resulted in the evolution of specific structural and functional characteristics which provided a selection advantage, at the same time conferring

those same genetic traits on other tissues and organs in a manner which was not necessarily advantageous, but at least didn't interfere with reproduction. This iterative process of selection for cell-population-specific phenogenetic traits probably gave rise to novel structures and functions by putting cells of different lineages in proximity to one another during development. This same evolutionary strategy is undoubtedly the cause of complex diseases due to failure of cellular cooperativity resulting from nonadaptively evolved genetic interrelationships that were missed by the fine filter of reproduction.

We will exemplify this process by looking at the evolution of the lung, a key organ for vertebrate evolution from water to land.

How and Why Did the Lung Evolve?

The structural origin of the lung is somewhat controversial. Darwin himself had speculated that the lung evolved from the fish swim bladder because they both are bladder-like structures that emanate from the esophagus (11). The homology has been put into question because the swim bladder is a dorsal structure whereas the lung is a ventral structure. Given the complete lack of evidence for air filled organs in chondrichthyans, and the isolated position of placoderms for which buoyancy organs of uncertain homology have been demonstrated, it is likely that the homologous pharyngeal swim bladder arose in the ancestors of early bony fish, and was predated by behavioral mechanisms for surface (water) breathing. However, as a basic outpouching structure the lung and swim bladder likely share common molecular motifs. For example, Parathyroid Hormone-related Protein (PTHrP), which is necessary for the formation of lung alveoli, is a stretch-regulated paracrine factor in the urinary bladder (34) and uterus (35) as well. Furthermore, PTHrP signals for lung development and homeostasis through its paracrine effect on leptin (36), a metabolic hormone. Leptin has recently been shown to accelerate limb development in the tadpole, linking metabolism to locomotion through a common endocrine mechanism; leptin may also link metabolism and locomotion to respiration (37), all of which are key steps in vertebrate evolution from water to land, suggesting that although there may not be a one-to-one relationship between the swim bladder and the lung anatomically, these structures may be evolutionarily homologous based on cell-molecular gene regulatory networks. Perhaps the scenario of puddles drying up (38) forced an exaptation of the swim bladder GRN to form the gas exchanger.

All vertebrate gas exchangers, including the swim bladder, express surfactant genes, yet the swim bladder is too large in diameter to necessitate surfactant as a surface tension reducing agent; it is more likely a lubricating agent (20) that prevents the walls of the swim bladder from adhering to one another. However it should be noted that physostomous fish must take a bite of air to inflate their swim bladders during development or else they will die, which may require the surface tension reducing activity of surfactant for this process.

Can Stretch-regulated PTHrP Signal Amplification Explain Lung Evolution?

Up to this point the explanation of how PTHrP has promoted the structural and functional phylogeny of the lung is based upon mechanisms relevant to lung physiology. However, the Baldwin Effect—the ability of organisms to genetically inherit traits through embryonic development—would account for the evolutionary retention and amplification of PTHrP signaling, because the capacity to increase the efficiency of gas exchange would be expected to confer a selective advantage. We have hypothesized that PTHrP signaling is amplified phylogenetically from colder, relatively inactive animals such as frogs, to high-activity, high body temperature, warm-blooded organisms such as mammals (28) due to the selection pressure for metabolic efficiency, which in turn selects for increased structural and functional efficiency of oxygenation. The complementary effects of PTHrP on alveolar structure and function are hypothesized to facilitate this process, as depicted in Figure 2.

It could be argued that the interrelationship between PTHrP signaling and the evolution of the lung are circular reasoning, i.e., that the increased surface area would naturally distend the parenchyma, leading to increased PTHrP signaling. Several aspects of this mechanism, however, suggest that the effect of PTHrP on the evolution of the gas exchange unit is causal: (i) PTHrP is necessary for alveolization (39–41); (ii) PTHrP is a gravisensor (42), suggesting that it facilitates adaptation to gravity; (iii) PTHrP is non-linearly affected by stretch (42,43), potentially explaining the so-called over-engineering of the bird lung (44); (iv) the PTHrP paracrine signaling pathway determines the myogenic and adipogenic fibroblast phenotypes of the alveolar interstitium (45–47), providing a mechanism for the phylogenetic transition from a muscle cell–dominated interstitium in amphibians and reptiles to the fat cell–dominated interstitium of mammals (23); and (v) PTHrP inhibition of fibroblast proliferation may cause the thinning of the interstitium (48,49).

