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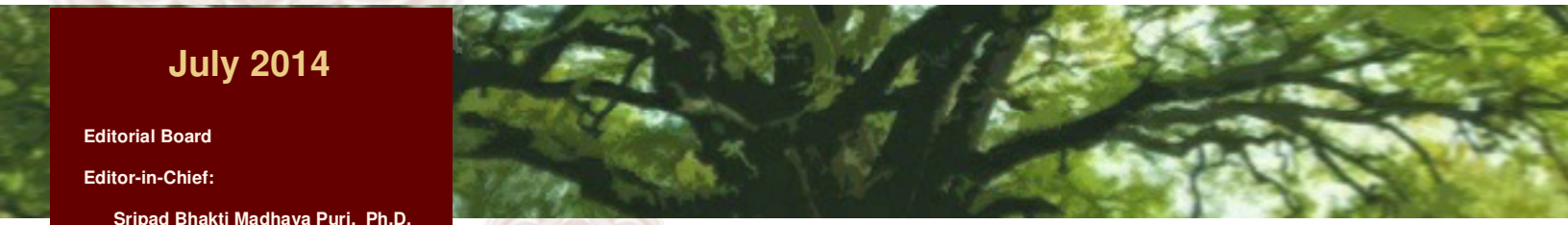

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Genetics, Epigenetics, Paragenetics Getting Closer to Life

Bhakti Madhava Puri, Ph.D.

Genetics

Gregor Mendel (1822-1884) was the first to explain that certain 'traits' were inherited in plants from one generation to the next. These would later become known as genes. Frederich Miescher in 1869 analyzed a substance from the nucleus of cells, which he therefore called nuclein. Further study of nuclein revealed that it contained elements like hydrogen, oxygen, nitrogen and phosphorous, with a specific ratio of nitrogen to phosphorous. Then in 1878 Albrecht Kossel determined that nuclein contained nucleic acid, from which he isolated five nucleobases (nitrogen compounds now referred to by the letters C, G, A, T, U representing cytosine, guanine, adenine, thymine, and uracil). It was also discovered that ribose, a sugar was present in the nuclein compound. What Miescher had isolated from the cell nucleus was actually what would latter be identified as DNA (Deoxy-ribo-Nucleic-Acid).

In 1888 the term chromosome was first suggested by von Waldeyer (1836-1921) to describe the carriers of these traits located in the nuclein. The name refers to the way they were identified using dyes, combining the Greek words chrome (color) and soma (body). Then in 1909 Wilhelm Johannsen coined the term 'gene' to refer to these traits. He also distinguished what he called the genotype to describe the genetic constitution of an organism, and the phenotype to describe the rest of the organism. Phoebus Levene in 1919 identified the nucleobase, sugar and phosphate that made up a unit called a nucleotide, which later X-ray diffraction patterns showed were regularly occurring in the strand of DNA.

Linus Pauling (1901 – 1994) proposed that the DNA structure was a triple helix in 1952, but this proved to be electrostatically unstable. The next year in 1953, James Watson (1928 - present) and Francis Crick (1916 – 2004) made their case for a double stranded DNA, following the discovery of Rosalind Franklin (1920 -1958). This is the model we use today.

While this chemical and structural analysis of genes proved to be of great importance in the study of the constituents of organisms, it missed the even more important role played by the living condition from which they were abstracted. The attempt to interpret only the molecular constituents of an organism, and the

chemical reactions associated with them is insufficient for describing the living or *in vivo* activity that actually occurs in a thriving organism. Take away the life of an organism and all the chemical reactions that were systematically occurring stop, despite all the same chemicals being present. In other words, it is not just a matter of chemical reactions producing life.

The hypothetical DNA theory, established by the historical study of DNA isolated and crystallized from an organism's nucleus, only gives us a chemical picture of what is going on in an organism. The actuality of the living organism's functionality is vastly underdetermined by such chemical descriptions. In order to determine how genes are functioning in their living environment, selected mutations by x-radiation or other means is used to establish what a particular gene is doing or not doing. The conception of genes established by this type of investigation was summarized in a paper by L. J.. Stadler in 1954, in which he gave what is appropriately called the operational definition of a gene [1].

Stadler writes:

> [O]perationally, the gene can be defined only as the smallest segment of the gene-string that can be shown to be consistently associated with the occurrence of a specific genetic effect.
>
> (1) It cannot be defined as a single molecule, because we have no experimental operations that can be applied in actual cases to determine whether or not a given gene is a single molecule"; (2) "it cannot be defined as an indivisible unit, because, although our definition provides that we will recognize as separate genes any determiners actually separated by crossing over or translocation, there is no experimental operation that can prove that further separation is impossible"; and (3) for similar reasons, it cannot be defined as the unit of reproduction or the unit of action of the gene-string, nor can it be shown to be delimited from neighboring genes by definite boundaries.
>
> The operational definition merely represents the properties of the actual gene, so far as they may be established from experimental evidence by present methods. The inferences from this evidence provide a tentative model of the hypothetical gene, a model that will be somewhat different in the minds of different students of the problem and will be further modified in the light of further investigation.

Further investigation came with the molecular structure of DNA being established along with a host of other discoveries brought about by molecular biologists. The complexities of the basic function of protein formation so vital to a cell was as much increased by such analysis, as simplified or made clearer for understanding. There are billions and trillions of atoms in a cell, all working together to keep it alive. Such a well organized system is not maintained by chemical reactions alone. R. A. Jorgenson writes [2]:

> "In modern terms, knowing the complete sequence of a chromosome does not allow us to precisely determine all of the many interdependent elements of a gene, including all those elements in *cis* that are necessary for the normal operation of a given gene that is associated with a specific genetic effect."

