The functional matrix hypothesis revisited. 1. The role of mechanotransduction

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The periodic incorporation of advances in the biomedical, bioengineering, and computer sciences allow the creation of increasingly more comprehensive revisions of the functional matrix hypothesis. Inclusion of two topics, (1) the mechanisms of cellular mechanotransduction, and (2) biologic network theory, permit this latest revision; presented here in two interrelated articles. In this first article, the several possible types of intracellular processes of mechanotransduction are described. These translate the informational content of a periosteal functional matrix stimulus into a skeletal unit (bone) cell signal. The correlation between the strengths of the endogenous electrical fields produced by muscle skeletal muscle activity, and those to which bone cells maximally respond are stressed. Further, a physical chain of macromolecular levers, connecting the extracellular matrix to the bone cell genome is described, suggesting another means of epigenetic regulation of the bone cell genome, including its phenotypic expression. (Am J Orthod Dentofac Orthop 1997;112:8-11.)

Introduction. This series of four articles is a cohesive and constructive perspective of “where we are now after all the dust has settled.” But, there is another important and I think key feature and that is a discussion of functional matrix-type studies (by different names, perhaps) in other biologic disciplines that otherwise we probably would quite unaware of.

In itself is a most noteworthy contribution, because most of us, in both the basic and clinical orthodontic sciences, are really not aware of advances in other relevant fields. We can learn! Then, at the end, there is a look at the future, and this goes conceptually beyond anything we presume to understand today. In all, Dr. Moss’s assessment of his own work as a revision is, I think, more of a scholarly elaboration, based on a broad quillwork of biologic understanding, now gleaned from a variety of other specialties.

There surely is room in our distinguished journal, which has a solid reputation for recognizing balance, for an introspective dissection of a biologic concept that has profound clinical meaning. When that concept is evaluated in the light of parallel biologic theory, uncovered from other diverse fields, it presents a perspective for orthodontic scholars available nowhere else.

There are countless Moss references on the functional matrix over the years. This is the one that will be referred to for decades to come, and the one graduate students now will discuss in their seminars.

One point I would have liked Dr. Moss to have addressed in greater depth in the final pages is how the functional matrix is involved in its own growth and development on how it is controlled. That is, how much genome and how do the provocative ideas of complexity and self-organization play into this?

Donald Elnow

This article is presented as a series of interrelated articles, of which this is the first. The second article contains both a comprehensive summary of this latest revision of the FMH as well as the reference list for both articles.

DEVELOPMENT OF THE FUNCTIONAL MATRIX HYPOTHESIS (FMH)

A decade’s study of the regulatory roles of intrinsic (genomic) and extrinsic (epigenetic) factors in cephalic growth evolved into the functional matrix hypothesis (FMH). This initial version, as augmented, and stressing epigenetic primacy (as defined in Moss and Herring), became peer-accepted as one explanatory paradigm.

Periodically, incorporation of advances in the biomedical, bioengineering, and computer sciences have created more comprehensively explanatory FMH versions. And recent work on two topics, cellular transduction of informational signals and
biologic cellular network theory, permit the presentation of this latest revision. 7,10

THE CONCEPTUAL AND ANATOMIC BASES OF THE REVISED FMH

A comprehensible revision of the FMH should indicate (a) those portions that are retained, extended or discarded, and (b) which prior deficiencies are now resolved.

Although the principal FMH concepts are either generally known or easily available, 7,11,13 three are of particular resonance for this revision.

The developmental origin of all cranial skeletal elements (e.g., skeletal units) and all their subsequent changes in size and shape (e.g., form) and location, as well as their maintenance in being, are always, without exception, secondary, compensatory, and mechanically obligatory responses to the temporally and operationally prior demands of their related cephalic nonskeletal cells, tissues, organs, and operational volumes (e.g., the functional matrices).

More precisely, the FMH claims that epigenetic, extraskeletal factors and processes are the prior, proximate, extrinsic, and primary cause of all adaptive, secondary responses of skeletal tissues and organs. 7 It follows that the responses of the skeletal unit (bone and cartilage) cells and tissues are not directly regulated by informational content of the intrinsic skeletal cell genome per se. Rather, this additional, extrinsic, epigenetic information is created by functional matrix operations.

The FMH postulates two types of functional matrices: periostea and capsular. 16,17 The former, typified by skeletal muscles, regulates the histologically observable active growth processes of skeletal tissue adaptation.

This new version deals only with the responses to periostea matrices. It now includes the molecular and cellular processes underlying the triad of active skeletal growth processes: deposition, resorption, and maintenance. Histologic studies of actively adapting osseous tissues demonstrate that (1) adjacent adaptational tissue surfaces simultaneously show deposition, resorption, and maintenance; (2) adaptation is a tissue process. Deposition and maintenance are functions of relatively large groups (cohorts, compartments) of homologous osteoblasts, never single cells; and (3) a sharp demarcation exists between adjacent cohorts of active, depository, and quiescent (resting) osteoblasts.