Ontogeny and Homeostasis

Stimulation of PTHrP and its receptor by alveolar wall distension coordinately increases surfactant production (50) and alveolar capillary blood flow (51)- referred to as ventilation(V)/ perfusion(Q) or V/Q matching. V/Q matching is the net result of the evolutionary integration of cell/molecular interactions by which the lung and pulmonary vasculature have functionally adapted to the progressive increase in metabolic demand for oxygen (52–54). The structural adaptation for gas exchange is 3-fold: (i), the decrease in alveolar diameter (55); (ii) the thinning of the alveolar wall (56); (iii) the maximal increase in total surface area (57). These structural adaptations could have resulted from the phylogenetic amplification of the PTHrP signaling pathway. PTHrP signaling through its receptor is coordinately stimulated by stretching the alveolar parenchyma (53). Binding of PTHrP to its receptor activates the cyclic AMP-dependent Protein Kinase A signaling pathway (58,59). Stimulation of this signaling pathway results in the differentiation of the alveolar interstitial lipofibroblast, characterized by increased expression of Adipose Differentiation Related Protein (ADRP) and leptin. ADRP is necessary for the trafficking of substrate for surfactant production (60), and leptin stimulates the differentiation of the alveolar type II cell (61). PTHrP affects the cellular composition of the alveolar interstitium in at least three ways: (i) it inhibits fibroblast growth (62), and stimulates apoptosis (63), resulting in septal thinning; (ii) stimulation of epithelial type II cell differentiation by leptin (47) can inhibit epithelial cell growth (64); (iii) leptin may up-regulate type IV collagen synthesis (65) reinforcing the alveolar wall.

Ontogeny and Phylogeny

Primordial lung endoderm and mesoderm differentiate into over 40 different cell-types. We know a great deal about growth factor signaling that determines these processes, and the downstream signals that alter nuclear read-out. And because a great deal of effort has been put into understanding the consequences of preterm birth in humans and model organisms, we also know how these mechanisms lead to homeostasis, or fail to do so, in which case the phenotype for chronic lung disease informs us of the mechanism of lung fibrosis.

Embryonic lung development is subdivided into branching morphogenesis and alveolarization, only the latter being plastic (66). Deleting the PTHrP gene results in failed alveolarization (41), inferring relevance of PTHrP to lung evolution, since alveolarization is the mechanism for vertebrate lung evolution (52–54). Because PTHrP and its receptor are highly conserved (67), and stretch-regulated (34–36), and provide a mechanistic link between the endoderm, mesoderm and vasculature (36,68), we are compelled to investigate its overall role in lung phylogeny and evolution.

The combined effects of i-iii in the previous section would lead to natural selection for progressive, concomitant decreases in both alveolar diameter and alveolar wall thickness

through ontogeny (69) and phylogeny (70), increasing the surface area-to-volume ratio of the lung. PTHrP turns off myofibroblast differentiation by inhibiting Gli (71,72), the first molecular step in the mesodermal Wingless/int (Wnt) pathway, and by inactivating β catenin (73), followed by activation of lymphocyte enhancer-binding factor (LEF-1), CCAAT/enhancer binding protein alpha (C/EBP α) and Peroxisome Proliferator Activated Receptor gamma (PPAR γ). The downstream targets for PPAR γ are adipogenic regulatory genes such as Adipocyte Differentiation Related Protein (ADRP) and leptin. PTHrP induces the lipofibroblast phenotype, first described by Vaccaro and Brody (74). This cell-type is expressed in the lungs of a wide variety of species, including both newborn and adult humans (75). They are found next to type II cells in the adepithelial interstitium (59), and are characterized by neutral lipid inclusions surrounded by ADRP, which mediates the uptake and trafficking of lipid from the lipofibroblast to the type II cell for surfactant phospholipid synthesis (76), and protects the alveolar acinus against oxidant injury (46). The concomitant inhibitory effect of PTHrP on both fibroblast and type II cell growth, in combination with PTHrP augmentation of surfactant production, would have the net effect of distending and “stenting” the thinning alveolar wall, synergizing with the up-regulation of PTHrP, and physiologically stabilizing what otherwise would result in an unstable structure that would collapse by the Law of Laplace (77).