Epigenetics – between genotype and phenotype

C. H. Waddington (1905 - 1975) first proposed the term "epigenetics" in 1942 to describe the region between the gene and the whole organism (phenotype) [3]. Today, what is called the epigenome refers to all the chromosomal modifications, DNA modifications, chromatin protein modifications and their complexes. It is the epigenome that determines both the expression of the genes and their inheritance. R. A. Jorgenson reports [4], "Many of these modifications appear to be "programmable" and to be "read out" to

influence chromosomal functions." Nobel laureate Barbara McClintock stated this revolutionary proposal more clearly in her Nobel lecture [5], "to determine the extent of knowledge the cell has of itself, and how it utilizes this knowledge in a 'thoughtful' manner when challenged."

	Chromatin	Chromosome
Definition	In the nucleus, the DNA double helix is packaged by special proteins (histones) to form a complex called chromatin. The chromatin undergoes further condensation to form the chromosome.	A compact structure of nucleic acids and protein found in the nucleus of most living cells, carrying genetic information in the form of genes.
Structure	Composed of nucleosomes-a complex of DNA and proteins (called histones). Represent DNA folded on nucleoproteins by a magnitude of 50. The chromatin fiber is app. 10 nm in diameter.	Chromosomes are condensed Chromatin Fibers. They are a higher order of DNA organization, where DNA is condensed at least by 10,000 times onto itself.
Appearance	Chromatin Fibers are Long and thin. They are uncoiled structures found inside the nucleus.	Chromosomes are compact, thick and ribbon-like. These are coiled structures seen prominently during cell division.
Pairs	Chromatin is unpaired.	Chromosome is paired.
Metabolic activity	Permissive to DNA replication, RNA synthesis (transcription) and recombination events.	Refractory to these processes.
Presence	Found throughout the cell cycle.	Distinctly visible during cell division (metaphase, anaphase) as highly condensed structures upto several thousand nm.
Visualization	Electron microscope (beads on string appearance)	Light microscope (classic four-arm structure when duplicated)

The difference between chromatin and chromosome. [6]

Paragenetics

In 1960 R. A. Brinks suggested that chromosomes possess a paragenetic function in addition to their genetic function [7]. The physical nature of the paragenetic function is characterized by the variety of forms or states of chromatin that can reside at any genetic locus. While the genetic function is stable, the paragenetic function is labile and programmable in ontogeny. It is this latter function that allows organisms to transfer informational macromolecules (RNA and proteins) in a systematic and regulated manner over what is known as the "RNA information superhighway." Given this capacity, organisms may be able to store information at numerous genetic loci in the form of paragenetic chromatin states, which can be reprogrammed during ontogeny or environmental stress [8]. This reprogrammable system could operate over the whole organism as a storage device, allowing it to make informed 'decisions' during growth and development, or in response to the environment. Such processing capacity could be considered a form of 'intelligence,' which also could be passed on to future generations.

The study of the flow of information within and between cells and organisms represents the cutting edge of modern biological research. While physical correlates of cognitive behavior in living organisms are being discovered, it does not spell reduction to such correlates. The electronic activity within the physical components of a radio, for example, may be minutely determined, but ultimately it is not merely the electrical activity that produces the intelligent speech that is heard. Only the intelligent person whose voice is being broadcast through the radio can explain that. Without the broadcaster, the radio would sit silently even though fully functional. An organism without its living agency also appears to be devoid of metabolic activity although all the chemical components are fully present.

How to connect life to matter will be the ultimate challenge that has to be met. This will prove to be a philosophical problem we hope to address in the near future.

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Darwin Under Siege from Embryology, Homology, and Genetics

Bhakti Vijnana Muni, Ph.D.

Introduction

Evolutionary Developmental biology seeks to search out the causal factors behind evolution and ontogeny. Modern biology recognizes that population genetics is not sufficient to explain species diversity. In addition, due to a failure in understanding the origin of structural novelties and body plans from mere genetics, a deeper approach that includes a systematic organic concept is necessary to realize a reasonable explanation of species diversity. Modern developmental biology faces questions well beyond both Darwin's idea of evolutionary mechanisms and the Modern Synthesis. Even developmental biology has become one of the strongest proofs against evolution of body plans.

Embryology: The strongest proof against evolution

Darwin had considered embryology to be one of the strongest proofs for evolution. He wrote, "Embryology is to me by far strongest single class of facts in favour of (evolutionary) change of form [1]," and, "Embryology in Chapter VIII is one of my strongest points I think [2]." Darwin got support from Weismann, Haeckel, Roux, etc. but by around 1900 ontogeny and evolution became separated due to a series of setbacks. The experiments of Driesch had disproved preformism and Roux's theory of *Entwicklungsmechanik* or developmental mechanics. Driesch became a vitalist and proposed the concept of entelechy. In this way Roux and Driesch represented two poles in developmental biology, viz. preformationism and epigenesis at the beginning of the 20th century.

