SPECIAL ARTICLE



# *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 be quite unaware of.* This 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 quiltword 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?

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0889-5406/97/$5.00 + 0 8/1/70662

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).[1] This initial version, as augmented,[2] and stressing epigenetic primacy (as defined in Moss[3] and Herring[4]), 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.[5,6] 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,[3-11,18] 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.[3] 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: periosteal 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 periosteal 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[1,2] 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)[6,19-21] 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[23]; e.g., the sum of all lower attributes (biophysical, biochemical, genomic) 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,[24-32] 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 *intra*cellular signal. Mechanotransduction[33] is one type of cellular signal transduction.[34-36] 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[37] and dynamic[38] 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.[39,40]

Osseous mechanotransduction is unique in four ways: (1) Most other mechanosensory cells are cytologically specialized, but bone cells are not; (2) one bone-loading stimulus can evoke three adaptational responses, whereas nonosseous processes generally evoke one; (3) osseous signal transmission is aneural, whereas all other mechanosensational signals use some afferent neural pathways[28,41]; 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 periosteal 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: ionic and mechanical.

*Ionic or electrical processes.* This involves some process(es) of ionic transport through the bone cell (osteocytic) 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.[10,42]

Although no consensual agreement exists, osteocytic, 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,[43-46] in many other cell types,[25] and significantly in fibroblasts.[47] When activated in strained osteocytes, they permit passage of a certain sized ion or set of ions, including $K^+$, $Ca^{2+}$, $Na^+$, and $Cs^+$.[46,48-50]

Such ionic flow may, in turn, initiate intracellular electrical events, for example, bone cell S-A channels may modulate membrane potential as well as $Ca^{2+}$ ion flux.[23,51] Other bone cell mechanically stimulatory processes have been suggested.[52]

Rough estimates of osteocytic mechanoreceptor strain sensitivity have been made,[10,53] and the calculated values cover the morphogenetically significant strain range of 1000 to 3000 $\mu\varepsilon$ in the literature.[54-56]

*Electrical processes.* These include several, nonexclusive mechanotransductive processes (e.g., electromechanical and electrokinetic), involving the plasma membrane and extracellular fluids. Electric field strength may also be a significant parameter.[57]

1. *Electromechanical.* As in most cells, the osteocytic plasma membrane contains voltage-activated ion channels, and transmembrane ion flow may be a significant osseous mechano-

transductive process.[58,59,60-62] It is also possible that such ionic flows generate osteocytic action potentials capable of transmission through gap junctions.[63]

2. *Electrokinetic.* Bound and unbound electric charges exist in bone tissue, many associated with the bone fluid(s) in the several osseous spaces or compartments.[42,64] It is generally agreed that electrical effects in fluid-filled bone are not piezoelectric, but rather of electrokinetic, that is, streaming potential (SP) origin.[42,65,66] The SP is a measure of the strain-generated potential (SGP) of convected electric charges in the fluid flow of deformed bone. The usually observed SPG of $\pm 2$ mV can initiate both osteogenesis and osteocytic action potentials.[66,67]
3. *Electric field strength.* Bone responds to exogenous electrical fields.[68] Although the extrinsic electrical parameter is unclear, field strength may play an important role.[69] A significant parallel exists between the parameters of these exogenous electrical fields[68,69] and the endogenous fields produced by muscle activity. Bone responds to exogenous electrical fields in an effective range of 1 to 10 μV/cm, strengths that are "*. . .on the order of those endogenously produced in bone tissue during normal (muscle) activity*"[70] (italics mine).

*Mechanical processes.* Although it is probable that the intracellular, transductive process discussed later does *not* initiate action potentials, it is an alternative means by which periosteal functional matrix activity may regulate hierarchically lower level bone cell genomic functions.

The mechanical properties of the extracellular matrix influence cell behavior.[71] Loaded mineralized bone matrix tissue is deformed or strained. Recent data indicate that a series of extracellular macromolecular mechanical levers exist, capable of transmitting information from the strained matrix to the bone cell nuclear membrane.

The basis of this mechanism is the physical continuity of the transmembrane molecule integrin. This molecule is connected extracellularly with the macromolecular collagen of the organic matrix and intracellularly with the cytoskekeletal actin. The molecules of the latter, in turn, are connected to the nuclear membrane, at which site the action of the mechanical lever chain previously noted initiates a subsequent series of intranuclear processes regulatory of genomic activity.[72-75] (See Shapiro et al.,[76] for vimentin, and Green[77] for a general discussion of biophysical transductions.)

It is suggested that such a cytoskeletal lever chain, connecting to the nuclear membrane, can provide a physical stimulus able to activate the osteocytic genome,[78] possibly by first stimulating the activity of such components as the cfos genes.[56,73,78-86]

It is by such an interconnected physical chain of molecular levers that periosteal functional matrix activity may regulate the genomic activity of its strained skeletal unit bone cells, including their phenotypic expression.

