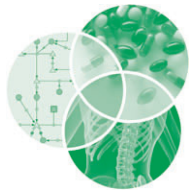
Advanced Review



The cell as the mechanistic basis for evolution

J.S. Torday*

The First Principles for Physiology originated in and emanate from the unicellular state of life. Viewing physiology as a continuum from unicellular to multicellular organisms provides fundamental insight to ontogeny and phylogeny as a functionally integral whole. Such mechanisms are most evident under conditions of physiologic stress; all of the molecular pathways that evolved in service to the vertebrate water–land transition aided and abetted the evolution of the vertebrate lung, for example. Reduction of evolution to cell biology has an important scientific feature—it is predictive. One implication of this perspective on evolution is the likelihood that it is the unicellular state that is actually the object of selection. By looking at the process of evolution from its unicellular origins, the causal relationships between genotype and phenotype are revealed, as are many other aspects of physiology and medicine that have remained anecdotal and counter-intuitive. Evolutionary development can best be considered as a cyclical, epigenetic, reiterative environmental assessment process, originating from the unicellular state, both forward and backward, to sustain and perpetuate unicellular homeostasis.

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IN THE BEGINNING

The traditional descriptive perspective for Physiology, as portrayed by Galen and Harvey, is like a set of Lego Blocks™, one biochemical process linked to another, until an entire assembly emerges. In contrast to that *ex post facto* narrative, a predictive mechanistic approach can be asserted, going as far as asserting that there are founding First Principles for Physiology that originated in and emanate from the unicellular stage of life.[1] Einstein's insight to Relativity Theory emerged from a dream in which he traveled in tandem with a light beam, seeing it as an integral particle and wave.[2] Similarly, viewing physiology as a continuum from unicellular to multicellular organisms provides fundamental insights to ontogeny and phylogeny as a functionally integral whole,[3] directly linking the external physical environment to the internal environment of physiology. And even extending beyond that, to the metaphysical realm by bearing in mind that the calcium waves that mediate consciousness in paramecia[4] form a continuous arc to the axons of our brains[5] as one and the same fundamental mechanism.

Life probably began much like the sea foam that can be found on any shoreline, since such lipids naturally form primitive soap bubble-like 'cells' when vigorously agitated in water. Such primitive cells provided a protected space for catalytic reactions that decreased and stabilized the internal energy state within the cell, from which life could emerge.[6] Crucially, that cellular compartment permits circumvention of the Second Law of Thermodynamics. That elusion of physical law is the essential property of life as self-referential, self-organizing, and self-perpetuating, always in flux, staying apace with, and yet continually separable from a stressful, ever-changing external environment.[7] That is the bargain we life forms have struck with Nature.

Even from the inception of life, rising calcium levels in the oceans[8] have driven a perpetual balancing selection for calcium homeostasis,[9] epistatically counter-balanced by lipid metabolism. Metaphorically, the Greeks called it Ouroboros (Figure 1), an ancient symbol depicting a serpent eating its own tail.

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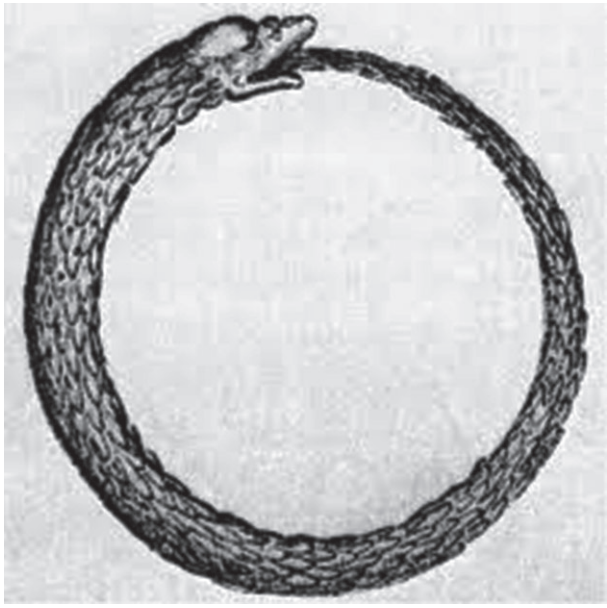

FIGURE 1 | Ouoroboros, an ancient symbol depicting a serpent eating its own tail.