Darwinian evolutionary embryology was primarily based upon the then prevailing concept of shared developmentally conserved stages, universality of the germ layers etc. which used the criterion of homology. The concept of embryological archetypes had dominated zoology after Darwin. But biologists like Francis Maitland Balfour and Walter Garstang came to think of Haeckel's interpretation as excessive by the beginning of 20th century [3]. Embryology had not revealed any ancestral patterns which could be used for the extrapolation from ontogeny to phylogeny. Balfour became aware of the features in the early ontogeny of secondary adaptations but he could not find there any proof of inheritance of ancestral characters. Garstang also became an important critic of recapitulation theory [4].

As evolutionary biologists did not find any scope for demonstrating recapitulation of phylogeny the only option was to pursue the theory that perhaps ontogeny could somehow lead to evolution. This became one of the dominant themes of the work of many embryologists who somehow tried to explain their findings through the Modern Synthesis. This included Garstang, Sir Gavin Rylands de Beer and Balfour [5]. De Beer came up with the idea of heterochrony (developmental changes in the timing of events, leading to changes in size and shape) as providing a central role for evolution. Heterochrony had three dimensions: (i) Pre-displacement and post-displacement, (ii) neoteny (the change in timing of developmental events: slower) and, (iii) hypermorphosis (the change in timing of developmental events: further). This was based upon the idea of rate genes, which is a Mendelian gene that controls the speed of a particular developmental process. For him novelties could occur at any stage of development. He criticized the Haeckelian idea that evolutionary modifications could occur only among adult characters as terminal additions to an ontogenic sequence. A different set of concepts were being born in the beginning of 20th century due to a realization of the importance of epigenesis [6].

Homology: One of the big problems to Evolution

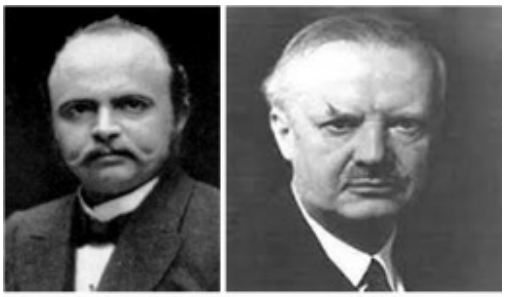

Fig. 1: Driesch and Spemann rejected the genetic concept of epigenesis

De Beer understood that homology was a big problem for evolution. Spemann (Fig.1) had also rejected Haeckel's recapitulation theory as well as Gegenbaur's evolutionary morphology and

phylogenetic trees. He rejected the genetic concept of homology as highly speculative. He took the example of regeneration of experimentally removed lenses in the eyes of amphibians. Even though the regenerated lens in the eye is less homologous than the older lens, it had developed from a different source than the original one. Due to having a separate origin it could not count as homologous based on the genetic definition of homology. An adult structure could be traced back to a developmental precursor but it has no correspondence to any structure in the egg. Thus it exists only potentially in the egg leading to a conclusion that the structure of the offspring is related to parental structure only ideally [7].

In this way experimental embryology revealed that homology was not so straightforward. De Beer accepted that developmental biology offered a substantial challenge to our understanding of the nature of homology [8]. For example, homologous structures need not develop from the same part of the egg or embryo. Further, they may not generate from the same germ layers. They need not be induced by the same organizers or induction processes. Homologous structures could arise by means of quite different developmental processes. It showed that early developmental stages of different species contained substantial differences. Development in the later stages, i.e. during the adult stages, reflected these differences in the early stages of development. The early differentiation was compatible with the differentiation of structures found in the adult stages. De Beer understood that it was futile to try to map sameness of structures by the sameness of a limited and particular set of genes. It is now understood that phenotypic structures that are controlled by identical genes can be non-homologous, homologous characters can be controlled by non-identical genes. De Beer said, "It is now clear that the pride with which it was assumed that the inheritance of homologous structures from a common ancestor explained homology was misplaced; for such inheritance cannot be ascribed to the identity of genes. [9]" He further said, "But if it is true that through the genetic code, genes code for enzymes that synthesize proteins which are responsible for the differentiation of the various parts in their normal manner, what mechanism can it be that results in the production of homologous organs, the same 'patterns', in spite of their not being controlled by the same genes? I asked this question in 1938, and it has not been answered. [10]"

Hourglass model is highly controversial

Experimental confirmation of the incorrectness of Haeckel's idea of the recapitulation theory has been reported abundantly. This idea was originally given by von Baer in 1828 as a guiding principle of comparative embryology and is well known as his third law [11]. It was an intuitive speculation of Darwin that animal morphology will be more conserved in the early stages of embryology and less in the adult forms. He thought this was the most reasonable and the most compelling evidence in favor of common descent. In that line of thinking natural selection will have the greatest opportunity to effect evolution in the adult stages and the least during the early embryological stages. In the earlier stages the adaptive possibilities would be less as they should be more pruned as a result of ancestral evolution or differentiation. Earlier embryological forms would represent the more necessary features and would afford lesser opportunities to diverge. Adult structures however should have more signs of species specific adaptations. However studies of morphological development did not confirm Darwin and von Baer. Studies show remarkable divergence between related species within the same phylum. This divergence can be seen both in the early and as well as in the later stages in development. These have very little apparent influence on adult morphology. This has been a saga that is completely against any Darwinian idea.