# *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, periosteal 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[87-91] that form an osseous CCN.[7,8,42] In these junctions, connexin 43 is the major protein.[92] Each osteocyte, enclosed within its mineralized lacuna, has many ($n$ ! 80) cytoplasmic (canalicular) 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 canalicular wall is filled macromolecular complexes.

Gap junctions are found where the plasma membranes of a pair of markedly overlapping canalicular processes meet.[93] In compact bone, the canaliculi cross "cement lines," and they form extensive communications between osteons and interstitial regions.[94] Gap junctions also connect superficial osteocytes to periosteal and endosteal osteoblasts. All osteoblasts are similarly interconnected laterally. Vertically, gap junctions connect periosteal osteoblasts with preosteoblastic cells, and these, in turn, are similarly interconnected.[95] Effectively, each CCN is a true syncytium.[87,91,93] Bone cells are electrically active.[7,58,85,95-101] In a very real sense, bone tissue is "hard-wired."[7,8,96]

In addition to permitting the intercellular transmission of ions and small molecules, gap junctions exhibit both electrical and fluorescent dye transmission.[63] Gap junctions are electrical synapses, in contradistinction to interneuronal, 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.[25,32] (See Carvalho et al.[102] for an opposite view.) A CCN is operationally analogous to an "artificial neural network," in which

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0889-5406/97/$5.00 + 0 8/1/70663

massively parallel or parallel-distributed signal processing occurs.[104-105] It computationally processes, in a multiprocessor network mode, the intercellular *signals* created by an electrical type of mechanotransduction of periosteal functional matrix *stimuli*. Subsequently the computed network output informational signals move hierarchically "upward" to regulate the skeletal unit adaptational *responses* of the osteoblasts.

Fortunately, the bases of connectionist theory are sufficiently secure to permit modeling of a biologically realistic osseous CCN.[106-110] It consists of a number of relatively simple, densely interconnected processing elements (bone cells), with many more interconnections than cells. It is useful that bone cells form a network because individual receptors cannot code unambiguously-only a population of cells can do so.[103]

In network theory, these cells are organized into "layers": an initial input, a final output, and one or more intermediate or "hidden" layers. Importantly, such networks need not be numerically complex to be operationally complex.[111] The operational processes are identical, in principle, for all bone cells in all layers. Regardless of the actual physiological stipulatory process, each cell in any layer may simultaneously receive several "weighted" inputs (stimuli). A weight is some quantitative attribute. In the initial layer, these represent the loadings. Within each cell independently, "... all the weighted inputs are then summed."[112] This sum is then compared, within the cell, against some liminal or threshold value. If this value is exceeded, an intracellular signal is generated, i.e., successful mechanotransduction occurs. This signal is then transmitted *identically* to all the "hidden" layer cells (adjacent osteocytes) to which each initial layer cell is connected by gap junctions (and there are many styles of connectivity). Next, similar processes of weighted signal summation, comparison, and transmission occur in these intermediate layers until the final layer cells (osteoblasts) are reached. The outputs of these anatomically superficial cells determines the site, rate, direction, magnitude, and duration of the specific adaptive response, i.e., deposition, resorption, and/or maintenance, of each cohort of osteoblasts.[113]

Information is not stored discretely in a CCN, as it is in a conventional, single CPU computer. Rather it is distributed across all or part of the network, and several types of information may be stored simultaneously. The instantaneous state of a CCN is a property of the state of all its cells and of all their connections. Accordingly, the informational representation of CCN is redundant, assuring that the network is fault or error tolerant, i.e, one or several inoperative cells causes little or no noticeable loss in network operations,[1-2] a matter of useful clinical significance.

The CCNs show oscillation, i.e., iterative reciprocal signaling (feedback) between layers. This attribute enables them to adjustively self-organize. This behavior is related to the fact that biologic CCNs are not preprogrammed; rather they learn by unsupervised or epigenetic "training,"[114] a process probably involving structural or conformational changes in the cytoskeleton.[83] The phenomena of both network "training" and "learning" are related to the suggested effects of the oscillatory nature of their strain history.[115] Accordingly, the structurally more complex network attributes and behavior of a CCN gradually or epigenetically self-organize and emerge during operation. These network attributes are not reducible, i.e., they are neither apparent nor predictable from a prior knowledge of the attributes of individual cells.

Gap junctions, permitting bidirectional flow of information, are the cytological basis for the oscillatory behavior of a CCN. All the osteoblasts of a cohort engaged in an identical adaptation process are interconnected by open gap junctions. The presence of sharp histological discontinuities between cohorts of phenotypically different osteoblasts is related to their ability to close gap junctions at the boundaries between such cohorts, and so prevent the flow of information.[116,117] Informational networks also can transmit inhibitory signals, a significant matter beyond present concerns.[118]

A skeletal CCN displays the following attributes: (1) Developmentally, it is an untrained self-organized, self-adapting and epigenetically regulated system. (2) Operationally, it is a stable, dynamic system that exhibits oscillatory behavior permitting feedback. It operates in a noisy, nonstationary environment, and probably uses useful and necessary inhibitory inputs. (3) Structurally, an osseous CCN is nonmodular, i.e., the variations in its organization permit discrete processing of differential signals. It is this attribute that permits the triad of histologic responses to a unitary loading event.

