Developmental gene regulatory networks—an insurmountable impediment to evolution

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Macroevolution requires that new developmental adaptations arise via random mutations that somehow provide a novel advantageous selectable trait. Developmental genetics research has documented that at the initial hierarchical levels of gene expression, it is nearly impossible to beneficially change the overall program by even single gene mutations without causing a major catastrophe. Another important aspect of the developmental genetics paradigm is the paradox of conserved protein sequence among top-level transcription factors combined with mutation intolerance. Extreme sequence conservation would seem to support common descent yet lack of mutability negates the fundamental mechanism of evolutionary change. In contrast, an Intelligent Design model predicts common code serving a general purpose in unrelated engineered systems.

Initial animal embryo cells are genetically identical and pre-packaged by the mother with maternal RNA, ribosomes, and proteins, which control the establishment of the body plan in the offspring embryo.[1–3] As the cells continue to divide over the process of embryogenesis, they are converted into different cell types, eventually resulting in skin, muscle, bone, connective tissues, nerve cells, etc, in a process called differentiation.

Embryogenesis was first experimentally investigated in the 19th century because of its fundamental importance to all of biology. Recent reviews show that the oocyte is polarized via a complex and redundant system of interactions between the cytoskeleton, several signalling pathways, and cell-to-cell communication. These issues are also of intense interest to assisted reproductive research and the assessment of embryo quality. Precisely when and how the cells of the mammalian embryo become committed to a specific cell type is of intense interest to stem cell researchers with evidence that it occurs as early as the 2 or 4 cell stage.[1–4]

Each differentiated cell employs specific parts of its genome, namely those genes and regulatory regions that are necessary to construct each specific cell type required by the developing embryo. Genes and regions of the genome that are not required at any stage of development are blocked by repressive chromatin states associated with DNA methylation and histone modifications.[5]

A complex control system exists which causes the embryonic cells to differentiate so that the appropriate body parts and organs will develop at the proper location in the developing body at the required time. This system must operate at a high level of control to insure the zygote develops into a complete functional organism consisting of many billions of differentiated cells that develop into functional organs and organ systems. The fates of individual cells and lineages are determined by a variety of genetic systems involving transcription factors, gene regulatory features (promoters, enhancers, and silencers), chromatin-modifying non-coding RNAs, as well as cytosine and histone modifications that accurately mark and dynamically designate its state in the developmental continuum.[6–8]

Many gene products, including proteins and a diversity of non-coding RNAs, are required for the development of a specific animal body plan and its many structures and organs. These gene products transmit information that influences how and when individual cells differentiate. These signals must interact with each other during embryological development in order to regulate both how cells and tissues are organized and assembled. The cell's many types of signalling molecules, such as hormones and cytokines, also coordinate and influence this cellular development. They form networks of coordinated systems that interact in ways analogous to how computer systems are designed to achieve the functional complexity of integrated circuits, hardware, and software required.[8]

When and how cell signalling molecules are transmitted often depends both on what signals from other molecules are received, and when they are received. This system, in turn affects the transmission of yet other signals—all of which must be properly integrated and coordinated in order to achieve the numerous specific time-critical functions required for organism development from a zygote to an adult.

Such organism and organelle specific genetic circuitry also guides the process of biomineralization resulting in skeletons and teeth as well as the generation of turtle and clam shells.[9] The coordination and integration of a plethora of signalling molecules ensure that the proper cellular differentiation

and organization of distinct cell types occurs during the development of a specific animal body plan, such as that of a mammal or insect.

