The functional matrix hypothesis revisited. 3. The genomic thesis

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Although the initial versions of the functional matrix hypothesis (FMH) theoretically posited the ontogenetic primacy of "function," it is only in recent years that advances in the morphogenetic, engineering, and computer sciences provided an integrated experimental and numerical data base that permitted recent significant revisions of the FMH—revisions that strongly support the primary role of function in craniofacial growth and development. Acknowledging that the currently dominant scientific paradigm suggests that genomic, instead of epigenetic (functional) factors, regulate (cause, control) such growth, an analysis of this continuing controversy was deemed useful. Accordingly the method of dialectical analysis, is employed, stating a thesis, an antithesis, and a resolving synthesis based primarily on an extensive review of the pertinent current literature. This article extensively reviews the genomic hypothesis and offers a critique intended to remove some of the unintentional conceptual obscurantism that has recently come to surround it. (Am J Orthod Dentofac Orthop 1997;112:338-42.)

"The whole plan of growth, the whole series of operations to be carried out, the order and site of synthesis and their co-ordination are all written down in the nucleic acid message."[1]

"Within the fertilized egg lies the information necessary to generate a diversity of cell types in the precise pattern of tissues and organs that comprises the vertebrate body."[2]

The initial version of the functional matrix hypothesis (FMH),[3-8] claiming epigenetic control of morphogenesis, was based on macroscopic (gross) experimental, comparative, and clinical data. Recently revised,[9,10] it now extends hierarchically from gross to microscopic (cellular and molecular) levels and identifies some epigenetic mechanisms capable of regulating genomic expression. This warranted revisiting our earlier analysis of the perennial genomic/epigenetic controversy.[11]

The epigenetic position of the FMH may seem quixotic when molecular genetics is the premier ontogenetic research paradigm. Indeed, most clinicians and experimentalists[11-14]—there are exceptions[15]—subscribe to the two epigraphs above, stated more succinctly as "genes make us, body and mind."[16]

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Nevertheless, a continuing countercurrent of dissent claims morphogenesis is regulated (controlled, directed) by epigenetic mechanisms and processes.[17-31] In addition, several new disciplines explicitly invoke epigenesis.[32-42]

The epigenetic/genomic problem is a dichotomy, and dialectics is one analytical method for its resolution. The method consists of the presentation of two opposing views, a thesis and an antithesis, and of a resolving synthesis. Such a dialectic analysis is presented here in two interrelated articles that respectively consider (1) the genomic thesis and (2) an epigenetic antithesis and a resolving synthesis. Because a comprehensive review of this problem would be encyclopedic, only selected relevant aspects of ontogeny (morphogenesis) and phylogeny (evolution) are considered here.

An Odontogenic Example of the Genomic/Epigenetic Dichotomy

Odontogenesis provides a comprehensible example. The widespread diagnostic use of vertebrate dental coronal morphology in zoological systematics, vertebrate paleontology, physical anthropology, and forensic odontology suggests to many a rigid genomic control of odontogenesis, as reflected in the temporally sequential, and spatially restricted, expression of the genomically regulated production of specific molecules as exhibited, for example, in murine molar development.[43]

Nevertheless, data exist strongly supportive of epigenetic regulation of odontogenesis. For exam-

ple, Chiclid fish are polyphyodont (have continuously replacing dental sets) and can exhibit pronounced dental phenotypic plasticity.[44] When the fish are fed on hard-shelled mollusks, the replacing teeth are large and molariform, but when soft food is fed, those teeth are gracile, conical, and nonmolariform. Experimentally in aquaria, the two phenotypic states may be repeatedly and arbitrarily alternated in succeeding dental generations by alternately changing the diet's consistency. Because each dental replacement cycle involves identical odontogenic stages, it is postulated that (1) mechanical forces, related to differential diet "hardness," generate epigenetic signals, mechanotransductively processed by dental papilla cells[9,10]; and (2) these signals control at least the temporal and spatial expression of genomic products related to the development of differential tooth form, such as size and shape.[45-47]

If the epigenetic/genomic dichotomy of odontogenetic regulation is unresolved, how much more so the complex topic of cephalic morphogenesis where, parenthetically, mechanical loadings also play a significant regulatory role.[15]