Certain simplifications exist in this article, as in most of the bone literature. It is assumed that bone cells are organized in only two dimensions, bone loadings occur only at discrete loci, and gradients of strain are not considered. However, biologic reality is otherwise. In a loaded three-dimensional bone volume, gradients of deformation must exist, and

each osteocyte probably senses uniquely different strain properties. Further, it is probable that each osteocyte is potentially able to transmit three different adaptational 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 insight.*

The morphogenetic primacy of periosteal functional matrices on their skeletal units is consensually accepted. As a muscular demand alters, e.g., myectomy, myotomy, neurectomy, exercise, hypertrophy, hyperplasia, atrophy, augmentation, or repositioning, the triad 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 periosteum, extrinsic physical loadings tend to deform bone tissue and to invoke skeletal unit (bone) adaptation responsive processes. A classic example is the regulation of coronoid process form by the temporalis muscle.[119] The tension in the tendon of this contracted muscle, transmitted through intertwined periosteal fibers inserted into subjacent bone, deforms the loaded skeletal unit.[120]

Although some periosteal osteoblasts may be directly stimulated,[121] extant data suggest osteocytic primacy in mechanosensory processes.[122] Anatomically, bone cells are competent mechanoreceptors. Their three-dimensional array of extensive canalicular cell processes is architecturally well-suited to sense deformation of the mineralized matrix.[123]

Although no one mechanical parameter reliably predicts all bone adaptational or remodeling responses,[124] *strain* probably plays the primary role[125-128] and is a competent stimulus.[51] The significant strain attribute may vary with specific conditions.[129] These include: (a) loading category-bone responds best to dynamic rather static loading[54]; (b) frequency-osteocytes may be physiologically "tuned" to the frequencies of muscle function,[130-132] tunings being analogous to those of specialized nonosseous sensory cells,[34,35] e.g., auditory hair cells; and (c) magnitude-relatively small microstrains ($\mu e$) (about $10^{-6}$ mm/mm), and strain magnitudes of $2000 \pm 1000$ $\mu e$, are morphogenetically competent.[55,56,129,133]

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

Skeletal muscle contraction is a typical periosteal functional matrix loading event,[13,14,16,120,134,135] 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 or activity *and implicate the dynamics of muscle contraction as the source of this energy band*" (italics mine).[68,132,136] Of particular significance to the FMH is the close similarity of muscle stimulus frequencies to bone tissue response frequencies.

## MECHANOTRANSDUCTION: 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-frequency harmonics of muscle dynamics are also those found to be morphogenetically competent (i.e., osteoregulatory); (d) normal skeletal muscle activity produces intraosseous 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 electrokinetic 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 lever) 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.[3,13,16-18,38,129,137]

In reality, it is probable that the ionic (electrical)

and mechanical (molecular lever) transductive processes in osteocytes are neither exhaustive nor mutually exclusive. While using differing intermediate membrane mechanisms or processes, they share a common final common pathway, i.e., they eventually produce signals regulatory of osteoblastic activity. Certainly in the ionic processes, and possibly in the molecular lever system mechanism, the transductive 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,[121] and then subsequent biologic computation in an osseous CCN.

## CONCLUSION

Where the original FMH version offered only verbal descriptions of periosteal matrix function and skeletal unit response, the addition to the FMH of the concepts 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 integrated description of the totality of the processes of epigenetic regulation of bone form than previously possible.