Myofibroblast Transdifferentiation as Evolution in Reverse

Lung development prepares the fetus for birth and physiologic homeostasis. Surfactant production in particular is crucial for effective gas exchange (78). Based on this functional linkage between lung development and homeostasis, we have generated data demonstrating that the underlying mechanisms of repair may recapitulate ontogeny. If lung fibroblasts are deprived of PTHrP, their structure changes (79): first, the PTHrP receptor is down-regulated, as are its down-stream targets ADRP and leptin: the decline in the lipofibroblast phenotype is mirrored by the acquisition of the myofibroblast phenotype, characteristic of fibrosis (80).

During the process of fetal lung development the mesodermal fibroblasts are characterized by Wnt (Wingless/int)/beta catenin signaling that determines the splanchnic mesodermal fibroblast (81). We have shown that during alveolarization, the generation of lung fluid up-regulates the PTHrP signaling pathway in the endoderm, causing the down-regulation of the Wnt/beta-catenin pathway (59,81), leading to the differentiation of the lipofibroblast. These cells dominate the alveolar acinus during fetal lung development, but are highly apoptotic in the post-natal lung (81,82), giving rise to the alveolar septa (81,83). Central to this paracrine determination of the mesodermal cell-types is the failure of the fibroblasts to terminally differentiate (84).

Phylogenetically, the swim bladder and frog lung interstitium are characterized by myofibroblasts; the lipofibroblast phenotype doesn't appear until the appearance of reptiles and mammals (52–54). The recapitulation of myofibroblasts during lung injury is consistent with the similarities between lung ontogeny and phylogeny, and with the molecular mechanisms of fibroblast transdifferentiation described above, and may, therefore, represent lung evolution in reverse.

A wide variety of factors can inhibit the normal paracrine induction of the lipofibroblast, and promote myofibroblast proliferation and fibrosis, including prematurity, barotrauma, oxotrauma, nicotine and infection. In all of these instances, injury of the epithelial type II cell can cause down-regulation of PTHrP (85), causing the mesodermal fibroblasts to default to the myofibroblast phenotype (50). Myofibroblasts cannot promote the growth and differentiation of the alveolar type II cell for alveolarization(43), and produce angiotensin II(86), which further damages the type II cell population (87).

The PTHrP receptor is present on the adipothelial fibroblasts (88). Stretching of the alveolus by fluid or air up-regulates both PTHrP ligand (89) and PTHrP receptor activity (36), promoting surfactant production by the type II cell, and lipofibroblast neutral lipid uptake, protecting them against oxidant injury (46). PTHrP receptor binding stimulates cyclic Adenosine Monophosphate (cAMP)-dependent Protein Kinase A expression, which determines the lipofibroblast phenotype. Treatment of the transdifferentiating myofibroblast either in vitro (90) or in vivo (91) with PPAR γ agonists blocks the transdifferentiation of the myofibroblast, preventing fibrotic injury (50,60).