The gene regulatory network model

The current approach to understanding developmental biology incorporates concepts of systems biology and centres around the idea that developmental gene regulatory networks (dGRNs) control the ontogeny of the body plan. In this paradigm, dGRNS are made up of transcription factors and regulatory modules (e.g. enhancers) that control the spatial and temporal expression of genes.[10–14] In reality, signalling pathways within and between cells serve as links between subcircuits in dGRNs.[10] Epigenetic mechanisms that modify chromatin structure and regulate gene expression are also directly involved in controlling dGRN activity as well.[6,7] In modelling these unfathomably complex systems, the secular scientific community typically only defines dGRNs as consisting of transcription factors and their regulatory modules.[10,13,14]

The pioneering researchers in the area of dGRNs were two now-deceased scientists at the California Institute of Technology—Eric Davidson and Roy Britten. Their work on gene regulatory networks was paradigm-shifting with tremendous impact in many different fields of biology. Their novel ideas were originally put forth in several theoretical papers between 1969 and 1971.[15–17] To explain development in multi-cellular organisms, they formulated a theory that proposed a model of developmental gene control by regulatory sequence found in the regions of the genome containing high copy DNA based on early observations of DNA sequence complexity in studies of reassociation kinetics. It was assumed that the genetic content was contained in low copy sequences that were surrounded in a sea of moderate to highly repetitive sequences. Thus, the logical conclusion was that the more highly repetitive sequences formed a controlling genetic matrix governing the protein-coding genes during development.

After these early years, Davidson and others went on to more fully elucidate the nature of dGRNs using the modern tools of molecular biology and eventually genomics with many exciting advances coming in the first decade of the 21st century.

The general idea that has emerged from the most recent studies of dGRNs in a variety of model organisms is that the dGRN is hierarchical in structure and can be thought of in a very simplified manner by considering transcription factors (TFs) to be nodes.[14,18] The dGRN is then composed of three sequential layers or categories of nodes as depicted in figure 1. The TFs at the most top levels (kernels) are general activators and involved in initiating overall regulatory

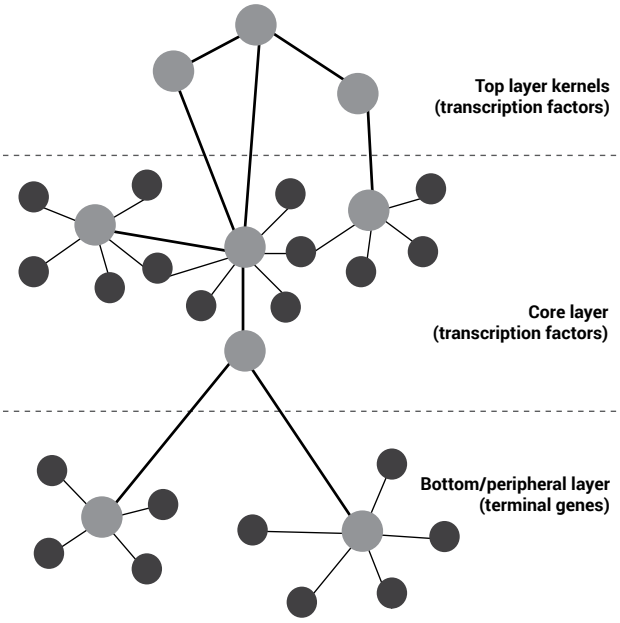

Figure 1. A simplified hierarchical schematic of a dGRN. The large grey nodes represent transcription factors (TFs) and their targets are represented by small grey nodes. The links (lines) represent the regulation of target genes by TFs. Links between the TFs are in bold. TFs typically regulate multiple target genes and themselves can be regulated by multiple TFs. Nodes with many links are often called hubs. The three tiers attempt to convey the hierarchical concepts of dGRNs described in the text. The top-level kernel TFs affect most other modules in the network and are typically associated with initiating subcircuits and cascades. The lowest layer contains genes downstream at the terminal end of a cascade which typically function more specifically in differentiation and also tend to be those specifically involved in phenotypic variability.

cascades. The TFs that comprise the middle nodes coordinate the transcription of many genes in combination with other TFs and would largely be involved with the activation or repression of genes related to growth, cell migration, shape, adhesion, and elasticity. The nodes at the lowest or outermost levels are considered to be at the periphery and would typically be indicative of the downstream developmental differentiation and much of the phenotypic variation we see among plant and animal kinds. For example, in humans, genetic variability in peripheral nodes would relate to skin colour, eye colour, height, hair-related traits, etc.