### REFERENCES

1. Moss ML. The functional matrix. In: Kraus B, Reidel R, editors. Vistas in orthodontics. Philadelphia: Lea and Febiger, 1962:85-98.
2. Moss ML. Twenty years of functional cranial analysis. Am J Orthod 1972;61:479-85.
3. Moss ML. Genetics, epigenetics and causation. Am J Orthod 1981;80:366-75.
4. Herring S. Epigenetic and functional influences on skull growth. In: Hanken J, Hall BK, editors. The skull I. Chicago: University of Chicago Press, 1993:153-206.
5. Moss ML. Integration of the functional matrix hypothesis and the finite element method: a new paradigm for the analysis of craniofacial growth. Le Journal de l'Odgewise 1987;15:7-94.
6. Moss ML. Finite element comparison of murine mandibular form differences. J Craniofac Genet Dev Biol 1988;8:3-20.
7. Moss ML. Bone as a connected cellular network: modeling and testing. Ann Biomed Eng 1991;19:17-9.
8. Moss ML. Alternate mechanisms of bone remodeling: their representation in a connected cellular network model. Ann Biomed Engineer 1991;19:655.
9. Moss ML. Advances in finite element modeling of orofacial growth: the integration of macroelement and boundary element methods with the functional matrix hypothesis. J Jpn Orthod Soc 1994;53:557-96.
10. Moss ML, Cowin SC. Mechanotransduction in bone. In: Lanza R, Langer R, Chick W, editors. Textbook of tissue engineering. New York: Springer Verlag, 1995 (in press).
11. Moss ML. Growth of the calvaria in the rat: the determination of osseous morphology. Am J Anat 1954;94:333-62.
12. Moss ML. A functional analysis of human mandibular growth. Am J Prosthet Dent 1960;10:1149-60.
13. Moss ML. The primacy of functional matrices in orofacial growth. Trans Br Soc Stud Orthod Dent Pract 1968;19:65-73.
14. Moss ML. Differential roles of the periosteal and capsular functional matrices in orofacial growth. Trans Eur Orthod Soc 1969;45:193-206.
15. Moss ML, Rankow R. The role of the functional matrix in mandibular growth. Angle Orthod 1968;38:95-103.
16. Moss ML, Salentijn L. The primary role of the functional matrices in facial growth. Am J Orthod 1969;55:566-77.
17. Moss ML, Salentijn L. The capsular matrix. Am J Orthod 1969;56:474-90.
18. Moss ML, Young R. A functional approach to craniology. Am J Phys Anthrop 1960;18:281-92.
19. Skalak R, Dasgupta G, Moss ML, Otten E, Dullemeijer P, Vilmann H. A conceptual framework for the analytical description of growth. J Theor Biol 1982;94:555-77.
20. Skalak R, Dasgupta G, Moss ML, Patel B, Sen K, Moss-Salentijn L. The application of the finite element method to the analysis of craniofacial growth and form. Am J Orthod 1985;87:452-72.
21. Moss ML, Moss-Salentijn L, Skalak R. Finite element modeling of craniofacial growth and development. In: Graber L, editor. Orthodontics: stepping stones to the future. St. Louis: CV Mosby, 1986:143-68.
22. McAlarney M, Dasgupta G, Moss ML, Moss-Salentijn L. Anatomical measurements in the study of craniofacial rat growth. J Craniofac Genet Dev Biol 1992;12:2-12.
23. Parter HH. Hierarchy theory: the challenge of complex systems. New York: G.Braziller, 1973.
24. Goldsmith P. Plant stems: a possible model system for the transduction of mechanical information in bone modeling. Bone 1994;15:249-60.
25. French AS. Mechanotransduction. Ann Rev Physiol 1992;54:135-52.
26. Kernan M, Cowan D, Zuker C. Genetic dissection of mechanoreception-defective mutations in Drosophila. Neuron 1994;2:1093-206.
27. Hamil, OP, McBride DW Jr. Mechanoreceptive membrane channels. Am Scientist 1995;83:30-7.
28. Hackney CM, Furness DN. Mechanotransduction in vertebrate hair cells: structure and function of the stereociliary bundle. Am J Physiol 1995;268:C1-13.
29. Fraser DJ, Macdonald AG. Crab hydrostatic pressure sensors. Nature 1994;371:383-4.
30. Olsson S, Hanson BS. Action potential-like activity found in fungal mycelia is sensitive to stimulation. Naturwissch 1995;82:30-1.
31. Cui C, Smith DO, Adler J. Characterization of mechanosensitive channels in Escherichia coli cytoplasmic cell membrane by whole-cell patch clamp recording. J Membr Biol 1995;144:31-42.
32. Wildren DC, Thain JF, Minchin P, Gubb I, Reilly A, Skipper Y, et al. Electrical signaling and systematic proteinase inhibitor induction in the wounded plant. Nature 1992;360:62-5.
33. Mayer EA, Sigra. transduction and inter-cellular communication. In: Walsh JH, Dockray GJ, editors. Gut peptides: biochemistry and physiology. New York: Raven Press, 1994:35-75.
34. Mantia J. Coding and processing of sensory information. In: Kandel ER, Schwartz JH, Jessel TM, editors. Principles of neural science. 3rd. ed. New York: Elsevier, 1991:339-40.
35. Martin J, Jessel TM. Modality coding in the somatic sensory system. In: Kandel ER, Schwartz JH, Jessel TM, editors. 3rd. ed. New York: Elsevier, 1991:341-52.
36. Wang N, Butler JP, Ingber DE. Mechanotransduction across the cell surface and through the cytoskeleton. Science 1993;260:1124-7.
37. Cleaver DE, Spooner BS. Impact of altered gravity on aspects of cell biology. Int Rev Cytol 1994;156:301-72.
38. van der Meulen MCH, Carter DR. Developmental mechanics determine long bone allometry. J Theor Biol 1995;172:323-7.
39. Martin TJ, Ng KW. Mechanisms by which cells of the osteoblastic lineage control osteoclast formation and activity. J Cell Biochem 1994;56:357-66.
40. Hill PA, Reynolds JJ, Meikle MC. Osteoblasts mediate insulin like growth factor-I and -II stimulation of osteoclast formation and function. Endocrinol 1995;136:124-31.
41. Moss-Salentijn L. The human tactile system. In: Nicholls HR, editor. Advanced tactile sensing for robotics. Chapter 4. Singapore: World Scientific Publishing, 1994.
42. Cowin SC, Moss-Salentijn L, Moss ML. Candidates for the mechanosensory system in bone. J Biomech Engineer 1991;113:191-7.
43. van der Laarse A, Ravesloot JH, Nelwelde PJ. Voltage, calcium and stretch activated ionic channels and intracellular calcium in bone cells. J Bone Miner Res 1992;7:S377-87.
44. Guggino ST, Talamousse D, Wagner JA, Snyder SH. Bone remodeling signaled by a dihydropyridine- and phenylalkylamine-sensitive calcium channel. Proc Nat Acad Sci 1989;86:2957-60.
45. Duncan R, Misler S. Voltage-activated and stretch activated $Ba^{2+}$ conducting channels in an osteoblast-like cell line (UMR 106). Fed Eur Biochem Soc 1989;251:197-201.
46. Keynes RD. The kinetics of voltage-gated ion channels. Q Rev Biophys 1994;27:339-41.
47. Stockbridge LL, French AS. Stretch-activated cation channels in human fibroblasts. J Biophys 1988;54:187-90.
48. Sachs F. Biophysics of mechanoreception. Membrane Biochem 1986;6:173-95.
49. Sachs F. Mechanical transduction in biological systems. CRC Rev Biomed Engineer 1988;16:141-69.
50. Sackin H. Mechanosensitive channels. Ann Rev Physio. 1995;57:333-53.
51. Hutter JJ, Hruska KA, Duncan RL. Human osteoblast like cells respond to