Modern developmental biology however coined a term called the 'pharyngula stage' or the 'phylotypic period' for the morphology that occurs in mid-embryogenesis in an attempt to revive evolutionary developmental biology in the era of post Haeckelian debacle. This is the hourglass model (Fig. 2) proposed by Denis

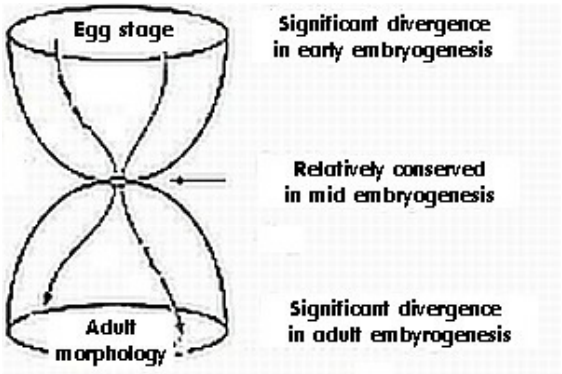

Fig.2: The Hourglass model is controversial

Duboule and Rudolf Raff in the 1990's. The hourglass model suggested that in the phylotypic period there is a relative conservation among different species of the same phyla during mid-embryogenesis. This meant that even though the morphological studies do indicate extensive divergence both in the early as well as the later stage, mid-embryogenesis coincides with a period of maximal similarity between all the species within each animal phylum.

This led evolutionary biologists towards even more speculative theories about gene-development nexus. One theory considered that as in the phylotypic stage there is substantial increase in the interactions between gene and developmental processes, any resulting evolutionary modification will be highly deleterious as a result of extensive and damaging side-effects. Another theory considered that as a result of increase in the highly coordinated and precise activity necessary for the growth and patterning during the phylotypic period, there would necessarily be a relatively conserved stage among the different species of the same phyla. The genomic organization of the Hox genes became a focal point for establishing these theories. Hox genes are an abbreviation of the term homeobox and are a group of related genes that control the body plan of an embryo along the anterior-posterior axis. These genes are necessary in determining the type of segment structures like legs, antennae and wings in fruit flies and also the number and structure of vertebrate ribs in humans that will form on a given segment after the embryonic segments have been formed.

The hourglass model is a kind of heterochronous concept which is concerned with the timing of embryological processes. Although support for the hourglass model was found in some studies from morphology as well as genetic sequence studies it has remained a controversial subject. Some studies claim the exact opposite, that there is a peaking of divergence at the phylotypic period and there is no temporal pattern of phenotypic conservation. E.g. Richardson et. al. wrote a paper entitled [12], "There is no highly conserved embryonic stage in the vertebrates: implications for current theories of evolution and development". They explain there that, "In view of the current widespread interest in evolutionary developmental biology, and especially in the conservation of developmental mechanisms, re-examination of the extent of variation in vertebrate embryos is long overdue. We find that embryos at the tailbud stage — thought to correspond to a conserved stage — show variations in form due to allometry, heterochrony, and differences in body plan and somite number. These variations foreshadow important differences in adult body form. Contrary to recent claims that all vertebrate embryos pass through a stage when they are the same size, we find a greater than 10-fold variation in greatest length at the tailbud stage. Our survey seriously undermines the credibility of Haeckel's drawings ... The wide variation in morphology among vertebrate embryos is difficult to reconcile with the idea of a phylo-genetically-conserved tailbud stage, and suggests that at least some developmental mechanisms are not highly constrained by the zootype. Our study also highlights the dangers of drawing general conclusions about vertebrate development from studies of gene expression in a small number of laboratory species."

Further Beninda Olaf et. al. report, "The phylotypic stage has never been precisely defined, or conclusively supported or disproved by comparative quantitative data. We tested the predictions of the 'developmental hourglass' definition of the phylotypic stage quantitatively by looking at the pattern of developmental timing variation across vertebrates as a whole and within mammals. For both datasets, the results using two different metrics were counter to the predictions of the definition: phenotypic variation between species was highest in the middle of the developmental sequence. This surprising degree of developmental character independence argues against the existence of a phylotypic stage in vertebrates. Instead, we hypothesize that numerous tightly delimited developmental modules exist during the mid–embryonic period… The onus is now clearly on proponents of the phylotypic stage to present both a clear definition of it and quantitative data supporting its existence [13]."

Hox genes, Developmental Biology and Evolution

Hox regulatory genes are also genes and this makes them a candidate for the same Mendelian rules as any other gene clusters. Some experiments also have been performed to demonstrate their Mendelian nature. For example the Rx homeobox gene of mice activates the process essential to the formation

of the eye and its bony orbit [14]. The debate produced by the Cambrian fossil record has given impetus to find mechanisms that can show that evolution could be a rapid process of organismal reorganization. This has produced a debate whether one define a species on the basis of genes, morphology, geography or reproductive isolation [15]. As Hox genes have an important role in the development of body plans, they are providing an appealing starting point for such studies in understanding the causal role in the regionalization of the body plans of all bilaterally symmetric animals. The speculation is rife that they may provide a new way to understand evolution in a redefined way. In this way it is speculated that it may come to the rescue of evolution by covering the lost ground for Modern synthesis which failed to integrate with developmental biology during the last century.