The Role of PPAR γ in Ontogeny and Repair

PTHrP induces lipofibroblast differentiation via the PKA pathway, which blocks Wnt signaling by inhibiting both glioma-associated oncogene homologue (Gli) and glycogen synthase kinase-3 β (GSK 3 β), and up-regulates the lipofibroblast phenotype- PTHrP receptor, ADRP, leptin, triglyceride uptake- by stimulating PPAR γ expression (50,59). Based on the minimalist idea that development culminates in homeostasis, disruption of homeostasis may lead back to developmental motifs (79). This occurs in various lung diseases (92–95), and by focusing on the continuum from development to homeostasis we can select treatments that are more consistent with promoting cellular reintegration than stopping inflammation. For example, Bronchopulmonary Dysplasia can be induced by over-distending an otherwise healthy but immature newborn baboon lung (96). Changing the homeostatic balance of the alveolus by knocking out surfactant protein genes B, C, or D leads to alveolar remodeling that is either grossly flawed (B) or less than optimal (C, D) physiologically. Interfering with cell-cell signaling blocks lung development (97), usually resulting in parenchymal simplification. Conversely, replacing missing developmental elements can re-establish lung development (98), homeostasis (98) and structure (99).

Repair recapitulates ontogeny because it is programmed to express the cross-talk between epithelium and mesoderm through evolution (8). This model is based on three key principles: (i) the cross-talk between epithelium and mesoderm is necessary for homeostasis; (ii) damage to the epithelium impedes the cross-talk, leading to loss of homeostasis and re-adaptation through myofibroblast proliferation; and (iii) normal physiology will either be re-established, or cell/tissue remodeling/altered lung function may occur, and/or fibrosis will persist, leading to chronic lung disease. The cell-molecular injury affecting epithelial-mesenchymal cross-talk recapitulates ontogeny (in reverse), providing effective diagnostic and therapeutic targets.

Bone as another example of PTHrP signaling and adaptation

PTHrP determines bone morphology through cell-cell signaling between (100). As indicated above, PTHrP is a stretch-sensitive gene, and therefore its expression in bone would be affected by physical force (101). Phenomenologically, the effect of physical force on bone morphology has been codified as Wolff's Law since the turn of the 20th century (102). In the absence of PTHrP signaling the developing bone does not calcify (103), and in adult animals lack of PTHrP causes decalcification (104), leading to osteoporosis. However, this mechanism may also be relevant to evolutionary adaptation to gravitational force. For example, we have shown experimentally that when bone cells are placed in 0 $\times$ g PTHrP gene expression decreases (105), returning to normal levels of expression within hours when the cells are put back in unit gravity. Similarly, when the bones of rats flown in space were analyzed for PTHrP expression (105), we found that PTHrP expression was decreased in the weight-bearing bones (tibia, femur), but not in the non-weight-bearing bones (parietal), indicating the local nature of the effect on the unweighted bone. These observations are consistent with experiments in which bone decalcification is observed when rats are subjected to hindlimb unweighting (106).

Based on contemporary thought in biology and medicine, osteoporosis is considered a disease of estrogen deficiency because it is so commonly seen in post-menopausal women (107). This is another example of how an evolutionary approach would offer a broader perspective on the mechanisms of osteoporosis.

Evolutionary Biology as the Conceptual Basis for Preventive Medicine

By approaching the problem of human disease from an evolutionary perspective, we no longer have to start with Oslerian pathophysiology and work our way backwards to an understanding of health. By elucidating the mechanisms by which complex structures and their attendant functions have evolved at the cell/molecular level, we can identify key candidate genes and pathways that have evolved to generate them. For example, the identification of the PTHrP paracrine pathway as the mechanism for the evolution of the alveolus has elucidated a number of cell-molecular mechanisms mediated by ligand-receptor interactions that would not have resulted from a standard pathophysiologic model: PTHrP/PTHrP receptor; Wnt/ β catenin; PPAR γ ; LEF-1; leptin/leptin receptor. The PTHrP receptor has been shown to be functionally polymorphic in a Japanese population (108). PPAR γ and the leptin receptor are also polymorphic, and have been implicated in the relationship between obesity and decreased pulmonary lung function (109,110). LEF-1/tcf, which regulates PPAR γ , signals through platelet activator inhibitor-1(PAI-1), the most polymorphic of all the human genes (111), and it is associated with a number of chronic lung diseases (112). With such a priori knowledge of the molecular mechanisms for the determinants of physiologic structure and function we will be able to effectively prevent chronic diseases in the future, potentially eliminating 75% of the health care costs in the U.S. alone.