mechanical strain with increased bone matrix protein production independent of hormonal regulation. Endocrinol 1995;136:528-35.
52 Harrigan TP, Hamilton JJ. An analytical and numerical study of the stability of bone remodeling theories: dependence on microstructural stimulus. J Biomech 1992;25:477-88.
54. Lanyon LE. Functional strain as a determinant for bone remodeling. Calcif Tiss Int 1984;36:S56-S61.
54. Lanyon LE, Rubin CT. Static vs dynamic loads as an influence on bone remodeling. J Biomech 1984;17:897-905.
55. Rubin LE, Lanyon LE. Limb mechanisms as a function of speed and gait: a study of functional strains in the radius and tibia of horse and dog. J Exp Zool 1985;101:187-211.
56. Rubin LE, Lanyon LE. Regulation of bone formation by applied loads. J Bone Jt Surg 1984;66A:397-402.
57. McLeod KJ. Microelectric measurement of low frequency electric fields effects in cells and tissues. Bioelectromagnetics 1992;(suppl):161-78.
58. Chesnoy-Marchais D. Fritsch J. Voltage-gated sodium and calcium currents in rat osteoblasts. J Physiol 1988;398:291-311.
59. Massari R, Bingmann D, Korenstein R, Tetsch P. Membrane potential of rat calvaria bone cells: dependence on temperature. J Cell Physiol 1990;144:1-11.
60. Jan LY, Jan YN. Tracing the roots of ion channels. Cell 1992;69:715-8.
61. Ravesloot JH, van Horsen RJ, Ypey DL, Niweide PJ. High-conductance anion channels in embryonic chick osteogenic cells. J Bone Miner Res 1991;6:355-63.
62. Ferrier J, Grygorczyk C, Grygorczyk R, Kesthely A, Lanyon F, Xia SL. Ba$^{2}$-induced action potentials in osteoblastic cells. J Membrane Biol 1991;123:255-9.
63. Stehlmacher K, Brummer F, Dusing R, Bingmann D. Dye and electric coupling between osteoblasts-like cells in culture. Calcif Tissue Int 1993;53:63-66.
64. Weinbaum S, Cowin S, Zeng Y. A model for the excitation of osteocytes by mechanical loading induced bone fluid shear stresses. J Biomech 1994;27:339-60.
65. Cross D, Williams WS. Streaming potential and the electromechanical response of physiologically moist bone. J Biomech 1982;15:777-95.
66 Pollack SR, Salzstein R, Pienkowski D. Streaming potentials in fluid filled bone. Ferroelectrics. 1984;60:297-309.
67. Turner CH, Forwood MR, Otter MW. Mechanotransduction in bone: do bone cells act as sensors of fluid flow? FASEB J 1994;8:875-8.
68. McLeod KJ, Rubin CT. Frequency specific modulation of bone adaptation by induced electric fields. J Theor Biol 1992;154:335-96.
69. Brighton CT, Okereke E, Pollack S, Clark CC. In vitro bone-cell response to a capacitatively coupled electrical field: role of field strength, pulse pattern and duty cycle. Clin Orthop Rel Res 1992;285:255-62.
70. McLeod KJ, Donahue HJ, Levin PH, Fontaine M-A, Rubin CT. Electric fields modulate bone cell function in a density-dependent manner. J Bone Miner Res 1993;8:937-84.
71. Halliday NL, Tomasek JJ. Mechanical properties of the extracellular matrix influence fibronectin fibril assembly in vitro. Exp Cell Res 1995;217:109-17.
72. Clark EA, Brugge JS. Integrins and signal transduction pathways: the road taken. Science 1995;268:233-9.
73 Watanabe H, Miake K, Sasaki J. Immunohistochemical study of the cytoskeleton of osteoblasts in the rat calvaria. Acta Anat 1993;147:14-23.
74. Hughes DE, Salter DM, Dedhar S, Simpson R. Integrin expression in human bone. J Bone Miner Res 1993;8:527-33.
75. Richardsen A, Parsons JT. Signal transduction through integrins: a central role for focal adhesion. Bioessays 1993;15:229-36.
76. Shapiro F, Cahill C, Malatantis G, Nayak RC. Transmission electron microscopic demonstration of vimentin in rat osteoblast and osteocytic cell bodies and processes using the immunoblot technique. Anat Rec 1995;241:39-48.
77. Green PB. Connecting gene and hormone action to form, pattern and organogenesis: biophysical transductions. J Exp Botany 1994;45:1775-88(Special Issue).
78. Jones DB, Bingmann D. How do osteoblasts respond to mechanical stimulation? Cells Mater 1991;1:329-40.
79. Machwate M, Jullienne A, Moukhtar M, Marie PJ. Temporal variation of c-fos proto-oncogene expression during osteoblast differentiation and osteogenesis in developing rat bone. J Cell Biochem 1995;57:62-70.
80. Petrov AG, Usherwood PN. Mechanosensitivity of cell membranes: ion channels, lipid matrix and cytoskeleton. Eur Biophys J 1994;23:1-18.
81. Yamagishita M. Function of proteoglycans in the extracellular matrix. Acta Path Jpn 1993;43:283-93.
82. Oldberg Å-J. Extracellular matrix molecules and their receptors: an overview with special emphasis on periodontal tissues. Crit Rev Oral Biol Med 1991;2:323-53.
83. Dayhoff JE, Hameroff SR, Lahoz-Beltra R, Swenberg CE. Intracellular messenger systems in neuronal learning: adaptive models. Int Jt Conf Neural Networks 1992;1:73-8.
84 Ingber DE. The riddle of morphogenesis: a question of solution chemistry or molecular cell engineering. Cell 1993;72:1243-52.
85. Haskin C, Cameron I. Physiological levels of hydrostatic pressure alter morphology and organization of cytoskeleta and adhesion proteins in MG-63 osteosarcoma cells. Biochem Cell Biol 1995;71:27-35.
86. Sadoshima J, Takahashi T, Jahn L, Izumo S. Roles of mechano-sensitive ion channels, cytoskeleton and contractile activity in stretch-induced immediate-early gene expression and hypertrophy of cardiac myocytes. Proc Nat Acad Sci USA 1992;89:9905-9.