The Ouroboros embodies self-reflexivity or cyclicity, especially in the sense of something constantly recreating itself. Just like the mythological Phoenix, it operates in cycles that begin anew as soon as they end. Critically, the basic cell permits the internalization of factors in the environment that would otherwise have destroyed it—oxygen, minerals, heavy metals, micro-gravitational effects, and even bacteria—all facilitated by an internal endomembrane system that compartmentalized those factors within the cell, making them useful.[10] These membrane interfaces are the biologic imperative that separates life from non-life-'Good walls make good neighbors.'[11]

THE ADVENT OF MULTICELLULARITY

Unicellular organisms have dominated the Earth for most of its existence. Far from static, these organisms were constantly adapting.[12] From them, the simplest cyanobacteria evolved first, producing oxygen and carbon dioxide that modified the nitrogen-filled atmosphere.[13] The rising levels of atmospheric carbon dioxide, largely generated by volcanoes and metamorphic degassing,[14] acidified the oceans by forming carbonic acid, progressively leaching more and more calcium from rock into the ocean waters. A period of low atmospheric oxygen eventually forced migration of life from sea to land.[15,16]

The existence of a protected compartment within such primitive 'cells' allowed for the formation of the endomembrane system, giving rise to chemiosmosis, or the generation of bioenergy through the partitioning of ions within the cell, like a battery.[17] Early in this progression, the otherwise toxic ambient calcium concentrations within primitive cells had to be lowered by forming calcium channels, composed of lipids embedded within the cell membrane,[18] and the complementary formation of the Endoplasmic Reticulum, an internal membrane system for the compartmentalization of intracellular calcium[19] (Figure 2). Ultimately, the advent of cholesterol synthesis facilitated its incorporation into the cell membrane of eukaryotes, differentiating them (our ancestors) from prokaryotes (bacteria), which are devoid of cholesterol. This process was contingent on an enriched oxygen atmosphere, since it takes eleven oxygen molecules to synthesize one cholesterol molecule.[20] The cholesterol-containing cell membrane thins out, critically increasing oxygen transport, enhancing motility through increased cytoplasmic streaming, and was also conducive to endocytosis, or cell eating.[3] All three of these processes are the cardinal characteristics of vertebrate evolution.[21] At some point in this progression of cellular complexity, impelled by oxygen promoting metabolic demand, the evolving physiologic load on the system resulted in Endoplasmic Reticulum Stress, periodically causing the release of toxic levels of calcium into the cytoplasm of the cell. The counterbalancing, or epistatic mechanism was marked by the advent of the Peroxisome,[22] an organelle that utilizes lipids to buffer such excess calcium. That mechanism ultimately became homeostatically fixed, further promoting the movement of ions into and out of the cell. Importantly, the internalization of the external environment by this mechanism reciprocally conveyed functional biologic information about the external surroundings, and promoted intracellular communication—what Claude Bernard referred to as the *Internal Milieu*.[23] Walter B. Cannon later formulated the concept that biological systems are designed to 'trigger physiological responses to maintain the constancy of the internal environment in face of disturbances of external surroundings,' which he termed homeostasis.[24] He emphasized the need for reassembling the data being amassed for the components of biological systems into the context of whole organism function. Hence, in 1991, Weibel, Taylor, and Bolis tested their theory of 'Symmorphosis,' the idea that physiology has evolved to optimize the economy of biologic function[25] interestingly, the one exception to this otherwise ubiquitous theory was the lung, which they discovered was 'over-engineered,' but more about that later.