Constraints of the FMH

Initially, the FMH 7 provided only qualitative narrative descriptions of the biologic dynamics of cephalic growth, at the gross anatomic level, and it had two explanatory constraints: methodologic and hierarchical.

1. Methodologic constraint. Macroscopic measurements, which use the techniques of point mechanics and arbitrary reference frames, e.g., roentgenographic cephalometry, permitted only method-specific descriptions that cannot be structurally detailed. This constraint was removed by the continuum mechanics techniques of the finite element method (FEM) 6,18,22 and of the related macro and boundary element methods. 9,22

This penultimate FEM revision added objective, reference-frame-invariant, fine-grained, and conceptually integrated descriptions of the quantitative aspects of localized cephalic growth kinematics to the earlier qualitative (phenomenologic) descriptions of growth dynamics. 4,6,9

2. Hierarchical constraint. However, even that version's descriptions did not extend "downward" to processes at the cellular, subcellular, or molecular structural domains, or extend "upwards" to the multicellular processes by which bone tissues respond to lower level signals. All prior FMH versions were "suspended" or "sandwiched" as it were, between these two hierarchical levels.

Explicitly, the FMH could not describe either how extrinsic, epigenetic FM stimuli are transduced into regulatory signals by individual bone cells, or how individual cells communicate to produce coordinated multicellular responses.

At the lower cellular or molecular levels, another problem exists. Almost uniformly, experimental and theoretical studies of bone adaptation consider only the unicellular, unimolecular, or unigenomic levels. Accordingly, their results and derivative hypotheses generally are not extensible to higher multicellular, tissue, levels.

Consequently, in prior FMH versions, significant disjunctions exist between the descriptions at each of the several levels of bone organization. Such a hiatus is implicit in hierarchical theory in which the attributes of successively higher levels are not simply the sum of lower level attributes. Rather, at each higher level, new and more complex structural and operational attributes arise that cannot be predicted, even from a complete knowledge of those of the lower levels; e.g., the sum of all lower attributes (biophysical, biochemical, genonomic) of a bone cell cannot predict the higher attributes of a bone tissue.

At present, no unitary hypothesis provides a comprehensive, coherent and integrated description
of all the processes and mechanisms involved in bone growth, remodeling, adaptation, and maintenance at all structural levels. This newest FMH version, presented herein, transcends some hierarchical constraints and permits seamless descriptions at and between, the several levels of bone structure and operation from the genomic to the organ level. It does so by the inclusion of two complementary concepts: (1) that mechanotransduction occurs in single bone cells, and (2) that bone cells are computational elements that function multicellularly as a connected cellular network.

It is useful to present the database and derivative theories, supportive of the inclusion of these two concepts individually in a series of two coordinated articles: the first on mechanotransduction and the second on connected cellular networks.

Mechanotransduction

All vital cells are “irritable” or perturbed by and respond to alterations in their external environment. Mechanosensing processes enable a cell to sense and to respond to extrinsic loadings, a widespread biologic attribute, by using the processes of mechanoreception and of mechanotransduction. The former transmits an extracellular physical stimulus into a receptor cell; the latter transduces or transforms the stimulus’s energetic and/or informational content into an intracellular signal. Mechanotransduction is one type of cellular signal transduction. There are several mechanotransductive processes, for example, mechanoelectrical and mechanochemical. Whichever are used, bone adaptation requires the subsequent intercellular transmission of the transduced signals.

Osseous Mechanotransduction

Static and dynamic loadings are continuously applied to bone tissues, tending to deform both extracellular matrix and bone cells. When an appropriate stimulus parameter exceeds threshold values, the loaded tissue responds by the triad of bone cell adaptation processes. Both osteocytes and osteoblasts are competent for intracellular stimulus reception and transduction and for subsequent intercellular signal transmission. Osteoblasts directly regulate bone deposition and maintenance and indirectly regulate osteoclastic resorption.

Osseous mechanotransduction is unique in four ways: (1) Most other mechanosensory cells are cytophysiologically specialized, but bone cells are not; (2) one bone-loading stimulus can invoke three adaptation responses, whereas nonosseous processes generally evoke one; (3) osseous signal transmission is neural, whereas all other mechanosensational signals use some other neural pathways; and, (4) the evoked bone adaptational responses are confined within each “bone organ” independently, e.g., within a femur, so there is no necessary “interbone” or organismal involvement.

This process translates the information content of a periostal functional matrix stimulus into a skeletal unit cell signal, for example, it moves information hierarchically downward to the osteocytes. There are two, possibly complementary, skeletal cellular mechanotransductive processes: ion and mechanical.

Ionic or electrical processes. This involves some process of ionic transport through the bone cell (osteocyte) plasma membrane. There is a subsequent intercellular transmission of the created ionic or electrical signals that, in turn, are computed by the operation of an osseous connected cellular network (CCN), as described in the second article in this series. That network’s output regulates the multicellular bone cell responses.

Although no consensus agreement exists, osteocyte, ionic-mechanotransduction may involve several, possibly parallel, cellular processes.