In general, the TFs associated with the upper nodes tend to be more highly similar in protein sequence among different taxa than those on the periphery. In addition, the general toolkit of upper-level TFs (give or take a few) in any given organism can be found at the most allegedly basal positions in the alleged evolutionary tree of life.[19,20] Thus, the information complexity of this system and its most basic components appeared suddenly in the scheme of life, and according

to evolutionists was responsible for the amazing burst of body plans and creatures found in the so-called Cambrian explosion.[21–23]

However, this sequence similarity or conservation among TFs from top-kernel-level nodes across the spectrum of life offers little consolation to the evolutionist. The chief problem for evolution is that TFs at both top and middle-level nodes are highly resistant to mutation or perturbation of their expression. Because of extensive hierarchical interconnectivity, if a change occurs in a TF that patterns the embryo, the alteration affects all of the downstream connections resulting in major developmental problems and is universally fatal. While the extreme sequence conservation of these proteins may seem to support the notion of macroevolutionary common descent, because the early phases of development depend so critically on the establishment of specific expression patterns, very little alteration is tolerated.

It is also interesting to note that evolutionary developmental biologists use the same terminology as used to describe man-made engineered computer systems, but deny that they were intelligently designed. The following is an excerpt from a recent 2017 review in which the author states:

> "We suggested that GRNs comprised four different components: (1) recursively wired subcircuits of genes responsible for patterning parts of the developing embryo, which we described as kernels; (2) small subcircuits that are easily co-opted to form particular developmental roles (such as Notch), which we termed 'plug-ins'; (3) switches which activated or deactivated particular subcircuits, which acted as input/output (I/O) switches in the GRN; and (4) the downstream differentiation gene batteries."[24]

The recursively wired kernels in the dGRN elegantly and sequentially define the spatial domains of specific regions in the developing embryo. Amazingly, while the subcircuits of specific gene sets are not reused elsewhere in the development program, the individual kernel-level genes themselves are ingeniously deployed again for other tasks. And in opposition to evolutionary theory, once the pathway is established early in development, the entire system is stubbornly resilient to mutational change. Extensive research on the developmental circuits of the sea urchin has documented how tightly controlled and orderly this process is, and "disarming any one of these subcircuits produces some development abnormalities."[25] Developmental sequences, once traversed, are locked down so they do not change at any later time. Embryos require embryo-specific control systems, and adults require adult-specific control systems.

Building new designs by mutations

To construct a fundamentally new animal design from a pre-existing design by mutations and selection requires numerous major alterations of the pre-existing developmental gene regulatory network that is established in a very early zygote stage. Furthermore, the research of developmental biologists has shown that constructing a new animal design would require thousands of coordinated mutations, yet even the slightest alteration in one or a few genes or their regulatory sequences inevitably produces catastrophic consequences.

As Davidson has documented, a dGRN that regulates body-plan development "is very impervious to change" and usually leads to "catastrophic loss of the body part or loss of viability altogether".[12] This observable consequence virtually always occurs if even one dGRN subcircuit is interrupted. Because most of these changes are always "catastrophically bad, flexibility is minimal, and since the subcircuits are all interconnected … there is only one way for things to work. And indeed the embryos of each species can develop in only one way."[12]

In his book, Intelligent Design proponent Stephen Meyer noted that "Davidson's work highlights a profound contradiction between the neo-Darwinian account of how new animal body plans are built and one of the most basic principles of engineering—the principle of constraints."[26]

As a result, "the more functionally integrated a system is, the more difficult it is to change any part of it without damaging or destroying the system as a whole".[26] Because this system of gene regulation controls animal-body-plan development in such an exquisitely integrated fashion, any significant alterations in its gene regulatory networks inevitably damage or destroy the developing animal. This now-proven fact creates critical problems for the evolution of new animal body plans and the new dGRNs necessary to produce them, preventing gradual evolution via mutation and selection from a pre-existing body plan and set of dGRNs.