87. Bennett MVL, Goodenough DA. Gap junctions: electronic coupling and intercellular communication. Neurosci Res Prog Bull 1978;16:373-485.
88. Schirrmacher K, Schmitz J, Winterhager E, Traub O, Brummer F, Jones D, et al. Characterization of gap junctions between osteoblast-like cells in culture. Calcif Tiss Int 1992;51:285-90.
89. Jones SJ, Gray C, Sasaki H, Arora M, Boyde A, Gwynne R, et al. The incidence and size of gap junctions between bone cells in rat calvaria. Anat Embryol 1993;187:343-52.
90. Goodlie R, Green C. The incidence and size of gap junctions between bone cells in rat calvaria. Anat Embryol 1993;187:343-52.
91. Civitelli R. Cell-cell communication in bone. Calcif Tiss Int 1995;56:S29-33.
92. Minkoff R, Randles VR, Parker SB, Herzberg EL, Laing JG, Beyer JC. Gap junction proteins exhibit early and specific expression during intramembranous bone formation in the developing chick mandible. Anat Embryol 1994;190:231-41.
93. Rodan G. Introduction to bone biology. Bone 1992;13:S3-6.
94. Carter TA, Ashrafi SH, Weber DF. Canalicular communication in the cortices of human long bones. Anat Rec 1988;212:336-44.
95. Doty S. Cell-to-cell communication in bone tissue. In:Davidovitch Z, editor. The biological mechanisms of tooth eruption and root resorption. Birmingham: EBSCO Media, 1989.61-9.
96. Nowak R. Cells that fire together, wire together. J NIH Res 1992;4:60-4.
97. Bingmann D, Tetsch D and Fritsch J. Membranangeneschaften von Zellen aus Knochenexplantation. Z Zahnarztl Implantol 1989;6:277-81.
98. Bingmann D, Tetsch D, Fritsch J. Membrane properties of bone cells derived from calvaria of newborn rats (tissue culture). Pflugers Arch 1989;S412:R14.
99. Luckman A, Fritsch J, Babsan S. Short term effects of PTH on cultured rat osteoblasts: changes in membrane potential. Am J Physiol 1986;251:C483-90.
100. Ferrier J, Ward Kesthely AW, Hamblin F, Ross S. Further analysis of spontaneous membrane potential activity and hyperpolarization response to parathyroid hormone in osteoblast-like cells. J Cell Physiol. 1987;130:433-51.
101. Hohman EL, Elde RP, Rysavy JA, Einzig S, Gebhard RL. Innervation of periosteum and bone by sympathetic vasoactive intestine peptide-containing fibers. Science 1986;232:868-71.
102. Carvalho RS, Scott JE, Suga DM, Yen EH. Stimulation of signal transduction pathways in osteoblasts by mechanical strain potentiated by parathyroid hormone. J Bone Miner Res 1994;9:999-1011.
103. Edin BB, Trulsson M. Neural network analysis of the information content in population responses from human periodontal receptors. SPIE 1992;1710:257-6.
104. Denning PJ. Neural networks. Am Sci 1990;88:426-8.
105. Martino RL, Johnson CA, Suh EB, Trus BL, Yap TK. Parallel computing in biomedical research. Science 1994;265:902-8.
106. Dayhoff E. Neural network architecture. New York: van Nostrand Reinhold, 1990.
107. Zornetzer SF, Davis J, Lau C. An introduction to neural and electronic networks. San Diego: Academic Press, 1990.
108. Grossberg S. Neural networks and artificial intelligence. Cambridge: MIT Press, 1988.
109. Hinton GE, Anderson JA. Parallel models of associative memory. Hillsdale: Lawrence Erlbaum, 1989.
110. McClelland JL, Rumelhart DE. Parallel distributed processing. In: Psychological and biological models. 2 vol. Cambridge: MIT Press, 1987.
111. Kupferman I. Neural networks: they do not have to be complex to be complex. Behav Brain Sci 1992;15:767-8.
112. Wasserman PD. Neural computation. In: Theory and practice. New York: Nostrand Reichold, 1989.
113. Parfitt AM. Osteonal and hemi-osteonal remodeling: the spatial and temporal framework for signal traffic in adult human bone. J Cell Biochem 1994;55:273-86.
114. Fritzke B. Growing cell structures: a self-organizing network for unsupervised and supervised learning. Neural Networks 1994;7:1441-60.
115. Carter DR. The regulation of skeletal biology by mechanical stress histories. In: Skalak R, Fox CF, editors. Tissue engineering. New York: Alan R Liss, 1988:175-9.
116. Kaye F, Hodgins MB. Communication compartments in hair follicles and their implication in differentiation control. Development 1992;114:589-93.
117. Kodama R, Eguchi G. The loss of gap junctional cell-to-cell communication is coupled with dedifferentiation of retinal pigmented epithelial cells in the course of transdifferentiation into the lens. Int J Dev Biol. 1994;38:357-64.
118. Marotti G, Ferretti M, Muglia MA, Palumbo C, Palazzini S. A quantitative evaluation of osteoblast-osteocyte relationships on growing endosteal surface of rabbit tibiae. Bone 1992;13:363-8.
119. Horowitz SL, Shapiro HH. Modifications of mandibular architecture following removal of temporalis muscle in the rat. J Dent Res 1951;30:276-80.
120. Moss ML, Moss-Salentijn L. The muscle-bone interface: an analysis of a morphological boundary. Monograph 8. Craniofacial Series. Ann Arbor: Center for Human Growth and Development, University of Michigan;59-72.
121. Harrigan TP, Hamilton JJ. Bone strain sensation via transmembrane potential changes at surface osteoblasts: loading rate and microstructural implications. J Biomech 1993;26:185-200.