Stretch-activated channels. Several types of deformation may occur in strained bone tissue. One of these involves the plasma membrane stretch-activated (S-A) ion channels, a structure found in bone cells, in many other cell types, and significantly in fibroblasts. When activated in strained osteocytes, they permit passage of a certain sized ion or set of ions, including K⁺, Ca²⁺, Na⁺, and Cs⁺. Such ion flow may, in turn, initiate intracellular electrical events, for example, bone cell S-A channels may modulate membrane potential as well as Ca²⁺ ion flux. Other bone cell mechanically stimulatory processes have been suggested.

Rough estimates of osteocyte mechanoreceptor strain sensitivity have been made, and the calculated values cover the morphogenetically significant strain range of 1000 to 3000 µε in the literature.

Electrical processes. These include several, nonexclusive mechanotransductive processes (e.g., electromechanical and electrophoretic), involving the plasma membrane and extracellular fluids. Electric field strength may also be a significant parameter.
The functional matrix hypothesis revisited. 2. The role of an osseous connected cellular network

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Intercellular gap junctions permit bone cells to intercellularly transmit, and subsequently process, periosseal functional matrix information, after its initial intracellular mechanotransduction. In addition, gap junctions, as electrical synapses, underlie the organization of bone tissue as a connected cellular network, and the fact that all bone adaptation processes are multicellular. The structural and operational characteristics of such biologic networks are outlined and their specific bone cell attributes described. Specifically, bone is "tuned" to the precise frequencies of skeletal muscle activity. The inclusion of the concepts and databases that are related to the intracellular and intercellular bone cell mechanisms and processes of mechanotransduction and the organization of bone as a biologic connected cellular network permit revision of the functional matrix hypothesis, which offers an explanatory chain, extending from the epigenetic event of muscle contraction hierarchically downward to the regulation of the bone cell genome. [Am J Orthod Dentofac Orthop 1997;112:221-6.]

The first article in this series considered the implications for the functional matrix hypothesis (FMH) of the ability of bone cells to carry out intracellular mechanosensation and transduction and intercellular communication. In this article, we will consider the implications for the FMH of the inclusion of connectionist network theory.

BONE AS AN OSSEOUS CONNECTED CELLULAR NETWORK (CCN)

All bone cells, except osteoclasts, are extensively interconnected by gap junctions,7,12 that form an osseous CCN.28,42 In these junctions, connexin 43 is the major protein.92 Each osteocyte, enclosed within its mineralized lacuna, has many (n = 1-80) cytoplasmic (canaliculi) processes, =15 μm long and arrayed three-dimensionally, that interconnect with similar processes of up to 12 neighboring cells. These processes lie within mineralized bone matrix channels (canaliculi). The small space between the cell process plasma membrane and the canaliculi wall is filled with a macromolecular complex.

Gap junctions are found where the plasma membranes of a pair of markedly overlapping canaliculi processes meet.53 In compact bone, the canaliculi cross "cement lines," and they form extensive communications between osteons and interstitial regions.62 Gap junctions also connect areolar osteocytes to periosseal and endosteal osteoblasts. All osteoblasts are similarly interconnected laterally. Vertically, gap junctions connect periosseal osteoblasts with preosteoblastic cells, and these, in turn, are similarly interconnected.92 Effectively, each CCN is a true syncytium.4,5,9,92 Bone cells are electrically active.5,58,85,9,13 In a very real sense, bone tissue is "hard-wired."9,92

In addition to permitting the intercellular transmission of ions and small molecules, gap junctions exhibit both electrical and fluorescent dye transmission.69 Gap junctions are electrical synapses, in contradistinction to innervational, chemical synapses, and, significantly, they permit bidirectional signal traffic, e.g., biochemical, ionic.

Mechanotransductively activated bone cells, e.g., osteocytes, can initiate membrane action potentials capable of transmission through interconnecting gap junctions. The primacy of ionic signals rather than secondary messengers is suggested here, because, although bone cell transduction may also produce small biochemical molecules that can pass through gap junctions, the time-course of mechanosensory processes is believed to be too rapid for the involvement of secondary messengers.25,31 (See Carvalho et al.12 for an opposite view.) A CCN is operationally analogous to an "artificial neural network," in which
each osteocyte probably senses uniquely different strain properties. Further, it is probable that each osteocyte is potentially able to transmit three different adaption signals, in three different directions—some stimulatory and some inhibitory. However, these processes have not yet been adequately modeled. The role of periosteal functional matrices: new insights.

The morphogenetic primacy of periosteal functional matrices on their skeletal units is consensually accepted. As a muscular demand alters, e.g., myectomy, myotomy, myectomy, exercise, hypertrophy, hyperplasia, atrophy, augmentation, or repositioning, the trial of active bone growth processes correspondingly adapts the form of its specifically related skeletal unit.

Presently excluding the stimulation of neural afferents in muscle, tendon, and periostium, extrinsic physical loadings tend to deform bone tissue and to invoke skeletal unit (bone) adaptive response processes. A classic example is the regulation of coroid process form by the temporalis muscle. The tension in the tendon of this contracted muscle, transmitted through intertwined periosteal fibers inserted into subjacent bone, deforms the loaded skeletal unit.