Harold Morowitz is a proponent of the concept that the energy that flows through a system also

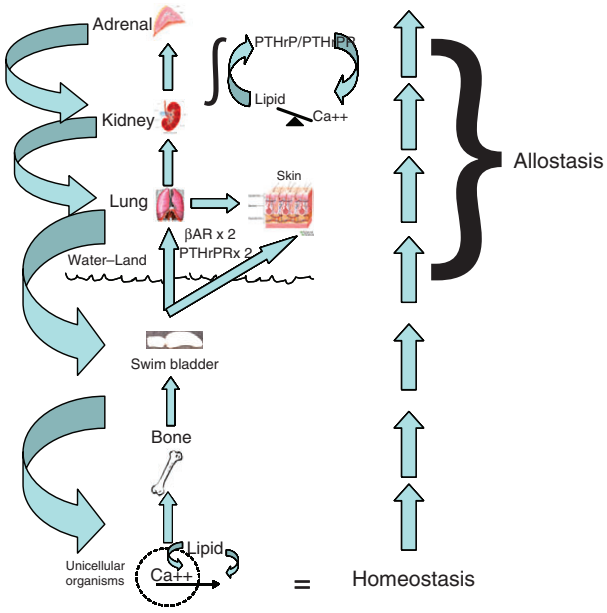

FIGURE 2 | Lipid-calcium homeostasis to complex physiology. The ontogenetic and phylogenetic integration ($\int$) of calcium–lipid homeostasis, from unicellular organism incorporation of lipid into the plasmalemma to multicellular organism calcium/lipid epistatic homeostasis fostered the Evolution of metazoans. This figure, focuses on the specific stress of the water–land transition on the Evolution of a wide variety of organs- bone, lung, skin, kidney, adrenal- resulting from the duplication of the PTHrP Receptor gene in fish, followed by the βAdrenergic Receptor (βAR) gene, culminating in integrated physiology, or allostasis (on far right). Internal selection was mediated through selection pressure on homeostatic mechanisms mediated by paracrine cell–cell interactions; as vertebrates adapted to land, the PTHrP signaling mechanism iteratively allowed for physiologic adaptations to air breathing (skin, lung), prevention of dessication (skin, kidney) and 'fight or flight' (adrenal). The blue arrows on the far left signify how evolved traits refer back to their antecedents, or are *exapted*.

helps organize that system.[26] Others [27] have likewise derived a general model for allometry (the study of the relationship of body size to shape, anatomy, physiology, and behavior). They proposed a mathematical model demonstrating that metabolism complies with the 3/4 power law for metabolic rates (i.e., the rate of energy use in mammals increases with mass with a 3/4 exponent). Back in 1945, Horowitz[28] hypothesized that all of biochemistry could be reduced to hierarchical networks, or 'shells.' Based on these decades of study, investigators acknowledge that there are fundamental rules of physiology, but they do not address either how or why these rules have evolved.

As eukaryotes thrived, they experienced increasing pressure for metabolic efficiency in competition with their prokaryotic cousins. They are hypothesized to have ingested bacteria via endocytosis, which were assimilated as mitochondria,[29] providing more bioenergy to the cell for homeostasis. Eventually, eukaryotic metabolic cooperativity between cells gave rise to multicellular organisms, which were effectively able to compete with prokaryotes. As Simon Conway Morris has archly noted, 'Once there were bacteria, now there is New York.'[30] Bacteria can function as pseudo-multicellular organisms through such behavioral traits as quorum sensing and through biofilm formation. The subsequent counterbalancing selection by cellular growth factors and their signal-mediating receptors in our ancestors facilitated cell–cell signaling, forming the basis for eukaryotic metazoan evolution. It is this same process that is recapitulated each time the organism undergoes embryogenesis.[3]

This cellular focus on the process of evolution serves a number of purposes. First, it regards the mechanism of evolution from its unicellular origins as the epitome of the integrated genotype and phenotype. This provides a means of thinking about how and why multicellular organisms evolved, starting with the unicellular cell membrane as the common source for all evolved complex traits .[31] Further, it offers a discrete direction for experimentally determining the constituents of evolution based on the ontogeny and phylogeny of cellular processes.[1,3] For example, it is commonplace for evolutionists to emphasize the fact that any given evolved trait had its antecedents in an earlier phylogenetic species as a pre-adapted, or exapted trait .[32] These ancestral traits can then subsequently be cobbled together to form a novel structure and/or function.[33] Inescapably, if followed to its logical conclusion, all metazoan traits must have evolved from their unicellular origins.