Developmental biologists openly recognize these clear problems for the standard evolutionary synthesis. The problem as elaborated by Davidson, noted that neo-Darwinian evolution erroneously assumes that all microevolutionary processes equate to macroevolutionary mechanisms, thus producing the false conclusion that the "evolution of enzymes or flower colors can be used as current proxies for study of evolution of the body plan".[12] Typical evolutionary research programs involve studying genetic variation within a species or genus involving inter-fertile natural populations or populations from controlled crosses. From a developmental systems biology perspective, the genes or regulatory features involved in such variability lie at the peripheral nodes and do not explain novel body plans associated with macroevolution. Davidson notes that the standard evolutionary synthesis

"erroneously assumes that change in protein-coding sequence is the basic cause of change in [the] developmental program; and it [also] erroneously assumes that evolutionary change in body-plan morphology occurs by a continuous process".[12] Davidson also aptly notes that "these assumptions are basically counterfactual" because the "neo-Darwinian synthesis from which these ideas stem was a pre-molecular biology concoction focused on population genetics and adaptation natural history".[12] Neo-Darwinism in any form does not provide a mechanistic means of changing the genomic regulatory systems that drive embryonic development of the body plan. Alternating the peripheral differentiation process associated with observable variability is an entirely different scenario from building a new form of animal life by changing the fundamental structure of a resilient dGRN.

Is saltational evolution the answer?

An interesting trend among developmental biologists is that due to the severe problems that the stability of dGRN structure and function present to the standard neo-Darwinian (modern synthesis paradigm), many tend to gravitate towards a hopeful monster type of evolutionary scenario. This idea started well before the era of genomics and molecular biology with the writings of Richard Goldschmidt during a career that spanned from 1900 to 1958.[27] He was ahead of his time in that he promoted a view of physiological genetics emphasized by the dynamics associated with the products of genes such as enzymes, hormones, or inducing substances. He also believed that the concept of genes as discrete units was not as cut and dried as the leading Darwinists of the day believed. Most importantly, he proposed that if evolution was to be properly understood, it had to be directly linked to developmental processes with the timing and quantity of the product of a gene being key elements.

Goldschmidt astutely believed that 'microevolutionary' research which merely studied the distribution of variation within interbreeding taxa, did not provide answers to the bigger problems of discontinuity and unbridgeable gaps associated with macroevolution. Harvard paleontologist Stephen Gould also knew this to be true due to the clearly observable discontinuity between animal forms in the fossil record. In fact, Goldschmidt's ideas were revived by Gould. In a 1977 article titled 'The return of hopeful monsters', Gould stated that as "a Darwinian, I wish to defend Goldschmidt's postulate that macroevolution is not simply microevolution extrapolated, and that major structural transitions can occur rapidly without a smooth series of intermediate stages".[28]

These ideas, initially promoted by Goldschmidt and later revived by Gould, were originally based on homeotic mutations observed in fruit fly developmental genes that gave four wings instead of two and caused legs to develop in place of antennas (figure 2). Of course, these are detrimental effects providing no benefit to the fly. These genetic aberrations cause displaced body parts due to mutations in key genes involved in embryo patterning.[29]

Modern developmental biologists typically still adhere to a form of saltational macroevolution because of the inherent evolutionary developmental problems associated with mutations and the pervasive evidence of fossil record discontinuity. However, they now propose that the evolutionary mechanism itself is related to changes in the regulatory structure of dGRNs, not mutations within the kernel level or core transcription factor genes themselves.[10,11,30]

Because these internal nodes in the dGRN are so impervious to change, it is believed that somehow subcircuits in dGRNs themselves have been co-opted, re-purposed, or as some say, 'rewired', to create new highly different phenotypes.[31,32] Of course, this has never been observed at the level needed to account for large macroevolutionary changes—it is only a hopeful inference. The alteration of a developmental regulatory sequence, especially enhancer elements, has been observed to contribute to differential patterns in peripheral gene expression associated with phenotypic variability within a genus or species.[33] However, it has never been shown to occur in the re-patterning of internal dGRN nodes to produce a fundamentally new or different type of creature required to explain macroevolution. Furthermore, if developmental subcircuits could somehow