Although some periosteal osteoblasts may be directly stimulated, extant data suggest osteocyte primacy in mechanosensitive processes. Anatomically, bone cells are competent mechanoreceptors. Their three-dimensional array of extensive canalicular cell processes is architecture well-suited to sense deformation of the mineralized matrix.

Although no one mechanical parameter reliably predicts all bone adaptational or remodeling responses, strain probably plays the primary role and is a competent stimulus. The significant strain attribute may vary with specific conditions. These include: (a) loading category—bone responds best to dynamic rather static loading; (b) frequency-osteocytes may be physiologically “tuned” to the frequencies of muscle function; (c) tunings being analogous to those of specialized nonsensory sensory cells, e.g., auditory hair cells, and (d) magnitude—relatively small strains of 0.002 to 1000 με are morphogenetically competent.

Although it is reasonably presumed that mechanosensitive processes, of both the ionic and mechanical type, involve the plasma membrane of the osteocyte soma or canalicular processes, the receptive, and subsequent transductive, processes are neither well understood nor consensually agreed on.

Skeletal muscle contraction is a typical periodical functional matrix loading event, and frequency is one of its critical parameters. Although the fundamental frequency of contracting muscle is about 2 Hz, other strain-related harmonics of 15 to 40 Hz exist.

These higher-order frequencies, significantly related to bone adaptational responses, are...present within the [muscle contraction] strain energy spectra regardless of animal activity and implicate the dynamics of muscle contraction as the source of this energy band (italics mine).

Of particular significance to the FMH is the close similarity of muscle stimulus frequencies to bone tissue response frequencies.

MECHANOSENSORY TRANSDUCTION: A TENTATIVE SYNTHESIS

The previously mentioned data suggest that the ability of periosteal functional matrices to regulate the adaptive responses of their skeletal units by ionic mechanotransductive processes is related to several factors. These are that (a) normal muscle function strains attached bone tissue intermittently; (b) the dynamics of skeletal muscle contraction fit rather nicely with the energetic requirements for bone cell responsiveness; (c) the range of specific strain-sensitive harmonics of muscle dynamics are also those found to be morphogenetically competent (i.e., osteogenetically); (d) normal skeletal muscle activity produces intracortical electric fields on the order of extrinsic fields found to be similarly morphogenetic; and, (e) bone cells may be stimulated by two mechanisms—directly by strain-activated plasma membrane channels and indirectly by electrokineetic phenomena.

These factors strongly suggest a rather precise matching of significant operational characteristics between a contracting skeletal muscle stimulus and the ability of loaded bone cells to transduce this into signals capable of regulating their adaptive responses. In a phrase, bone appears to be closely “tuned” to skeletal muscle, i.e., skeletal units are tuned to their periosteal functional matrices.

When both the ionic membrane and the mechanical (molecular level) transductive processes are conceptually and operationally combined with the data of both electric field effects and of contraction frequency energetics, they provide a logically sufficient biophysical basis of support for the hypothesis of epigenetic regulation of skeletal tissue adaptation.

In reality, it is probable that the ionic (electrical)
and mechanical (molecular level) transduction processes in osteocytes are neither exhaustive nor mutually exclusive. While using different intermediate membrane mechanisms or processes, they share a common final common pathway, i.e., they eventually produce signals regulating osteoclastic activity. Certainly in the ionic processes, and possibly in the molecular level system mechanism, the transduction process(es) also cause a transplasma membrane ionic flow(s), creating a signal(s) capable of intercellular transmission to neighboring bone cells through gap junctions, and then subsequent biologic computations in an osseseous CCN.

CONCLUSION

Where the original FMD version offered only verbal descriptions of pericellular matrix function and skeletal unit response, the addition to the FMD of the effects of mechanotransduction and of computational bone biology offers an explanatory chain extending from the epigenetic event of skeletal muscle contraction, hierarchically downward, through the cellular and molecular levels to the bone cell genome, and then upward again, through histologic levels to the event of gross bone form adaptational changes. Analyzing size and shape changes by reference frame-invariant, finite element methods produces a more comprehensive and integrative description of the totality of the processes of epigenetic regulation of bone form than previously possible.

REFERENCES

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 osteomorphogenetic 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 Dentofacial 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."

"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."

The initial version of the functional matrix hypothesis (FMH) claiming epigenetic control of morphogenesis, was based on macroscopic (gross) experimental, comparative, and clinical data. Recently revised, 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."

The epigenetic position of the FMH may seem quixotic when molecular genetics is the premier osteomorphogenetic research paradigm. Indeed, most clinicians and experimentalists—there are exceptions—subscribe to the two epigraphs above, stated more succinctly as "genes make us, body and mind." Nevertheless, a continuing countercurrent of dissent claims morphogenesis is regulated (controlled, directed) by epigenetic mechanisms and processes. In addition, several new disciplines explicitly invoke epigenesis. 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 beencyclopedic, only selected relevant aspects of ontogeny (morphogenesis) and phylogeny (evolution) are considered here.