As Hox genes play an important role in developmental biology they seem to link the developmental processes with evolutionary mechanisms governed by genetic changes by bringing them closer to epigenesis. In spite of all such claims the speculations are more heuristic than factual. Genetic research does not really aim at the study of development as such, but has rather aimed strictly to the study of their role set against a constant developmental background [16]. The Hox genes are sometimes called 'master genes' or master controllers. This is meant to portray their crucial role as developmental switches. They trigger large numbers of downstream genes in the generation of complex structures like the eye. But such approaches are criticized for too much hedging. Epigenesis is the antithesis of preformationism. Waddington had tried to produce a non-mystical and non-vitalistic account of development by combining epigenesis with genetics, an account which he called epigenetics. Although gene regulation is important to development, it is at the same time not all that important. This fact began to become clear after the experiments of Driesch (Fig. 1). Due to these considerations (failure to integrate epigenesis with genetics) Hox genes became a good candidate to explain the genetic basis of developmental biology. It has been found however that the experimental manipulation of these genes only produces some grotesque or monstrous features. E.g. in Drosophila the antennae were converted into legs, or it effectively transformed the third thoracic segment into another second thoracic segment, or produced a second set of wings instead of halteres. Because of these the idea of Hox genes as master controllers gained some air. But it has subsequently been understood upon reflection that they are not so much master controllers but act more like efficient micromanagers. Hox genes are themselves subject to regulation as all other genes by cellular processes like cell-cell signaling. Even the so called ability of Hox genes to produce large scale rearrangements of body plans is more context dependent. E.g. Akam says, "When it comes to the downstream targets of the Hox genes, context is everything, in particular, which other transcription factors are present in the same cell will be a key factor determining the outcome of Hox gene action." [17]

This made the Hox gene's epithet of being master controller an overestimation and resulted in the overselling of Hox genes in development. This also indicates its limitations in effecting any evolutionary changes. E.g. Budd proposes along the lines of the concept of the genetic assimilation of Waddington that homeobox genes are used post hoc to streamline developmental processes once gradual morphological change has occurred. He calls this model 'homeotic takeover' which may be more amenable to the concept of natural selection [18]. It significantly downgrades the estimated capacities of Hox genes and brings the whole question of evolution significantly closer to same parameters which the Mendelian genes have already been subjected to.

Mueller points out that the origin of novelty and its role in the evolution of phenotypic complexity represents an evolutionary problem that had been sidelined by the Modern Synthesis due to its focus on variation and population dynamics. Major theoretical consequences of the evo-devo conjecture depend on the properties of developmental systems. An understanding of the problems faced by evolutionary biology has led to the proposal that organismal evolution progressed from a pre-Mendelian world to the Mendelian one that we study today [19]. Modern organisms are Mendelian in the sense that genotype and phenotype are inherited in a close correlation, and development is under program-like genetic control. The developmental mechanists pin their hopes on the existence of a hypothetical pre-Mendelian world where a much looser connection between genotype and phenotype would have prevailed. This could have permitted the generation of multiple forms from single genotypes depending on environmental influences [20]. These speculations are examples of typical tendency

of reverting to excessive hedging. More hedging is seen when they try to justify that the generic physical properties of cells, cell aggregates and tissues would have been the decisive determinants of biological form before the advent of genetic programs which would have frozen the forms only later. This significantly complicates living processes placing them in an unknown territory of which there is no evidence of existence.

Limitation of Genetics in explaining evolution

Many scientists like J. A. Shapiro, McClintock, Zent-Györgyi, George Wald and Anthony Flew overcame any linear, mechanical and digital concept of cell based on the concept of gene that arose within post Mendelian biology. The concept of gene had gained a realist interpretation in terms of segments within the DNA molecule in the 20th century. But Ruth Hubbard wrote a book named, 'Exploding the Gene Myth' [21] where she explained, "The myth of the all-powerful gene is based on flawed science that discounts the environmental context in which we and our genes exist." Shapiro has compared the attempts of the geneticist in trying to explain life in terms of genes with that of a man searching for the keys under a lamppost [22]. McClintock clearly saw that the genome was a member of the cellular apparatus which was itself subject to cellular control [23]. As the concept of "gene" has evolved into a more dynamic and inclusive conception, any oversimplified understanding of living organisms in terms of only discrete interacting molecules does not have any actual explanatory significance. Living organisms are dynamically complex functional entities which are irreducible to simple mechanical-chemical descriptions [24, 25]. This means that all our evolutionary views have become flawed as randomness and necessity will never be able to explain evolution. The growing realization of the sentient concept of the cellular phenomenon is squarely bringing the question of evolution into the domain of cognitive control. McClintock for example came to consider organisms as subjective beings from her research [26]. The major realization within post-Mendelian biology is that Mendel's experiments managed to bring to light only some partial truth in terms of genes but the whole truth is proving to be an organic-systematic sentient concept.

Conclusions

The problem of organismal form is an ancient dispute among different kinds of philosophers and scientists. Aristotle explained that the soul is the first principle of living organisms and distinguished them from non-living objects like rocks which have no soul [27]. The dispute between pre-formationism and epigenesis, for instance in Roux and Driesch, represent the two poles of the developmental argument which demand all our attention even now. Due to the theoretical analysis of experimental results scientific understanding has already led biologists to undertake a deeper level of theoretical and ontological underpinning. No mechanization like the evo-devo and developmental systems theory has any realistic solution. With the growing realization of the limitations of these approaches the time is now ripe for the biological sciences to explore a more cognitive role in genetic assimilation and developmental integration. According to Vedanta there is no evolution beyond a species. This is confirmed in the fossil records which show sudden appearance of forms and stasis. The Vedantic idea is that there is conservation of species identity in Nature. Essentially Nature is living or biocentric and is a far cry from the chemical understanding of 20th century. The sensory capacities of organisms provide the basis for a very important new paradigm for biology. Organisms are regarded as intelligent and sentient. Our hope is that scientists pursuing the 21st century evolutionary biology will become conversant with these principles of life and will be able to see biology from that new and fresh light.