Conclusions

Classically, the ways in which investigators have tested and probed physiologic principles has been by studying pathology, or pathophysiology (113)- in large part because of the ethical issues involved in human experimentation. This approach has led to understanding of human disease, but only as it presents as an emergent and contingent problem. It does not lend itself to understanding the cell-molecular etiology for complex diseases. However, with access to the entire human genome (114), we can now frame such pathologic questions as a hierarchical subset of the continuum from adaptation to maladaptation, and from maladaptation to disease. By taking such a broad-based evolutionary biologic tack, in the future we can expand our horizons regarding normal and abnormal physiologic conditions, offering a much more robust, integrated way of thinking about processes of homeostasis, repair and aging. If nothing else, this approach will provide a more functionally dynamic set of genetic targets than the standard annotated gene compendiums generated through population-based genetic analyses or Systems Biology. By thinking about the genetic antecedents of physiology and pathology, we can begin developing the scientific basis for Preventive Medicine.

Acknowledgments

Grant Support: National Institutes of Health Grants HL055268 (J.S. Torday) and HL075405 (V.K. Rehan)

Abbreviations

ADRP

Adipocyte Differentiation Related Protein

C/EBP α

CCAAT/enhancer binding protein alpha

cAMP	cyclic Adenosine Monophosphate
Gli	glioma-associated oncogene homologue
GSK 3β	glycogen synthase kinase-3beta
GRN	gene regulatory network
LEF-1	lymphocyte enhancer-binding factor
PAI-1	platelet activator inhibitor-1
PTHrP	Parathyroid Hormone-related Protein
PPARγ	Peroxisome Proliferator Activated Receptor gamma
Wnt	Wingless/int

Reference List

1. Dobzhansky T. American Biology Teacher 1973;35:125.
2. Raff, R. The Shape of Life: Genes, Development and the Evolution of Animal Form. The University of Chicago Press; Chicago: 1996.
3. Bonner, RT. First Signals: The Evolution of Multicellular Development. Princeton University Press; Princeton: 2000.
4. Thompson, D. On Growth and Form. University of Cambridge Press; Cambridge: 1917.
5. Ohno, S. Evolution by Gene Duplication. Springer-Verlag; New York: 1970.
6. Kipling, R. Just So Stories. Doubleday and Company; Garden City: 1902.
7. Torday JS, Rehan VK. FASEB J 2007;21:2640. [PubMed: 17766328]
8. Torday JS, Rehan VK. Am J Physiol Lung Cell Mol Physiol 2007;292:L608. [PubMed: 17085519]
9. Brenner S. Biosci Rep 2003;23:225. [PubMed: 15074543]
10. Fisher, RA. The Genetical Theory of Natural Selection. Clarendon Press; Oxford: 1930.
11. Darwin, CR. The Origin of Species. J Murray; London: 1859.
12. Macklem PT. Am J Respir Crit Care Med 2004;169:438. [PubMed: 14766660]
13. West GB, Brown JH, Enquist BJ. Science 1997;276:122. [PubMed: 9082983]
14. Smith E, Morowitz HJ. Proc Natl Acad Sci U S A 2004;101:13168. [PubMed: 15340153]
15. Behe MJ, Snoke DW. Protein Sci 2004;13:2651. [PubMed: 15340163]
16. Wagner GP. Nat Rev Genet 2007;8:473. [PubMed: 17486120]
17. Hood L. Mech Ageing Dev 2003;124:9. [PubMed: 12618001]
18. Gould SJ, Vrba ES. Paleobiology 1982;8:4.
19. Allaby, M. The Concise Oxford Dictionary of Zoology. The Oxford University Press; Oxford: 2008.
20. Daniels CB, et al. Physiol Biochem Zool 2004;77:732. [PubMed: 15547792]
21. Clements JA, Nellenbogen J, Trahan HJ. Science 1970;169:603. [PubMed: 5426782]
22. Gould SJ. Science 1983;219:439. [PubMed: 17742803]
23. McGowan SE, Torday JS. Annu Rev Physiol 1997;59:43. [PubMed: 9074756]