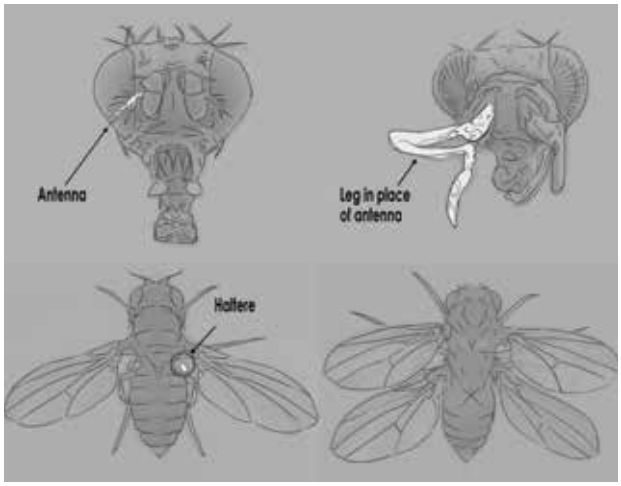

Figure 2. Mutations in top-level developmental homeotic genes involved in embryonic patterning result in misplaced body parts as vividly documented in *Drosophila* (fruit fly). The upper panel shows legs growing in place of antennae. The lower panel shows an extra abdominal segment with an extra set of wings. Because the haltere (an organ involved in flight stability) is missing in the four-winged mutant, these aberrations prohibit flight. Mutations such as these are ultimately lethal.

be coopted or repurposed or re-wired to foster evolution, this does not explain how or where the original developmental information arose in the first place. For all practical purposes, developmental biologists have yet to propose a viable mechanism for saltational evolution to occur.

In consideration of the interaction and complexity of dGRNs, one of the few researchers that has tackled the developmental conundrum is Michael Lynch. Like his colleagues in developmental genetics, Lynch admits that the modern Darwinian synthesis offers no credible solution.[34] He states, "Although numerous investigators assume that the global features of genetic networks are moulded by natural selection, there has been no formal demonstration of the adaptive origin of any genetic network" and "the mechanisms by which genetic networks become established evolutionarily are far from clear".[34] So what is the alternative model proposed by Lynch that might account for the origination of fundamentally new complex genetic networks that would propel evolution? Amazingly, he puts forth a neutral model evolutionary idea on a grand scale where genomes and their complex interdependent networks stochastically evolve through mutations and random genetic drift. Lynch claims:

> "… many of the qualitative features of known transcriptional networks can arise readily through the non-adaptive processes of genetic drift, mutation and recombination, raising questions about whether natural selection is necessary or even sufficient for the origin of many aspects of gene-network topologies."[34]

Needless to say, Lynch's ideas are pure hopeful speculation and the many problems with the neutral model of evolution have been discussed at length previously in this journal.[35–37]

Saltationist hyper-evolution in creation science?

Hopeful monster-style evolution is not just the playground of secular developmental geneticists. Surprisingly, a form of rapid saltational evolution with direct implications on our discussion of dGRNs has been proposed recently within the young-earth creationist community.[38] The basis of this idea stems from the acceptance by some geologists that the stratigraphic boundary marking the end of the Genesis global Flood is at the top of the Cretaceous. This becomes problematic as most mammal fossils are located above this boundary in the Paleogene and Neogene. Thus, it is believed that the crown mammal groups found in these sediments were the result of punctuated equilibrium-style diversification from a limited number of mammal groups on the ark which were then somewhat 'miraculously' entombed in localized post-Flood watery catastrophes the world over in the short space of just a few hundred years. Kurt Wise, who is a creationist paleontologist and a former graduate student of saltation-promoting evolutionist Stephen Gould, is a leading proponent of this idea who states that this "suggests a remarkably complete post-Flood fossil record, with most biostratigraphic gaps probably no more than decades in length".[39] Like his secular colleagues, Wise can pinpoint no mechanism to underpin his ideas and in fact promotes a more rapid form of hyper-evolution that even evolutionists find credible. University of Akron evolutionist and vocal creationist critic Joel Duff states:

> "Kurt Wise has taken the hyper-evolution rapid-speciation young-earth model of the origin of biological diversity and pushed it nearly to its logical end. Consistent with his ideas about the possible origin of whales from walking ancestors, he lists seals and sea lions together with bears as having a common ancestor on the ark."[40]

While not the purpose of this report, many previous papers have discussed at length the geological and paleontological shortcomings of placing the post-Flood boundary at the Cretaceous-Paleogene.[41–51] Bolstering these efforts is a recent research report by geologist Tim Clarey using large-scale global stratigraphic geologic data sets.[52] These comprehensive results "collectively establish that the Flood/post-Flood boundary had to have been much higher in the Cenozoic rock record".[52] As noted by Clarey, "the advocates for a K-Pg boundary end to the Flood have backed themselves into a corner by giving themselves only about 100 years of time for the entire Tertiary system to be deposited in a series of local catastrophes". And, "This is why Wise is advocating evolutionary saltation to explain the mammal record in the Tertiary. He has to. How else do you explain the mammalian fossil record of the Tertiary?" Clearly, neither the findings of complexity and stability in dGRNs nor the global geologic record support the contentions of those attempting to unnecessarily integrate Gould-style evolution into the creation model.

Increasing developmental complexity with eco-evo-devo

Organisms live in a dynamic world where symbiosis and phenotypic plasticity are now being shown to be the rules, not the exceptions.[53] Unfortunately for the evolutionist, these new layers of complexity raise more questions than answers. Not only are organisms dependent on their own internal dGRNs for development, but layers of interactive complexity also exist that are related to other organisms and complex networks of sensory inputs and responses. Secular biologists are now calling this new, and somewhat broad field, ecological evolutionary development or eco-evo-devo.[53,54]

Developmental plasticity is the ability of an embryo to adjust and change its form based on environmental

cues detected by complex sensory networks and adaptive programs built into the organism. A single genome can provide the differentiation specifications to provide a variety of adaptive forms, physiologies, and phenotypes. Through epigenetic modifications to the genome, many of these traits can also be inherited for multiple succeeding generations—giving offspring a fast track on adapation.[55]

Directly related to the concept that an organism both requires and dynamically responds to external inputs for development is the concept of developmental symbiosis—a harmonized process requiring a symbiotic interaction. For all practical purposes, there are no germ-free organisms in nature and many of these intimate interactions are required for development. For example, the seeds of orchids will not germinate without a specific type of fungus.[56] The proper developmental patterning regarding axis orientation in a nematode requires the presence of a specific type of bacterium.[57] The intestines of mammals and fish require gut microbiota to complete their proper development.[58–60] If the developmental complexities inherent to dGRNs within an organism's own genome were not enough to completely invalidate evolution, the fact that organisms require other organisms (having their own dGRNs) to develop properly, buries the concept of macroevolution even deeper in the abyss of unreality.

Summary

At the very core of the validity of models for macroevolution is how organisms develop. Any form of Darwinian evolution requires that new developmental adaptations arise via random mutations that somehow provide a novel advantageous selectable trait. Decades of developmental genetics research in a wide variety of organisms has documented in detail the fact that once an embryo begins to develop along a certain trajectory, mutations in top and mid-level transcription factor genes in the hierarchy model of regulation described by Davidson cause fatal catastrophe in the program. This mutation-intolerant obstacle poses a complete barrier for the modern Darwinian synthesis, the neutral model, and saltational evolution.

Another important aspect of the developmental genetics paradigm is the paradox of conserved protein sequence among top-level transcription factors combined with their intolerance of mutation. It is quite a quandary for the evolutionist—extreme conservation of sequence would seem to support common descent yet lack of mutability negates the fundamental requirement of evolutionary change. An Intelligent Design model, however, would predict that common code serving a general common purpose would be found among unrelated engineered systems that were the work of the same Creator—exactly as we find in man-made systems.

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