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Dissecting Reductionism in Immunology

Bhakti Niskama Shanta, Ph.D.

Biology is Spurning Reductionism

To understand how life works, scientists must rely upon simplification (idealized models and deterministic concepts), both in terms of analysis and explanation. René Descartes introduced reductionism by explaining that the world can be considered to be a clockwork mechanism. According to Descartes, to understand a whole we have to study the parts and with that knowledge of parts we can reassemble each component to recreate the whole. Descartes' 'clockwork universe' is the foundation of the Newtonian mechanistic approach and scientists, including biologists, use this approach to understand reality. Following this approach, scientists are looking for an objective representation (by reducing the whole to its simplest components) of an extremely complex reality.

Influenced by this guiding vision biologists try to explain life in terms of physical and chemical properties of individual components of the body of a living organism. Reductionism can be classified in three categories: (1) ontological reductionism (every system is composed of certain fundamental elements), (2) epistemological reductionism (laws and theories of a complex system can be obtained from laws and theories of a lower level system), and (3) methodological reductionism (knowledge of complex systems can be grasped by studying their individual constituents). Reductionism is commonly practiced as an analytical methodology to explore molecular and cellular processes in biology. The method of dissecting biological systems into their constituent parts has been successful in developing a catalog of the chemical constituents of numerous living processes. However, this reductionism is reaching its limits and such approach cannot address the complexity of either a smallest functional cell [1] or a complex human brain [2]. An increasing number of scientists argue that biological systems cannot be conceived by Descartes' clockwork model. Biological systems cannot be grasped either by the determinism of Newtonian mechanics or by random systems analysis of statistical mechanics [3]. The properties of a protein are not equal to the sum of the properties of each amino acid. In a living cell proteins can distinctively catalyze a chemical reaction or identify an antigen not only because their amino acids are arranged in a particular manner, but also because their three-dimensional structure and function are controlled by sentient living cell. The empirical evidence in 21st century biology confirms Aristotle's statement, "The whole is more than the sum of its parts" and the claims of Immanuel Kant [4]:

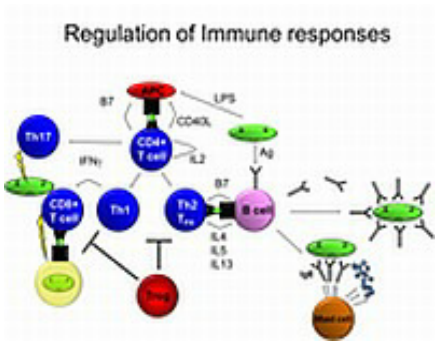
Immanuel Kant

"*there will never be a Newton of the blade of grass, because human science will never be able to explain how a living being can originate from inanimate matter*"

Glitch of Reductionism in Immunology

Biologists believe that biological systems are composed exclusively of atoms and molecules (without consciousness and soul) and it is feasible to explicate life by physicochemical properties of their individual constituents, down to the atomic level. Francis Crick stated, "The ultimate aim of the modern movement in biology is to explain all biology in terms of physics and chemistry" [5] and for more than half century this mindset guided the research in molecular biology. However, in this mindset biologists misconceive biological systems, which are complex, functional wholes. The dynamic integrated relationship of biological systems cannot be understood by study of their individual constituent parts. The complex nature of a functional whole also cannot be realized or predicted from the properties of the individual, isolated components. [6]

In biology textbooks cells and organelles are explained in the form of their constituents. Physiology and psychology are explained as an outcome of biochemistry and neurophysiology, respectively. Similarly, immunology is also described in terms of molecular properties of antibodies, T-cell receptors, major histocompatibility complex molecules, cytokines and proteasomes. Studying these narrations, students get the impression that living organisms are mere physicochemical systems. However, advancement in biology confirms that such an approach has a detrimental influence on biomedical research, and especially on drug discovery and vaccine development. In this approach there is an intentional predominance



of physical explanation over biological explanation [7] and it is presumed that the complete nature of a higher level can be understood form the properties of a lower level.

Nagel [8] explained that reductionism tries to elucidate the details of a higher level theory through the theory of lower levels, and it presumes that axioms and laws of the higher level can be inferred from the theory of lower levels. Therefore, reductionism is completely dependent on the feasibility of interlinking the terms of lower level and higher level theories, and on the viability of rationally obtaining laws from the lower level theory that are valid for the higher level theory. [9] The reductionist approach of dissecting the immune system into its parts breaks the dynamic integrated relationship of the parts and in the process the vital irreducible characteristics of the immune system as a whole are destroyed. It is an attempt to understand the immune system by examining its constituents in isolation and without interference from the environment. Although this type of simplistic approach is useful for immunologists to gain some knowledge of different mechanisms at work in individual parts of the immune system, but by following this approach immunologists cannot achieve a sufficient rational explanation for the functioning of the complex immune system as a whole. The dynamic integrated relationship between the constituents of a biological system cannot be realized by totaling the characteristics of its isolated parts. [6] Several aspects of immunogenicity depend upon biological potential (like, immunoglobulin gene repertoire, self-tolerance, the production of cytokines, and various cellular and regulatory processes) of host organism, but we cannot find that when the parts are examined discretely. Moreover, a reductionistic approach cannot explain how these aspects of the immune system are controlled in a host organism to produce neutralizing antibodies. [10] Antibodies also work in an integrative manner and the neutralizing synergy between various antibodies cannot be reduced to the straightforward addition of effects of constituent molecules in isolation. [11] The methods based on a reductionistic approach cannot plausibly have power over the immune system and hence are inefficient for development of vaccines.