24. Maronde E, Stehle JH. Trends Endocrinol Metab 2007;18:142. [PubMed: 17374488]
25. Gericke GS. Med Hypotheses 2006;66:92. [PubMed: 16183208]
26. Wickramasinghe SN. Comp Biochem Physiol Comp Physiol 1993;104:63. [PubMed: 8094661]
27. Arendt D. Theory Biosci 2005;124:185. [PubMed: 17046355]
28. Torday JS, Rehan VK. Am J Respir Cell Mol Biol 2004;31:8. [PubMed: 15208097]
29. Mayr E. Science 1961;134:1501. [PubMed: 14471768]
30. Kuhn, T. The structure of scientific revolutions. University of Chicago Press; Chicago: 1962.
31. Jacob F. Science 1977;196:1161. [PubMed: 860134]
32. Moczek AP. Bioessays 2008;30:432. [PubMed: 18404691]
33. King N, Hittinger CT, Carroll SB. Science 2003;301:361. [PubMed: 12869759]
34. Yamamoto M, Harm SC, Grasser WA, Thiede MA. Proc Natl Acad Sci U S A 1992;89:5326. [PubMed: 1376916]
35. Daifotis AG, Weir EC, Dreyer BE, Broadus AE. J Biol Chem 1992;267:23455. [PubMed: 1385422]
36. Torday JS, Rehan VK. Am J Physiol Lung Cell Mol Physiol 2002;283:L130. [PubMed: 12060569]
37. Crespi EJ, Denver RJ. Proc Natl Acad Sci U S A 2006;103:10092. [PubMed: 16782821]
38. Romer AS. Science 1967;158:1629. [PubMed: 4862529]
39. Lanske B, Kronenberg HM. Crit Rev Eukaryot Gene Expr 1998;8:297. [PubMed: 9807698]
40. Ramirez MI, Chung UI, Williams MC. Am J Respir Cell Mol Biol 2000;22:367. [PubMed: 10696074]
41. Rubin LP, et al. Dev Dyn 2004;230:278. [PubMed: 15162506]
42. Torday JS. Adv Space Res 2003;32:1569. [PubMed: 15000128]
43. Torday JS, Torres E, Rehan VK. Pediatr Pathol Mol Med 2003;22:189. [PubMed: 12746170]
44. Weibel, ER.; Taylor, C.; Bolis, L. Principles of Animal Design. Cambridge University Press; Cambridge: 1998.
45. Sanchez-Esteban J, et al. J Appl Physiol 2001;91:589. [PubMed: 11457769]
46. Torday JS, Torday DP, Gutnick J, Qin J, Rehan V. Pediatr Res 2001;49:843. [PubMed: 11385147]
47. Torday JS, et al. Am J Physiol Lung Cell Mol Physiol 2002;282:L405. [PubMed: 11839533]
48. Boros LG, Torday JS, Paul Lee WN, Rehan VK. Mol Genet Metab 2002;77:230. [PubMed: 12409271]
49. Chen HL, et al. J Biol Chem 2002;277:19374. [PubMed: 11897779]
50. Torday JS, Rehan VK. Pediatr Res 2007;62:2. [PubMed: 17515838]
51. Gao Y, Raj JU. Am J Physiol Lung Cell Mol Physiol 2005;289:L60. [PubMed: 15749740]
52. Maina JN. Anat Rec 2000;261:25. [PubMed: 10700733]
53. Maina JN. Adv Anat Embryol Cell Biol 2002;163III
54. Maina JN. J Anat 2002;201:281. [PubMed: 12430953]
55. Daniels CB, Orgeig S. News Physiol Sci 2003;18:151. [PubMed: 12869615]
56. Massaro D, Massaro GD. Am J Respir Cell Mol Biol 2003;28:271. [PubMed: 12594052]
57. Weibel ER, Taylor CR, Hoppeler H. Respir Physiol 1992;87:325. [PubMed: 1604056]
58. Rubin LP, Kifor O, Hua J, Brown EM, Torday JS. Biochim Biophys Acta 1994;1223:91. [PubMed: 8061059]
59. Torday JS, Rehan VK. Pediatr Res 2006;60:382. [PubMed: 16940239]
60. Rehan VK, Wang Y, Patel S, Santos J, Torday JS. Pediatr Pulmonol 2006;41:558. [PubMed: 16617452]
61. Rehan V, Torday J. Cell Biochem Biophys 2003;38:239. [PubMed: 12794266]
62. Maioli E, Fortino V, Torricelli C, Arezzini B, Gardi C. Exp Dermatol 2002;11:302. [PubMed: 12190938]
63. Ortega A, et al. J Am Soc Nephrol 2006;17:1594. [PubMed: 16672315]
64. Adamson IY, Hedgecock C, Bowden DH. Am J Pathol 1990;137:385. [PubMed: 1696785]
65. Wolf G, Ziyadeh FN. Contrib Nephrol 2006;151:175. [PubMed: 16929141]
66. Warburton D, et al. Mech Dev 2000;92:55. [PubMed: 10704888]
67. Papasani MR, et al. Endocrinology 2004;145:5294. [PubMed: 15297442]