EVOLUTION, CELLULAR-STYLE

Moving forward in biologic space and time, how might such complex biologic traits have come about? Physiologic stress must have been the primary force behind such a generative process, transduced by changes in the homeostatic control mechanisms of cellular communication.[1] Mechanistically, when physiologic stress occurs in any complex organism, it increases blood pressure, causing vascular wall shear stress, particularly in the microvascular beds of visceral organs. Such shear stress generates Reactive Oxygen Species (ROS), specifically at sites of greatest vascular wall friction. ROS are known to damage DNA, RNA, and protein, and to particularly do so at those sites most affected by the prevailing external stress. This can result in context-specific gene mutations, and even gene duplications, all of which can profoundly affect the processes of evolution [1] by favoring selection for such adaptations. So it should be borne

in mind that such genetic changes are occurring within the integrated structural–functional context of specific tissues and organs. However, understanding the biochemical processes facilitating the genetics equips a profound and testable mechanism for understanding the entire aggregate of genetic changes as both modifications of prior genetic lineages, and yet 'fit enough to survive' in their new form.

Over evolutionary time, such varying modifications of structure and function would iteratively have altered various internal organs.[1] These divergences would either successfully conform to the conditions at hand, or failing that, would cause yet another round of damage-repair. So either an existential solution was found, or the organism became extinct; either way, such physiologic changes would have translated into both Phylogenetic and Ontogenetic evolution.[3] Such an evolutionary process need not be unidirectional. In the forward direction, developmental mechanisms recapitulate phylogenetic structures and functions, culminating in homeostatically controlled processes. And in the reverse direction, the best illustration lies with the genetic changes that occur under conditions of chronic disease, usually characterized by simplification of structure and function. For example, scarring mechanisms are typified by fibroblastic reversion to their primordial signaling pathway.[34] This sustains the integrity of the tissue or organ through the formation of scar tissue, albeit suboptimally, yet allowing the organism to reproduce before being overwhelmed by the ongoing injury-repair.

THE WATER–LAND TRANSITION AND VERTEBRATE EVOLUTION

Nowhere are such mechanisms of molecular evolution more evident than during the water–land transition (Figure 2). Net rises in oxygen and carbon dioxide in the Phanerozoic atmosphere over the course of the last 500 million years partially dried up the oceans, lakes, and rivers,[15,16] forcing organisms to adapt to land by remodeling tissues and organs, or else become extinct. There were two known gene duplications that occurred during this period of terrestrial adaptation—the Parathyroid Hormone-related Protein (PTHrP) Receptor,[35] and the β Adrenergic Receptor (βAR).[36] The cause of these gene duplications can be reconstructed based on their effects on vertebrate physiology. PTHrP is necessary for a variety of traits relevant to land adaptation—ossification of bone, skin barrier development, and the formation of alveoli in the lung.[37] Bone had to ossify to maintain the integrity of skeletal elements under the stress of higher gravitational forces on land compared to relative buoyancy in water. PTHrP signaling is necessary for calcium incorporation into bone. It is known from the fossil record that there were at least five attempts to breach land by fish ancestors based on fossilized skeletal remains. Those events must have been accompanied by the evolution of visceral organs, based on both *a priori* reasoning, and the fact that the genes involved in skeletal development are also integral to the morphogenesis of critical internal organs, particularly PTHrP.[37] In the aggregate, the net effect of shear stress on PTHrP-expressing organs like bone, lung, skin, and kidney would have precipitated the duplication of the PTHrP Receptor, facilitating the evolution of those progeny best suited for adaptation to land.[3] These were the founders of the subsequent terrestrial species.