Naive Reductionism in Vaccine Development

A vaccine which can imitate the natural immune response towards an infectious pathogen and permanently safeguard against infection by the same pathogen is considered a good vaccine. [12] In vaccine development research, scientists try to develop synthetic vaccines using the molecular data obtained from the reductionistic knowledge of immunology. Such studies presume an oversimplified reality and hence are insufficient to mimic dynamic integrated biological phenomena or an organic whole. To know what causes protection against a particular disease in the immune system reductionists try to deactivate antibodies or cytotoxic T-cell responses. To find a physico-chemical explanation for the specific binding reactions they dissect antigenic sites and cellular receptors. To unravel the mechanism for how an individual neutralizing antibody manages to attach to the target antigen they study the antigen-antibody complexes using X-ray crystallography. Such approaches are useful for amassing significant data and information for structural links of different interactions during infectivity neutralization. However, using the same data and information immunologists cannot explain: (1) how immune system as a whole puts an end to the capability of pathogen to infect its host, and (2) how vaccination can produce the necessary neutralizing antibodies. [13]

Due to a growing familiarity with molecular structure of antigenic sites recognized by antibodies and T-cell receptors, immunologists believe that it is possible to discover vaccines using the molecular design strategies found in structure-based drug design. [14] In that approach they overlook the uniqueness of the relationship between a drug and its receptor or target molecule. In a procedure called 'rational design' they make an attempt to modify the structure of the drug slightly to achieve an improvement in its biological function. This simplistic mechanistic view in *abiology* imposes an unjustifiable attention on a single cause to understand the functioning of an intricate biological system. The commonly practiced linear causality explanations in physics and chemistry are insufficient to address the network and circular causality of an organic whole. The immensely complex organic whole does not allow *abiology* to unravel all the causal relations of a functional dynamic integrated biological phenomenon. [15] Due to a misunderstanding, reductionists falsely believe that causality is a relationship between two chemicals/objects or between a structure and a function. In reality, causality is a relationship between successive events and *abiology* cannot establish a unique causal relationship between the structure and the function of a biomolecule in an organism. In living organisms a single chemical structure of a biomolecule can execute many different functions and also one function can be produced by several different chemical structures. [16] *Abiology* can at best hunt for correlations and

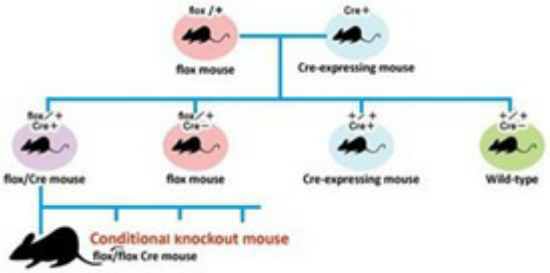
not causal relationships between a structure and a biological function. [17] A naive 'rational design' of biomolecules in vaccine development research cannot establish a relationship between 'neutralization of an infectious agent' (biological function) and the structure of an antibody molecule. High-throughput screening, combinatorial chemistry, genomics, proteomics, bioinformatics and so on are based on unmitigated reductionism and therefore have failed to live up to the expectations in terms of production of the new drugs. [18] Drug discovery is driven by only a belief that *in vitro* cell cultures and computer models can help us understand life and therefore ignores the fact that in such an approach there is no congruence between *in vitro* assays and the *in vivo* systems. Moreover, a monotonous application of animal models as a substitute for clinical studies in human disease has also continually proven derisory. [19] Hence, such oversimplified reductionistic approaches underestimate the patients and life in general. [20]

Evolutionary Biology Imposes an Extreme Reductionistic View

Evolutionary biology proclaims that during the course of evolutionary history natural selection can select a particular molecular structure that is responsible for a specific function with survival advantage. [21] In an organism different mechanisms with several different genes and gene products may be responsible for the appearance of the same function and vice versa. For example, using genome data scientists found that two enzyme functions are associated with seven different folds each [22] and several biochemical functions can be carried out by proteins with the same fold (even by members of a single homologous family). [23] In such situations natural selection (only looking for a function with survival advantage) has no means to distinguish between different molecular structures. Therefore, instead of structural explanation, this *abiology* attempts to provide a functional explanation for the present biological structures on the basis of survival advantages in the past. Like a man looking-for-keys-under-the-lamppost evolutionists try to analyze DNA sequences with the belief that a linear causal succession connects the sequence of a gene to the biological function of the product of that gene. This simplistic stance overlooks the role of cellular and extra-cellular environment, and of regulatory genes when its claim that gene sequence is exclusively the cause of the manifestation of a co-linear protein sequence. Ignoring the role of the sentient cell, this view further believes that the three-dimensional structure of the protein is solely determined by the protein sequence only. Immune system crystallographic studies of several antigen-antibody complexes show that a considerable amount of induced mutual adaptation of the two partners takes place at the time of the binding process. Some oversimplified reductionistic analyses try to represent the binding site images as two-dimensional flat areas at the surface of proteins, or even one-dimensional linear sequences forming continuous epitopes without any conformational characteristics. In reality, antigenic specificity cannot be explained even by three-dimensional structures, because, in such binding processes, time (the fourth dimension) also plays a crucial role. [24] It is now well established that even at a cellular level all biological functions are well regulated from within by a sentient cell. [25] The three-dimensional structure of an epitope is only a time slice image of processes within an integrated dynamic whole, and therefore, the structure and function of a binding site cannot be studied separately. Hence, the protein tertiary structure cannot be predicted from sequence information alone. [26]