68. Jiang B, et al. J Cardiovasc Pharmacol 1998;31(Suppl 1):S142. [PubMed: 9595423]
69. Meyrick, B.; Reid, L. Development of the Lung. Marcel Dekker; New York: 2008. p. 135-214.
70. Liem KF. American Zoologist 1988;28:739-759.
71. Eisenmann DM. WormBook 2005;1. [PubMed: 18050402]
72. Kaesler S, Luscher B, Ruther U. Biol Chem 2000;381:545. [PubMed: 10987360]
73. Hino S, Tanji C, Nakayama KI, Kikuchi A. Mol Cell Biol 2005;25:9063. [PubMed: 16199882]
74. Vaccaro C, Brody JS. Anat Rec 1978;192:467. [PubMed: 736269]
75. Rehan VK, et al. Exp Lung Res 2006;32:379. [PubMed: 17090478]
76. Schultz CJ, Torres E, Londo C, Torday JS. Am J Physiol Lung Cell Mol Physiol 2002;283:L288. [PubMed: 12114189]
77. Miskovitz P. Arch Phys Med Rehabil 2003;84:303. [PubMed: 12601667]
78. Bourbon, J. Pulmonary Surfactant: Biochemical, Functional, Regulatory, and Clinical Concepts. CRC Press; London: 1991.
79. Demayo F, et al. Am J Physiol Lung Cell Mol Physiol 2002;283:L510. [PubMed: 12169568]
80. Hinz B, et al. Am J Pathol 2007;170:1807. [PubMed: 17525249]
81. Shannon JM, Hyatt BA. Annu Rev Physiol 2004;66:625. [PubMed: 14977416]
82. Bruce MC, Honaker CE, Cross RJ. Am J Respir Cell Mol Biol 1999;20:228. [PubMed: 9922213]
83. Bostrom H, et al. Cell 1996;85:863. [PubMed: 8681381]
84. Hu E, Tontonoz P, Spiegelman BM. Proc Natl Acad Sci U S A 1995;92:9856. [PubMed: 7568232]
85. Mason RJ. Respirology 2006;11(Suppl):S12. [PubMed: 16423262]
86. Wang R, et al. Am J Physiol 1999;276:L885. [PubMed: 10330045]
87. Wang R, et al. Am J Physiol 1999;277:L1158. [PubMed: 10600886]
88. Urena P, et al. Endocrinology 1993;133:617. [PubMed: 8393771]
89. Torday JS, Sanchez-Esteban J, Rubin LP. Am J Med Sci 1998;316:205. [PubMed: 9749564]
90. Rehan VK, et al. Am J Physiol Lung Cell Mol Physiol 2005;289:L667. [PubMed: 15951329]
91. Rehan VK, Wang Y, Patel S, Santos J, Torday JS. Pediatr Pulmonol 2006;41:558. [PubMed: 16617452]
92. Chilosi M, et al. Respir Res 2006;7:95. [PubMed: 16813649]
93. deLemos RA, Coalson JJ. Clin Perinatol 1992;19:521. [PubMed: 1526070]
94. Holgate ST, et al. Proc Am Thorac Soc 2004;1:93. [PubMed: 16113419]
95. Voelkel NF, Vandivier RW, Tudor RM. Am J Physiol Lung Cell Mol Physiol 2006;290:L209. [PubMed: 16403941]
96. Coalson JJ. Semin Perinatol 2006;30:179. [PubMed: 16860157]
97. Ingram JL, Bonner JC. Curr Mol Med 2006;6:409. [PubMed: 16900664]