The Genomic Thesis

The genomic thesis holds that the genome, from the moment of fertilization, contains all the information necessary to regulate (cause, control, direct) (1) the intranuclear formation and transcription of mRNA and (2) importantly, without the later addition of any other information, to regulate also all of the intracellular and intercellular processes of subsequent, and structurally more complex, cell, tissue, organ, and organismal morphogenesis[1,2,48]: succinctly, "all (phenotype) features are ultimately determined by the DNA sequence of the genome."[49]

In this thesis, morphogenesis is but the predetermined reading-out of an intrinsic and inherited genomic organismal blueprint[48,49,50,51,52] where, in addition to molecular synthesis, the genome also regulates the geometric attributes of cell, tissue, organ, and organismal size, shape, and location. For example, "specific patterns of gene regulation (cause, control, regulate, determine) the mechanisms by which a fertilized egg divides and progresses through the various decision points to yield groups of cells that are first determined to become and then actually differentiate to become specialized tissues of the right dimension and in the proper location."[53]

The genomic thesis originated with classical (chromosomal) Mendelian genetics.[54,55] Combined with the empirical data of animal breeders, it earlier provided a theoretical basis for certain human eugenic theories proposing reproductive inhibition for individuals with "undesirable and genetically (chromosomally) regulated" medical and social conditions: a policy that eventually reached historical genocidal depths.[56,57]

Later, the blending of the classical chromosomal and vertebrate paleontological disciplines created the neo-Darwinian synthesis, a currently accepted paradigm of phylogenetic regulation.[58]

Recently, molecular (gene) genetics extended the claims of the thesis to the regulation of all aspects of ontogeny (i.e., of "growth and development"). The mega-human genome project,[59,60,61] called "the ultimate triumph of genetics,"[48] explicitly intends to: (1) describe the complete human genome; (2) demonstrate genomic controls of all developmental processes, at all structural levels, from the subcellular to the organismal; and, (3) in a societal context, possibly lead to some type of neoeugenics.

Many human activities now are claimed to be genomically regulated: e.g., psychological behavior[62]; personality[63]; alcohol and drug abuse[64]; chronobiological cyclic behaviors[65]; smoking, obesity, alcoholism, drug abuse, food-binging—indeed any attention-deficiency disorder,[66] among many others. The further suggestion of genomic control of intelligence generates prodigious, biomedical controversy in the social sciences and politics.[67] And note the frequent popular press reports of the "discovery" of yet another "gene" that "controls" yet another developmental, physiological, psychological, or sociological event, process, or state.

The Biologic Bases for the Genomic Thesis

While comprehensively considered elsewhere,[48,49,53] a brief review is useful. The somatic cells of an individual metazoan inherit two classes of molecular information: (1) an identical diploid DNA and (2) the maternal cytoplasmic constituents of the egg: e.g., mitochondria, cytoskeleton, membranes. Only approximately 10% of the genome seems related to phenotypic ontogenesis, whereas the human genome has approximately 100,000 genes, "well over 90% ... does not encode precursors to mRNAs or any other RNA."[53] With regard to individual phenotypic structural attributes, while all somatic cells commonly share approximately 5000 different polypeptide chains, each specific cell type is characterized only by approximately 100 specific proteins. And it is claimed that "these quantitative (protein) differences are related to dif-

ferences in cell size, shape and internal architecture."[53]

The encoding 10% of the DNA exists in two families; the vastly preponderant "housekeeping" genes and the nonabundant "structural" genes. The former regulate the normal molecular synthesis of agents involved in (1) the common energetic (metabolic, respiratory) activities of all cells and, (2) the specific activities of special cell types (e.g., neurons, osteoblasts, ameloblasts etc.).[52,68]

These genes also regulate the synthesis of the specific molecular gene products, whose presence, absence, or abnormal molecular configuration are associated with the (human) pathologic conditions said to have a unitary genetic cause—the so-called Mendelian disorders and the "single-gene disorders with nonclassic inheritance,"[52] such as Marfan syndrome, achondroplasia, osteogenesis imperfecta, and Duchenne muscular dystrophy, among many others.[52] For some, such "disorders provide the model on which the program of medical genetics is built."[59] In such conditions the absence of a normal type, or the presence of a structurally abnormal type, of a specific biochemical or molecular structural entity is sufficient to initiate the cascade of subsequent abnormal developmental pathways, eventuating in a specific pathological state.