122. Aarden EM, Burger EH, Nijweide PJ. Function of osteocytes in bone. J Cell Biochem 1994;55:287-99.
123. Lanyon LE. Osteocytes, strain detection, bone modeling and remodeling. Calcif Tiss Int 1993;53:S102-6.
124. Brown TD, Pedersen DR, Gray ML, Brand RA, Rubin CT. Perioseal remodeling: a combined experimental and analytic approach. J Biomech 1990;23:893-905.
125. Cowin SC. Strain assessment by bone cells. In: Skalak R, Fox CF, editors. Tissue Engineering. New York: Alan R. Liss, 1988:181-7.
126. Cowin SC. Bone biomechanics. Boca Raton: CRC Press, 1989a.
127. Cowin SC. A resolution restriction for Wolff's law of trabecular architecture. Bull Hosp Jt Dis 1989;49:205-12.
128. Rubin LE, McLeod KJ, Bain SD. Functional strains and cortical bone adaptation: epigenetic assurances of skeletal integrity. J Biomech 1990;25:43-54.
129. Martin RB, Burr DB. Structure, function and adaptation of compact bone. New York: Raven Press, 1989.
130. McLeod KL, Rubin CT. The effect of low-frequency electrical fields on osteogenesis. J Bone Jt Surg 1992;74A:920-9.
131. Turner CH. Functional determinants of bone structure: beyond Wolff's law of bone transformation. Editorial. Bone 1992;13:403-9.
132. Rubin CT, Donahue HJ, Rubin JE, McLeod KJ. Optimization of electric field parameters for the control of bone remodeling: exploitation of an endogenous mechanism for the prevention of osteopenia. J Bone Miner Res 1993;8:S573-81.
133. Turner CH, Forwood MR, Rho J Y, Yoshikawa T. Mechanical loading thresholds for lamellar and woven bone formation. J Bone Miner Res 1994;9:87-97.
134. Moss ML. A theoretical analysis of the functional matrix. Acta Biotheor 1969;18: 195-202.
135. Moss ML. Functional cranial analysis of the mandibular angular cartilage in the rat. Angle Orthod 1969;39:209-14.
136. Rodriguez AA, Agre JC, Knudston ER, NG A. Acoustic myography compared to electromyography during isometric fatigue and recovery. Muscle Nerve 1995;18: 188-92.
37. Moss ML. The functional matrix hypothesis and epigenetics. In: Graber TM, editor. Physiologic principles of functional appliances. St Louis: CV Mosby, 1988:3-4.