An Odontogenic Example of the Genonomic/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 that many rigid genomic control of odontogenesis, as reflected in the temporally sequential, and spatially restricted, expression of the genetically regulated production of specific molecules as exhibited, for example, in marine molar development.

Nevertheless, data exist strongly supportive of epigenetic regulation of odontogenesis. For exam-
pie, Chilid fish are polyphyletic (have continuously replacing dental sets) and can exhibit pronounced dental phenotypic plasticity. 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, mechanotransduces process by dental papilla cells, and (2) these signals control at least the temporal and spatial expression of genetic products related to the development of differential tooth form, such as size and shape.

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

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 latter 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. Succinctly, “all (phenotype) features are ultimately determined by the DNA sequence of the genome.”

In this thesis, morphogenesis is the predetermined reading-out of an intrinsic and inherited genomic organismal blueprint 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.

The genomic thesis originated with classical (chromosomal) Mendelian genetics. 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.

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

Recently, molecular (gene) genetics extended the claims of the thesis to the regulation of all aspects of ontogeny (i.e., “growth and development”). The mega-human genome project, called “the ultimate triumph of genetics,” 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 organism; and, (3) in a societal context, possibly lead to some type of neoneugenes.

Many human activities now are claimed to be genomically regulated: e.g., psychological behavior; personality; alcohol and drug abuse; chromobiliary cyclic behaviors; smoking, obesity, alcoholism, drug abuse, food-binging—indeed any attention-deficiency disorder, among many others. The further suggestion of genomic control of intelligence generates prodigious, biomedical controversy in the social sciences and politics. 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, a brief review is useful. The somatic cells of an individual metazoon inherit two classes of molecular information: (1) an identical diploid DNA and (2) the maternal cytoplasmic constituents of the egg: e.g., mitochondria, cytoskeleton, membrane. 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.” 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.153

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, amnioblasts 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.54 For some, such "disorders provide the model on which the program of medical genetics is built."59 In such cases 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, eventually 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 fibers, provides a corresponding skeletal tissue analogy. Here, alterations in the genetically 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 synthesis underlying the formation of such elemental (molecular) skeletal tissue "building blocks" does not substantiate the Putzrath 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.56-77

A characteristic article22 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 superfamilies. 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."71,72

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."73

Specific orthodontic implications of the genomic thesis include claims that "poorly coordinated-determined 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 dentoalveolar 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."72

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.78-91 Over time, many alternate, often differing, definitions appeared.24-25 Earlier, they were macroscopic in scale and considered only the extrinsic, extraorganismal environment, such as food, light, temperature, and radiation.59 Nineteenth century physiology added the intrinsic, intraorganismal "milieu interieur," 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 (intraorganismic) 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. While a genomic thesis claims that each higher level is achieved by the predetermined activity of the genomic information, an epigenetic thesis suggests that hierarchical complexity results from the functioning of epigenetic processes and mechanisms, as described in the disciplines of developmental mechanics, self-organization, complexity, and chaos, 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.

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 genonomically 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.

Causation. From this vast topic, 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 ectodermal 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. 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 what deals with only the molecular level of structural organization. The genomic hypothesis proposes no pathways from molecules to morphogenesis. 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 syndactyly, 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 NXXD13."

In the genomic thesis morphogenesis is reduced to molecular synthesis.

The Classification of Causation

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 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-friendly 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. This conclusion foreshadows the resolving synthesis of this dichotomy, presented in the companion article, which also contains the comprehensive bibliography.

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The functional matrix hypothesis revisited. 4. The epigenetic antithesis and the resolving synthesis

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In two interrelated articles, the current revision of the functional matrix hypothesis extends to a reconsideration of the relative roles of genic and of epigenetic processes and mechanisms in the regulation (control, causation) of craniofacial growth and development. The dialectical method was chosen to analyze this matter, because it explicitly provides for the fuller presentation of a genomic thesis, an epigenetic antithesis, and a resolving synthesis. The latter two are presented here, where the synthesis suggests that both genomic and epigenetic factors are necessary causes, that neither alone is also a sufficient cause, and that only the two, interacting together, furnish both the necessary and sufficient cause(s) of ontogenesis. This article also provides a comprehensive bibliography that introduces the several new, and still evolving, disciplines that may provide alternative viewpoints capable of resolving this continuing controversy; repetition of the present theoretical bases for the arguments on both sides of these questions seems nonproductive. In their place, it is suggested that the group of disciplines, broadly termed Complexity, would most likely amply repay deeper consideration and application in the study of ontogenesis. (Am J Orthod Dentofac Orthop 1997;112:410-7)

"...It is a fallacy that the genome, the totality of DNA molecules, is the main repository for developmental information; i.e. that there exists a genetic program, or blueprint, theoretically capable of creating an entire organism."98