A physical analogy is the construction of a building wall where either the proportions of the concrete are incorrect or an insufficient number of metal reinforcing rods are used. In both cases, eventual structural collapse is possible. Substitution of intercellular proteoglycans, and of collagen fibrils, provides a corresponding skeletal tissue analogy. Here, alterations in the genomically regulated processes of molecular synthesis can produce an eventual "structural collapse" at the hierarchically higher level of a macroscopic bone. Anticipating an antithesis, note here that the claim of genomic control of the molecular syntheses underlying the formation of such elemental (molecular) skeletal tissue "building blocks" does not substantiate the further claim that the genome regulates the growth and development (the size, shape, location and histological composition) of the gross anatomical bone.

The Genomic Thesis in Orofacial Biology

There is extensive support for the genomic thesis in the orofacial biology literature, with most genetic studies of cephalic or cranial morphogenesis explicitly or implicitly assuming genomic regulation of each anatomical structure.[69-77]

A characteristic article[12] claims that prenatal craniofacial development is controlled by two interrelated, temporally sequential, processes: (1) initial regulatory (homeobox) gene activity and (2) subsequent activity of two regulatory molecular groups: growth factor families and steroid/thyroid/retinoic acid super-family. For example, "homeobox genes coordinate the development of complex craniofacial structures" and in "both normal and abnormal development, much of the regulation of the development of virtually all of the skeletal and connective tissue of the face is dependent on a cascade of overlapping activity of homeobox genes."[12]

It is claimed that regulatory molecules can (1) "alter the manner in which homeobox genes coordinate cell migration and subsequent cell interactions that regulate growth" and (2) be involved in the "genetic variations causing, or contributing to, the abnormal development of relatively common craniofacial malformations ... perhaps modifying Hox gene activity."[52]

Specific orthodontic implications of the genomic thesis include claims that "poorly coordination-ordinated control of form and size of structures, or groups of structures (e.g., teeth and jaws) by regulator genes should do much to explain the very frequent mismatches found in malocclusions and other dentofacial deformities." And "single regulatory (homeobox) genes can control the development of complex structures ... indicating that single genes can determine the morphology of at least some complex structures," including "how characteristic noses or jaws are inherited from generation to generation."[52]

Critical Definitions

Clarification of this dichotomy is assisted by defining the present use of four terms: epigenetics, hierarchy, emergence, and causation.

Epigenetics. Several millennia ago epigenesis described the process(es) by which increasing structural complexity gradually arose from an originally unstructured mass, for example the stages of in vivo chick development or the gradual appearance of a pattern during weaving on a loom.[78-81] Over time, many alternate, often differing, definitions appeared.[22,82] Earlier, they were macroscopic in scale and considered only the extrinsic, extraorganismal environment, such as food, light, temperature, and radiations.[83] Nineteenth century physiology added the intrinsic, intraorganismal *milieu interieur,*[84] such as hormones, blood gases, nutrients, and ions.

Epigenetics, as defined here, includes (1) all of the extrinsic (extraorganismal) factors impinging on

vital structures, including importantly mechanical loadings and electroelectric states and (2) all of the intrinsic (intraorganismal) biophysical, biomechanical, biochemical, and bioelectric microenvironmental events occurring on, in, and between individual cells, extracellular materials, and cells and extracellular substances.

Hierarchy. Biological structures are hierarchically organized, with structural and functional complexity increasing "upward" from the ever-expanding family of subatomic particles to protons, electrons, atoms, molecules, subcellular organelles, and on to cells, tissues, organs, and organisms.[48] While a genomic thesis claims that each higher level is achieved by the predetermined activity of the genomic information, an epigenetic antithesis suggests that hierarchical complexity results from the functioning of epigenetic processes and mechanisms,[30] as described in the disciplines of developmental mechanics,[85,86] self-organization,[87] complexity, and chaos,[88,89,90,91] among others,—topics considered further in the following epigenetic antithesis.

Emergence. This phenomenon occurs in all natural hierarchies. It consists of the appearance, at each successively higher and structurally and/or operationally more complex level, of new attributes or properties, not present in the lower levels, whose existence or functions could not in any way be predicted, even from a complete knowledge of all of the attributes and properties of any or all of the preceding lower organizational levels.[92-94]

For example, full knowledge of all the attributes and properties of an osteocyte does not permit prediction of the attributes and properties of any type of bone tissue. And full knowledge of all attributes and properties of all constituent bone tissue types does not permit prediction of the form (size and shape), growth, or functions of a macroscopic "bone."