98. Kunig AM, et al. Am J Physiol Lung Cell Mol Physiol 2006;291:L1068. [PubMed: 16829629]
99. Bland RD. Biol Neonate 2005;88:181. [PubMed: 16210840]
100. Shimizu H, Yokoyama S, Asahara H. Dev Growth Differ 2007;49:449. [PubMed: 17661739]
101. Broadus AE, Macica C, Chen X. Ann N Y Acad Sci 2007;1116:65. [PubMed: 18083922]
102. Frost HM. Angle Orthod 2004;74:3. [PubMed: 15038485]
103. Lanske B, et al. J Clin Invest 1999;104:399. [PubMed: 10449432]
104. Miao D, et al. J Clin Invest 2005;115:2402. [PubMed: 16138191]
105. Torday JS. Advances in Space Research 2008;32:1569. [PubMed: 15000128]
106. Patterson-Buckendahl P, Globus RK, Bikle DD, Cann CE, Morey-Holton E. Am J Physiol 1989;257:R1103. [PubMed: 2589536]
107. Gambacciani M, Vacca F. Minerva Med 2004;95:507. [PubMed: 15785435]
108. Heishi M, et al. Biol Pharm Bull 2000;23:386. [PubMed: 10784412]
109. Tankersley CG, et al. J Appl Physiol 1998;85:2261. [PubMed: 9843551]
110. Weiss ST, Shore S. Am J Respir Crit Care Med 2004;169:963. [PubMed: 14742299]
111. Oh CK. Chem Immunol Allergy 2005;87:85. [PubMed: 16107765]

112. Hizawa N, et al. Clin Exp Allergy 2006;36:872. [PubMed: 16839401]
113. Bryan CS. Ann Intern Med 1994;120:682. [PubMed: 8135454]
114. Collins FS. Genome Res 2001;11:641. [PubMed: 11337461]

Figure 1. Solving for Evolutionary Principles Independent of Chronologic Time

By regressing Gene Regulatory Networks (GRNs) mediating cell-cell interactions relevant to structure/function across ontogeny and phylogeny against chronologic time and phenotype, we can generate a family of parallel lines, or simultaneous equations. Using this approach, we can 'solve' for the underlying evolutionary principles involved independently of chronologic time, making the biologic processes self-referential.

Figure 2. Evolutionary-Developmental Origins of the Lung

At the top (A) is the cellular mechanism of alveolar development. In the middle (B) is an epigenetic paracrine cellular mechanism for alveolar evolution, showing how oxygen and stretch may have 'driven' a series of cellular interactions for lung evolution from the advent of the lipofibroblast to leptin production, causing increased surfactant, which allowed for the decreased alveolar diameter, increasing the surface-to-volume ratio and increased gas exchange. At the bottom (C) is a descriptive genetic pathway for the same process shown.