As a result of such positive selection pressure for PTHrP signaling, its genetic expression ultimately evolved in both the pituitary[38] and adrenal cortex of land vertebrates,[39] further stimulating adrenocorticotrophic hormone and corticoids, respectively, in response to the stresses of land adaptation. This pituitary–adrenal cascade would have amplified the production of adrenaline, since corticoids produced in the adrenal cortex pass through the microvascular arcades of the adrenal medulla on their passage to the systemic bloodstream. This flow of corticoids through the medullary labyrinth enzymatically stimulates the rate-limiting step in adrenaline synthesis, catechol-O-methyltransferase, or COMT. Positive selection pressure for this functional trait may have resulted from cyclic periods of hypoxic stress, as follows. Episodes of intermittently large increases and decreases in atmospheric oxygen over geologic time, known as the Berner Hypothesis,[40] may have triggered lapses in the capacity of the lung to oxygenate efficiently, forcing alternating antagonistic adaptations to hyperoxia and hypoxia as a result. The periodic increases in oxygen sufficiency gave rise to increased body size, whereas the subsequent bouts of hypoxia are the most potent vertebrate physiologic stressors known. Such intermittent periods of pulmonary insufficiency would have been alleviated by the increased adrenaline production, stimulating lung alveolar surfactant secretion,[41] transiently increasing gas exchange by facilitating the distension of the existing alveoli. The increased distention of the alveoli, in turn, would have fostered the generation of more alveoli by stimulating stretch-regulated PTHrP secretion,[42] which is both mitogenic for alveolarization,[43] and angiogenic for the alveolar capillary bed,[44] aided and abetted by its potent vasodilatory activity.[45] In the aggregate, this process would have allowed for the iterative evolution

of the alveolar bed through positive selection pressure for those members of the species most capable of increasing their PTHrP secretion, though the only 'fossil evidence' resides in the ontogeny and phylogeny of land vertebrates.[46]

And it is worthwhile highlighting the fact that the increased amounts of PTHrP flowing from the adrenal cortex may have caused the evolution of the capillary arcade system of the adrenal medulla.[47] Such pleiotropic effects typify the positive selection that has occurred during the evolutionary process, yet they are never seen as both evolutionarily and physiologically cohesive by the top-down descriptive perspective.

This scenario is also consistent with the duplication of the βARs.[36] The increase in their density within the alveolar capillary bed was necessary for relieving a major constraint during the evolution of the lung in adaptation to land—the βARs were a ubiquitous mechanism for blood pressure control in both the lung alveoli and the systemic blood pressure. The pulmonary system had limited capacity to withstand the swings in blood pressure to which other visceral organs were subjected, having evolved for maximum surface area-to-blood volume ratio.[25] PTHrP produced by the alveolar epithelium is a potent vasodilator,[45] so it served to compensate for this constraint on elevated blood pressure in the interim. But eventually the βARs had to evolve to coordinately accommodate both the systemic and local pulmonary blood pressure control within the alveolar space.

The glucocorticoid (GC) receptor evolved from the mineralocorticoid (MC) receptor during this same period through a third gene duplication.[48] Since blood pressure would have tended to increase during the vertebrate adaptation to land in response to gravitational demands, there would have been positive selection pressure to reduce the vascular stress caused by the blood pressure stimulation by the MC aldosterone during this phase of land vertebrate evolution. The evolution of the GC receptor would have placed positive selection on GC regulation by reducing the hypertensive effect of the MCs by diverting steroidogenesis toward cortisol production. In turn, the positive selection for cortisol production would have stimulated βAR expression, potentially explaining how and why the βARs superseded the blood pressure reducing function of PTHrP. It is these *ad hoc* existential interactions that promoted land adaptation through independent local blood pressure regulation within the alveolus. This integration of blood pressure control in the lung and periphery by catecholamines represents allostatic evolution.[49]

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

Moreover, increased episodes of adrenaline production in response to stress may have fostered the evolution of the central nervous system. Peripheral adrenaline mediates and limits blood flow through the blood–brain barrier, which would have caused increased adrenaline and noradrenaline production within the evolving brain. Both adrenaline and noradrenaline promote neuronal development. It might even be speculated that this cascade led to human creativity and problem solving as an evolved expression of that same axis as an alternative to 'fight or flight,' since it is well-known that learning requires stress.[50]

The duplication of the βAR gene may also have been instigated by the same intermittent cyclical hypoxia resulting in the process of lung adaptation, subsequently facilitating independent blood pressure regulation within the alveolar microvasculature; both of these mechanisms would have been synergized by the evolution of the GCs during this transition.