Biological Mechanisms and Processes Defined

This article continues the dialectical analysis of the roles of genomic and epigenetic processes and mechanisms in the control of craniofacial growth and development. Previously a genomic thesis was outlined and several critical terms were defined.99 The dialectic process concludes here with an epigenetic antithesis and a resolving synthesis, following two additional definitions: (1) A process is a series of actions or operations that lead toward a particular result. (2) A mechanism is the fundamental physical or chemical process(es) involved in, or responsible for, an action, reaction, or other natural phenomenon.107 That is, mechanisms underlie processes. For example, loading a femur is an epigenetic process; the possible resultant modification(s) of bone cell DNA (for example by methylation101,102), or of chondrocytic DNA (for example as reflected in differential regulation of biosynthetic pathways103), are epigenetic mechanisms. Similarly, the specific steps of the activation and deactivation of appropriate portions of the bone cell genome, associated with the trio of possible osteoblastic responses to loading (deposition, resorption, or maintenance of bone tissue) are further examples of epigenetic mechanisms that control the genome. In this sense, the original versions of the functional matrix hypothesis (FMH) described only epigenetic processes,104 whereas recent revisions also described epigenetic mechanisms.105 The fundamental correctness of earlier FMH descriptions is supported by more recent research.106,107

The Epigenetic Antithesis

Some of the principal strengths of this antithesis come from precise definitions of what a gene is and is not. For example: (a) "gene. The unit of heredity; one or more nucleic acid sequences incorporating information necessary for the generation of a particular protein or RNA product"108; and, (b) "enough is known about the genetic machinery...[to know]...that this is virtually the only kind of information which polynucleotide molecules are inherently capable of containing; nothing there
at all about which proteins will be expressed in which cells at what time and in what quantities."

The genomic thesis is denied because it is both reductionist and molecular, that is, descriptions of the causation (control, regulation) of all hierarchically higher and structurally more complex morphogenetic processes are reduced to explanations of mechanisms at the molecular (DNA) level. For example, the genomic thesis of craniofacial ontogenesis passes directly from molecules to morphogenesis; directly from DNA molecules to adult gross morphology, ignoring the role(s) of the many epigenetic processes and mechanisms competent to control (regulate, cause) the large number of intervening, and increasingly more structurally complex, developmental stages particularly, and there are additional similarly reductionist views of odontogenesis.

The epigenetic antithesis, detailing both processes and mechanisms, is integrative, seeking to clarify the causal chain between genome and phenotype. Its goal is to identify and describe comprehensively the series of initiating biological processes and their related underlying (biochemical, biophysical) responsive mechanisms that are effective at each hierarchical level of increasing structural and operational complexity.

This article reviews some of the clinically significant epigenetic processes and mechanisms, existing at several organizational (structural, functional) levels, that regulate (direct, control, cause) cephalic and craniofacial (musculo-) skeletal morphogenesis.

Craniofacial Epigenetics

"Broadly speaking, epigenetics refers to the entire series of interactions among cells and cell products which leads to morphogenesis and differentiation. Thus all cranial development is epigenetic, by definition." This view is supported here, despite continued expressions of genomic regulation of craniofacial morphogenesis.

As previously noted, epigenetic factors include (1) all of the extrinsic, nonorganismal, macroenvironmental factors impinging on vital structures (for example, food, light, temperature), including mechanical loadings and electromagnetic fields, 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.

In terms of clinical orthodontics, and of the FMH, all therapy is applied epigenetics, and all appliances (and most other therapies) act as prothetic functional matrices. Clinical therapeutics includes a number of epigenetic processes, whose prior operations evoke a number of corresponding epigenetic mechanisms. These latter, in turn, underlie the observed processes of tissue adaptations by both skeletal units and functional matrices.

Epigenetic Processes and Mechanisms

In craniofacial morphogenesis, more is known presently about processes than about mechanisms. Despite this, it is no longer sufficient to note, for example, that otherwise undescribed epigenetic processes of "intrauterine environment" can regulate fetal mandibular growth. The future aim must be to elucidate the molecular, genomic, mechanisms whose activation underlies the adaptive growth processes of the mandibular functional cranial components (that is, of the mandibular skeletal units and their related functional matrices).

Loading

Many different epigenetic processes can evoke mechanisms capable of modifying DNA. At clinically significant structural levels, physical loading is unquestionably of the greatest importance. Among the numerous epigenetic factors influencing the vertebrate face is mechanical loading. It is useful to consider the epigenetic process of loading and some of the epigenetic mechanisms this process evokes.

*Loading per se.* Loads may be imposed at many structural levels. While clinical observations usually are macroscopic, the loadings act microscopically, at molecular and/or cellular levels. Loadings are able to regulate several alternative molecular (cellular) synthetic pathways (mechanisms) of many tissues, including bone, for example, the mechanical environment is important in maintaining the differentiated phenotype of bone cells. It should be noted that loading may be dynamic (for example, muscle contraction) or static (that is, gravity); and to be effective, loads may increase, decrease, or remain constant.