Emergence is not genomically controlled. Instead, the integrated activities of all the attributes in a given hierarchical level self-organize to produce the next higher level of complexity. In every real sense, biologic structures "build" themselves; that is, bones do not grow, they are grown. Epigenetic processes and mechanisms are regulatory (causal) of hierarchical organization and of emergence and self-organization.[95]

Causation. From this vast topic,[96] we consider only how the attributes of a given biologic structural level "cause" (control, regulate, determine) the attributes of the next higher level. For example, what causes osteogenesis on the ectofacial surface the left mandibular angular process of a given 14-year-old male? The genomic thesis holds that this process was predetermined; i.e, that individual's osteoblastic genome contained, at the moment of fertilization, all the information necessary to regulate where, when, for how long, in what direction, in what amount, and at what rates, bone formation and remodeling will occur in that individual, given the absence of disease and the presence of the usual and necessary extrinsic (environmental) factors, such as adequate nutrition, and the customary normal physiological states, such as are presumed to exist in physiology's hypothetical normal human.

The antithesis (and the FMH) suggests that epigenetic stimuli, created by operations of related functional matrices and their skeletal unit adaptive responses, create the "new" information sequentially, as mandibular ontogenesis proceeds.[9,10] All ontogenesis exhibits developmental "cascades," with multiple branching points where decisions are made between alternate developmental pathways. Such decisions are not predetermined by encoded genetic information, but instead are responses to some epigenetic stimulus(i). Hierarchy, emergence, and causation are topics of the greatest significance in any critique of the genomic hypothesis, because the scope and content of molecular genetics is precisely that; it deals with only the molecular level of structural organization. The genomic hypothesis proposes no pathways from molecules to morphogenesis.[30] Customarily, in craniofacial literature, the existence of two "facts" is stated: (1) that at the molecular level, a particular gene (or group of genes) exists and (2) that at some higher, macroscopic level, some clinical state of normal growth and development or of malformation and/or malfunction is observed. Without positing any specific mechanisms or processes at each intervening hierarchical level of the developmental cascade, it is simply stated that fact 1 is the cause of fact 2. For example, "it is demonstrated that synpolydactyly, an inherited human abnormality of the hands and feet, is *caused* [italics mine] by expansions of a polyalanine stretch in the amino-terminal region of HOXD13."[97]

In the genomic thesis morphogenesis is reduced to molecular synthesis.

The Classification of Causation[11]

There are four principal causes of ontogenesis: material (with what?), formal (by what rules?), efficient (how?), and final (why?). These may be categorized as either intrinsic (material and formal)

and extrinsic (efficient); final cause (teleology) is not considered further. Of importance, both material and formal causes are classified as prior causes, i.e., existing before the creation of some specific state or structure. Efficient cause is proximate; i.e., its operation immediately causes the creation of a new state or attribute. Material and formal causes are intrinsic because they reside within vital structure (either intracellularly or intercellularly); efficient causes are extrinsic—they represent the entire spectrum of epigenetic processes, mechanisms, and events capable of being imposed on vital structures.

In biology, material cause is represented by all the levels of cellular and intercellular materials, without reference to any specific structural (anatomical) arrangement. Formal cause is the genomic code, i.e., a series of "rules" or "laws." These act at the at the molecular level to regulate the initial creation of the constituents of material cause. Efficient cause(s) are the epigenetic factors, as defined above, whose actions immediately regulate the next developmental branching point.

A metaphor is helpful. Consider the use of a computer to prepare this manuscript. The material cause is the hardware: the computers, printers, disks, and papers. The formal cause is the software: a specific word processing program, both its apparent, user-friend form and, in reality, its ultimate expression in machine language code. No combination of hardware and software could ever write an article. Extrinsic, epigenetic input is required, i.e., the composition and input of the text itself. Both intrinsic causes must be present before (prior to) the textual input, whereas the extrinsic, epigenetic typing is immediately (i.e., proximately) followed by creation, on the hard disk, of the text itself.

Both prior (intrinsic) and proximate (extrinsic) causes are necessary causes; neither alone is a sufficient cause for the creation of this manuscript. Only the two integrated together furnish the necessary and sufficient cause.

In ontogenesis, genomic (intrinsic, prior) and epigenetic (extrinsic, proximate) factors are each a necessary cause, but neither alone is a sufficient cause. Only the interaction of both provides both the necessary and sufficient cause of morphogenesis.[11] This conclusion foreshadows the resolving synthesis of this dichotomy, presented in the companion article, which also contains the comprehensive bibliography.