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

THE CELLULAR APPROACH TO EVOLUTION IS PREDICTIVE

This reduction of the process of evolution to cell biology has an important scientific feature—it is predictive, in contrast to conventional physiology, which is post-dictive. For example, it may answer the untenable question as to why organisms return to their unicellular origins during their life cycles. Perhaps, as Samuel Butler surmised, 'a hen is just an egg's way of making another egg'[51] after all. It is worth considering the

hypothesis that since all complex organisms originated from the unicellular state, a return to the unicellular state is necessary in order to ensure the fidelity of any given mutation with all of the subsequently evolved homeostatic mechanisms, from its origins during phylogeny, through all the elaborating mutational permutations and combinations of that trait during the process of evolution.[1] One way of thinking about this concept is to consider that perhaps Haeckel's Biogenetic Law is correct after all—that ontogeny actually does recapitulate phylogeny. His theory has been dismissed for lack of evidence for intermediary steps in phylogeny occurring during embryonic development, like gill slits and tails. However, that transpired during an era when the cellular–molecular mechanisms of development were unknown. A testament to the existence of such molecular lapses is the term 'ghost lineage,' which fills such gaps in the fossil record euphemistically. We now know that there are such cellular–molecular physiologic changes over evolutionary time that are expressed in bone,[52] and are equally as important, if not more so in other organ systems.[53] In all likelihood, ontogeny must recapitulate phylogeny in order to vouchsafe the integrity of all of the homeostatic mechanisms that each and every gene supports in facilitating evolutionary development. Without such a 'fail-safe' mechanism for the foundational principles of life, there would inevitably be drift away from such First Principles, putting the core process of evolution in response to environmental change itself at risk of extinction. The only organism that comes to mind that may have been founded on another set of principles are viruses. S.J. Gould famously wondered whether an evolutionary 'tape' replayed would recapitulate?[54] In this construct, the answer would resoundingly be 'no,' since the fluctuations in carbon dioxide and oxygen do not occur now as they did when the atmosphere was in flux.

One implication of this perspective on evolution, starting from the unicellular state phylogenetically, being recapitulated ontogenetically, is the likelihood that it is the unicellular state that is actually the primary level of selection. The multicellular state, that which Gould and Lewontin called 'Spandrels,'[55] is merely a biologic 'agent' for monitoring the environment between unicellular stages in order to register and facilitate adaptive changes. This consideration can be based on both *a priori* and empiric data. Regarding the former, emerging evidence for epigenetic inheritance demonstrates that the environment can cause heritable changes in the genome, but they would only take effect phenotypically in successive generations.[56] This would suggest that it is actually the germ cells of the offspring that are being selected for.[56] The starvation model of metabolic syndrome may illustrate this experimentally. Maternal diet can cause obesity, hypertension, and diabetes in the offspring. But they also mature sexually at an earlier stage due to the excess amount of body fat. Though seemingly incongruous, this may represent the primary strategy to accelerate the genetic transfer of information to the next generation (positive selection), effectively overarching the expected paucity of food. The concomitant obesity, hypertension, and type II diabetes are unfortunate side-effects of this otherwise adaptive process in the adults. Under these circumstances, it can be surmised that it is the germ cells that are being selected for; in other words, the adults are disposable, as Kirkwood has opined.[57]

Hologenomic evolution theory[58] provides yet another mechanism for selection emerging from the unicellular state. According to that theory, all complex organisms actually represent a vast collaborative of linked, co-dependent, cooperative and competitive localized environments and ecologies functioning as a unitary organism toward the external environment. These co-linked ecologies are comprised of both the innate cells of that organism, and all of the microbial life that is cohabitant with it. The singular function of these ecologies is to maintain the homeostatic preferences of their constituent cells. In this theory, evolutionary development is the further expression of cooperation, competition, and connections between the cellular constituents in each of those linked ecologies in successive iterations as they successfully sustain themselves against a hostile external genetic environment. Ontogeny would then recapitulate phylogeny, since the integrity of the linked environments that constitute a fully developed organism can only be maintained by reiterating those environmental ecologies in succession toward their full expression in the organism as a whole.