Mechanical loading is known to influence gene expression. Of clinical (and FMH) interest, extrinsic musculoskeletal loading can rapidly change (1) both articular cartilage intercellular molecular syntheses, and mineralization; and (2) osteoblastic (skeletal unit) gene expression. Epigenetic loading processes include gravitational variations that evoke unique mechanisms of molecular synthesis.
**Extracellular matrix deformation.** Musculoskeletal tissue loading inevitably deform an extracellular matrix (ECM) that is not developmentally inert. Rather, in several ways, ECM regulates the formation, development, and maintenance of its included cells that synthesize the ECM. Further, ECM can regulate multilcellular tissue morphogenesis and contribute to genomic regulation of its enclosed cells.

**Cell-shape changes.** Tissue loading can also alter cell shape. This inevitably deform intracellular constituents, including the cytoskeleton. The epigenetic process of changing cell shape involves the epigenetic mechanisms of mechanotransduction of biophysical forces into genomic and morphogenetically regulatory signals.

Cell-shape change processes can also activate several other epigenetic mechanisms, for example, stretch-activated ion channels in cartilage and other mechanically initiated cell-signaling mechanisms.

There is recent orthodontic interest in the cell-shape change of nonskeletal cells.

Cell-shape change may lead to nuclear shape deformation. This, in turn, is a mechanism that can directly cause (regulate) a consequent alteration of the mechanisms of genomic activity.

**Epigenetic cell signaling processes.** Several loading processes can regulate genomic expression. One, previously described, begins with cellular mechanoreception and mechanotransduction of the loading stimulus into an intercellular signal that undergoes parallel processing within a connected cellular network of bone cells. The details of cell-signalling are reviewed extensively elsewhere.

**Chains of intracellular molecular levers.** A second epigenetic cellular process begins with deformation of the ECM. This matrix has an epigenetic regulatory role in morphogenesis, by virtue of integrin molecules that physically interconnect the several molecular components of the intracellular (cytoskeletal) and the extracellular environment (for cartilage). While the form (size and shape) of the cytoskeleton may be physically controlled by a broad spectrum of loadings, it responds identically to all.

The epigenetic mechanism evoked consists of a physical array of intracellular macromolecular chains, acting as levers, extending from the cell membrane to multiple specific sites on each chromosome. The molecular chain acts as an information transfer system between the extracellular environment and the genome, transmitting signals generated by deformations of the ECM directly to the intranuclear genome. Indeed, such informational transfer between cells and ECM is dynamic, reciprocal, and continuous.

**Other processes and mechanisms.** (1) DNA methylation is a potent epigenetic event. It is involved in many intracellular, extracellular, and intercellular mechanisms. It can introduce novel features of cellular function far removed from the classical Mendelian view of the gene, chromosome, and inheritance...with information flowing back to the DNA level and changing gene expression, the genome now being considered as a sophisticated response system and a carrier of information, a system activated by several epigenetic processes and mechanisms.

(2) There are numerous examples of yet other processes and mechanisms of epigenetic regulation of the genome.

(3) In addition, it has been shown that (botanical) epigenetic factors can impose metastable inheritable changes in the plant genome, a nontrivial matter not considered further here.

**Epigenetic Regulation of Higher Structural Levels**

In addition to the molecular and cellular processes and mechanisms noted, over a century ago the discipline of developmental mechanics (entwicklungsmechanik) established that the epigenetic process of extrinsic loadings play a major role in the regulation of bone tissue and bone organ growth, development, and morphology.

At the tissue level, there are several causal, strain-specific differences in bone tissue microstructure. Closely similar epigenetic mechanisms and processes are observed in the adaptational responses of all connective tissues, including cartilage, to loading.

At the organ level, the ability of the processes of motion and of articular function to regulate joint morphology is well-known, and, of course, physical activity processes regulate ontogaskeletal adaptational responses. Other epigenetic processes affecting bone tissue include local vascular factors.

**Regulation of functional matrices.** Periosteal functional matrices are under closely similar epigenetic control. Mechanical loads regulate skeletal muscle (periosteal functional matrix) phenotype; and chronic muscle stimulation can change its phenotype. Numerous studies establish the neurotrophic role of neural innervation in muscle genome regulation. It remains only to note the truism that, for muscloskeletal epigenetic factors, broadly termed function (or ex-
Exercise significantly control musculoskeletal growth, development, and maintenance of structural and physiological attributes.

A Resolving Synthesis

"It seemed that the next minute they would discover a solution. Yet it was clear to both of them that the end was still far, far off, and that the hardest and most complicated part was only just beginning."—Anton Chekov, The Lady with the Dog.

As the epigraph indicates, it is certain that no matter what arguments, theoretical constructs, and supporting experimental data are presented here, the prevailing tension between the genomic thesis and epigenetic antithesis will continue unabated. Nevertheless, a resolving synthesis will at least clarify the bases for continued discourse.

The fundamental argument of this resolving synthesis, based on an analysis of causation, was presented earlier and later amplified. It argues that morphogenesis is regulated (controlled, caused) by the activity of both genomic and epigenetic processes and mechanisms. Both are necessary causes; neither alone are sufficient causes; and only their integrated activities provides the necessary and sufficient causes of growth and development. Genomic factors are considered as intrinsic and prior causes; epigenetic factors are considered as extrinsic and proximate causes. The data supporting this synthesis are provided here and above.