Charles Darwin in his evolution theory advocated an extreme reductionistic view that the human ability to form and hold beliefs had evolved from purposeless chemicals and the lower animals. However, Darwin was concerned about the self defeating nature of his own theory: "With me the horrid doubt always arises whether the convictions of man's mind, which has been developed from the mind of the lower animals, are of any value or at all trustworthy. Would any one trust in the convictions of a monkey's mind, if there are any convictions in such a mind?" [27] In due course of time Darwin's *abiology* also produced a general consensus among scientists for an extreme reductionistic view that in a future based on gene analysis science can understand and control all the functions of living entities including psychological behavior. However, in reality what to talk about psychological behavior, even the simplest physiological functions like muscle contraction cannot be understood by simplistic reductionistic biochemical explanations such as the interaction between actin and myosin. [28] Biochemical pathways do not precede physiological functions and in reality they both take place at the same time. Therefore, biochemical explanation cannot

provide a causal rationalization for the physiological event. [21] Evolutionary biology also cannot provide functional explanations for why antibodies exist, how antibodies meet the requirements to shield host organism against infection and how the antibodies with such an ability to neutralize the infectivity of a pathogen are produced. [29] Evolutionary biology dogmatically believes that the majority of human diseases are caused by the interaction of several gene products. It is impossible for reductionists to identify all the gene products that are responsible for a specific biological function and moreover, genetic information of one gene is also dependent on other genes. The environment and both cellular and extracellular conditions also play a vital role in deciding the way the genetic information can be expressed. Genes in isolation cannot do anything including self-replication, because biological functions are carried out within a sentient organic whole and those complex functions are inimitable in isolation. Ignoring this, experiments based on 'single gene deletion' are continually conducted in *abiology* to comprehend complex genetic networks. To understand the task of



individual genes reductionists perform knockout experiments in mice by inactivating or removing a particular gene. It is observed in the studies that: (1) in many of these experiments knockout has no significant effect at all [30], (2) in some cases knockout has a completely unforeseen effect [31], and (3) disruption of the same gene can have diverse effects in different strains of mice. [32] Such diverse observations and a naive extrapolation of data from mice to other species only prove the impracticality of this approach. [19] Only an insignificant number of new drug targets are discovered based on human genome sequence data. [33] Gene therapy, stem-cell research, antisense technology and cancer vaccines failed to deliver results as per expectation [18] and moreover, they involve dangerous risks along with harmful side effects. [34]

Conclusion

In a living organism several events occur in which one object is hidden by another object that passes between it and the observer. For example, the antibody character of an immunoglobulin molecule can be sensed only when its corresponding antigen is known. Similarly, the epitope character of a set of amino acids in a protein can be ascertained only by recognizing an immunoglobulin that can bind to it. [35] Due to this several biological functions cannot be described by a structure identifiable before the interactions. Cell sentience plays a vital role at antigenic sites in a protein and antibody combining sites. Antibody specificity has significance only when cell's sentient faculty allows the antibody to discriminate and react differently with two or more epitopes. Therefore, the simplistic reductionistic models are too far from reality to be able to understand and predict the antigenic specificity. Sentience is essential in all biological functions and by dissecting a biological structure into its atomic constituents it is impossible to understand the organic whole. Vaccination completely depends on our immunological knowledge of sentient organisms. Inaccurate assumptions in simplistic models in reductionism can cause autoimmune disease by altering a beneficial immune reaction into a harmful one.

Several studies in molecular biology do not represent genuine biology. Present drug discovery research is also a purely protein chemistry because it is only concerned with molecular and atomic forces. It is misleading to include such studies under the field of immunology. In immunochemical research scientists only study the chemistry of interaction between a protein antigen and an antibody molecule (also a protein). Therefore it cannot represent a genuine biology. In this *abiology* immunologists are only making futile attempts to reduce biology to chemistry. The biological realm is driven by sentience and without that we cannot have functions like pathogen identification and elimination or the ability to discriminate self from non-self. Frontier biology acknowledges that even the smallest cells are sentient beings [36] and therefore advocates a change in the dogmatic reductionist stand of much of the old biological research. In the field of psycho-neuro-immunology scientists are studying how immunity is affected by the cognitive phenomenon of depression, stressors, and several other psychosocial aspects. The level of T-cells and responses to mitogens were found lower in students during the exam period. [37] People with a happy mood are found to have higher capability to fight off the cold when given a squirt of the rhinovirus. [38] Reductionistic theories produce a loss of faith in religion, which has a great influence on our health. Therefore, immunologists must heed the lesson from the conclusions of the *Encyclopedia of Medicine* [39]:

•"*The recent trend towards integration of religion and medicine has been stirred primarily by medical research demonstrating intimate and often complex relationships between religion and health… Over one hundred studies have now documented the high prevalence of religious coping among persons with a variety of diseases ranging from diabetes, kidney disease, heart disease, cancer, arthritis, and cystic fibrosis, to more general conditions such as chronic pain… Nearly 850 studies have now examined these associations, with between two-thirds and three-quarters of these finding that the religious person tends to be healthier and better able to cope with illness.*"

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