Another way to think about the notion of the unicellular state as the one being selected for is to focus on calcium signaling as the initiating event for all of biology. There is experimental evidence[59] that the increases in carbon dioxide that occurred during the Phanerozoic Era caused acidification of the oceans, causing leaching of calcium from the ocean floor. The rise in calcium levels can causally be linked to the evolution of the biota, and is intimately involved with nearly all biologic processes. For example, fertilization of the ovum by a sperm causes a wave of calcium, which triggers embryogenesis. These same sorts of processes continue throughout the life cycle, until the organism ultimately dies. There

seems to be a disproportionate investment in the zygote from a purely biologic perspective. However, given the prevalence of calcium signaling at every stage, on the one hand, and the participation of the gonadocytes in epigenetic inheritance on the other, the reality of the vectorial trajectory of the life cycle becomes apparent—it cannot be static, it must move either toward or away from change.

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

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

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

In retrospect, it should have come as no surprise that we have misapprehended our own physiology. Many discoveries in biomedicine are serendipitous, medicine is post-dictive, and the Human Genome Project has not yet yielded any of its predicted breakthroughs. However, moving forward, knowing what we now do, we should countenance our own existence as part of the wider environment … that we are not merely in this world, but literally of this world[1] … with an intimacy that we had never previously imagined.

This unicellular-centric vantage point is heretical, but like the shift from Geocentrism to Heliocentrism, our species would be vastly improved by recognizing this persistent, systematic error in self-perception. We are not the pinnacle of biologic existence, and we would be better stewards of the land and our planet if we realized it. We have learned that we must share resources with all of our biological relatives. Perhaps through a fundamental, scientifically testable, and demonstrable understanding of what we are and how we came to be so, more of us will behave more consistently with Nature's needs instead of subordinating them to our own narcissistic whims. As we become deeply aware of our true place in the biologic realm, such as we are already witnessing through our increasing recognition of an immense microbial array as fellow travelers as our microbiome, we may find a more ecumenical approach to life than we have been practicing for the last 5000 years.

BIOETHICS BASED ON EVOLUTIONARY ONTOLOGY AND EPISTEMOLOGY, NOT DESCRIPTIVE PHENOTYPES, AND GENES

By definition, a fundamental change in the way we perceive ourselves as a species would demand a commensurate change in our ethical behavior. Such thoughts are reminiscent of a comment in a recent biography of the British philosopher Derek Parfit in The New Yorker magazine, entitled 'How to be Good,' in which he puzzles over the inherent paradox between empathy and Darwinian Survival of the Fittest. These two concepts would seem to be irreconcilable, yet that is only because the latter is based on a false premise. Darwin's great success was in making the subject of evolution user friendly by providing a narrative that was simple and direct. Pleasing as it may be, it is at best, entirely incomplete. Think of it like the transition from Newtonian Mechanics to Einsteinian Relativity Theory. As much is learned about the unicellular world with its surprising mechanisms and capacities, new pathways must be imagined. It is clear that we

as humans are hologenomes, and all the other complex creatures are too. In fact, there are no exceptions. The reasons for this can only be understood properly through a journey from the 'Big Bang' of the cell forward, with all its faculties and strictures. By concentrating on cellular dynamics, an entirely coherent path is empowered. Tennyson's line about 'Nature, red in tooth and claw' is only the tip of what the iceberg of evolution really constitutes. As pointed out above, we evolved from unicellular organisms through cooperation, co-dependence, collaboration, and competition. These are all archetypical cellular capacities. Would we not then ourselves, as an example of cellular reiteration, have just those self-same and self-similar behaviors?

THE THEORY OF EVERYTHING (TOE)

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

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

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

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

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

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

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

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

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

CODA

In summary, by looking at the process of evolution from its unicellular origins, the causal relationships between genotype and phenotype are revealed, as are many other aspects of biology and medicine that have remained dogmatic, anecdotal, and counterintuitive. That is because the prevailing descriptive, top-down portrayal of physiology under Darwinism as tautologic.[60] In contrast to that, the cellular–molecular, middle-out approach is conducive to prediction, which is the most powerful test of any scientific concept. Though there is not a great deal of experimental evidence for the intermediate steps between unicellular and multicellular organisms

compared to what is known of ontogeny and phylogeny of metazoans, it is hoped that the perspectives expressed in this essay will encourage more such fundamental physiologic experimentation in the future.

In closing, rather than a refutation of Darwinian Evolution Theory, the position taken in this article is intended as a further extension of the Modern Synthesis.[61]

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