It is acknowledged that the validity of this dialectic synthesis is significantly dependent on the validity of its epigenetic antithesis. In turn, a defensible epigenetic antithesis should convincingly suggest some processes and/or mechanism(s) that can regulate (direct, control, cause) morphogenesis. It is argued here that these are provided by the newly emerging disciplines of complexity.

Complexity and self-organization

The theories of ontogeny and phylogeny currently are being significantly reinvigorated by the new and evolving science(s) of complexity that integrate topics from mathematics (for example, cellular automata, fractals, strange attractors), biology (for example, genetic algorithms, artificial life simulations, neural networks, emergence, adaptive systems, connectivity), and physics, while minimizing distinctions between them. Complexity theory (CT) also integrates specifically related topics in bioengineering and the computer sciences, for example, chaos, information, and hierarchical theories, fuzzy logic, as well as cytological mechanics and molecular (nano)mechanics.

Because epigenetic processes and mechanisms are best explained as examples of CT, a clearcut demonstration of the role of CT in craniofacial ontogeny, at some point, is both necessary and possible. But in this place only this brief, intuitive preview is possible. Because fairness to both the novelty and conceptual richness of CT requires a comprehensive presentation to make it generally intelligible, it will be substantively reviewed subsequently.

CT provides descriptions of the behavior of complex biological systems that exist as "ensembles" of several tissues and organs, and not as clusters of individual cells and extracellular substances. Such an ensemble (identical to a functional cranial component in the FMH) is termed here as a complex adaptive system (CAS), structurally arrayed as a vital continuum. This term is defined here as it is in the several analytical finite element methods (FFEM) recently introduced into orthodontics and physical anthropology.

CT provides compact, statistical descriptions of the collective growth behavior of such CAS complexes. During ontogeny, vital CAS exhibits the creation of robust, spontaneous, and emergent order.

An algorithm for control of such a CAS requires that it is able to alter itself in response to the (epigenetic) information produced by the system it is trying to control. In a CAS, minor changes in the epigenetic input can cause huge fluctuations in the morphological output.

CT, as it utilized information theory, assumed that a CAS processes information (both genomic and epigenetic) in a parallel, not a serial, manner. Where most previous biological theories of development were based on the methods of deterministic (genomically predetermined), classical mechanics, information theory, and CT, are probabilistic (epigenetically self-organized and emergent), and are based on the methods of statistical mechanics. It is probable that ontogeny involves nonlinear processes and is not fully predictable; that is, growth and development, to a significant extent, exhibit both random behaviors and frequent perturbations. To clarify this point, note that previous most biological models were studied as if they were linear. That is, when their mathematical formulas were graphed they looked like straight lines. Linear systems are predictable: the calculus shows the changes in their state, and statistics (especially regression analysis) reduces their data to a line. However, CT makes it clear that most biological systems are nonlinear and are not most correctly described by these mathematical techniques; nonlinear formulations are necessary.
The highly ordered morphological properties of adult complex biological systems (for example, functional matrices and skeletal units) result from the operation of a series of spontaneous and self-organized ontogenetic processes and mechanisms. Such emergent self-organizing events can create phenotypic variability under constant genetic and other extragenic and epigenetic conditions.

The operation of complexity can be suggested as follows. "Environmental factors thus play a decisive role in all ontogenetic processes. But it is the organism itself that, as an integrated system, dictates the nature of each and every developmental response... the living organism self-organizes on the basis of its own internal structuring, in continuous interaction with the environment in which it finds itself."[11]

CONCLUSIONS

Integration of pertinent advances in biomechanical and bioengineering permitted an ongoing revision of the functional matrix hypothesis. The first two articles in this series—by emphasizing the roles of a number of biophysical and biochemical factors in the regulation of morphogenesis, implicitly argued for the correctness of the fundamental epigenetic thrust of the FMM. However, because the conceptual tension between hypotheses suggesting the regulatory primacy of either genetic (genetic) or of epigenetic factors and/or processes in morphogenesis continues unabated, it seemed useful to reevaluate this nontrivial matter, using the dialectical method of presenting a thesis, an antithesis, and a resolving synthesis as illustrated in these two interrelated articles. I believe that the most appropriate conclusion permitted by the data bases at this time is to use the contemporary, manageable phrase... "it is a win-win situation." Again, using a popular phrase, genetic and epigenetic processes are "apples and pears." More correctly, they are examples of totally differing types of causation—genetic formal cause and epigenetic efficient cause. Individually both are necessary causes, but neither are sufficient causes alone. Together they provide both the necessary and sufficient causes for the control (regulation) of morphogenesis. Nevertheless, epigenetic processes and events are the immediately proximate causes of development, and as such they are the primary agencies. The fuller demonstration of exactly how epigenetic events carry out their roles will be considered elsewhere in the context of a review of the implications of complexity theory for the functional matrix hypothesis.

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