# *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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0889-5406/97/$5.00 + 0 8/1/79952

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

SPECIAL ARTICLES

# *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 genomic 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 later 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.[100] 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 methylation[101,102]), or of chondrocytic DNA (for example as reflected in differential regulation of biosynthetic pathways[103]), 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,[1-8] whereas recent revisions also described epigenetic mechanisms.[9,10] The fundamental correctness of earlier FMH descriptions is supported by more recent research.[104,105]

## 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 peptide or RNA product"[106]; 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

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0889-5406/97/$5.00 + 0 8/1/79951

at all about which proteins will be expressed in which cells at what time and in what quantities."[98]

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[13,18] particularly, and there are additional similarly reductionist views of odontogenesis.[17,22,60,107,108]

The epigenetic antithesis, detailing both processes and mechanisms, is integrative,[109] 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.[110]

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,[15,19,20,111] despite continued expressions of genomic regulation of craniofacial morphogenesis.[13,14]

As previously noted,[99] epigenetic factors include (1) all of the extrinsic, extraorganismal, 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 occuring 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 prosthetic 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.[112] The future aim must be to elucidate the molecular, genomic, mechanisms[101] 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.[113-116] 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."[118] 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.[117] Loadings are able to regulate several alternative molecular (cellular) synthetic pathways (mechanisms) of many tissues, including bone[118]; for example, the mechanical environment is important in maintaining the differentiated phenotype of bone cells.[102] 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.[119,120] Of clinical (and FMH) interest, extrinsic musculoskeletal loading can rapidly change (1) both articular cartilage intercellular molecular syntheses[121] and mineralization[122]; and (2) osteoblastic (skeletal unit) gene expression.[123,124] Epigenetic loading processes include gravitational variations that evoke unique mechanisms of molecular synthesis.[125]

*Extracellular matrix deformation.* Musculoskeletal tissue loading inevitably deforms 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.[126-129] Further, ECM can regulate multicellular tissue morphogenesis[130] and contribute to genomic regulation of its enclosed cells.[131]

*Cell-shape changes.* Tissue loading can also alter cell shape. This inevitably deforms intracellular constitutents, including the cytoskeleton.[132-134] The epigenetic process of changing cell shape invokes the epigenetic mechanisms of mechanotransduction of biophysical forces into genomic and morphogenetically regulatory signals.[135-138]

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.[139-142] There is recent orthodontic interest in the cell-shape change of nonskeletal cells.[143]

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.[140]

*Epigenetic cell signalling 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.[9,10] The details of cell-signalling are reviewed extensively elsewhere.[144]

*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).[145,127,128,146-148] While the form (size and shape) of the cytoskeleton may be physically controlled by a broad spectrum of loadings,[133,149] it responds identically to all.[150]

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.[146] 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.[9,10] Indeed, such informational transfer between cells and ECM is dynamic, reciprocal, and continuous.[151]

*Other processes and mechanisms.* (1) DNA methylation is a potent epigenetic event. It is involved in many intracellular, extracellular, and intercellular mechanisms.[101] 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,"[152,153] the genome now being considered as a sophisticated response system and a carrier of information,[154] a system activated by several epigenetic processes and mechanisms.[155] (2) There are numerous examples of yet other processes and mechanisms of epigenetic regulation of the genome.[113,115,156-159] (3) In addition, it has been shown that (botanical) epigenetic factors can impose metastable inheritable changes in the plant genome,[160-163] 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 (entwicklingsmechanik)[85,86] 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.[116,164-167]

At the tissue level, there are several causal, strain-specific differences in bone tissue microstructure.[168-171] Closely similar epigenetic mechanisms and processes are observed in the adaptational responses of all connective tissues, including cartilage, to loading.[164,165,172-175]

At the organ level, the ability of the processes of motion and of articular function to regulate joint morphology is well-known[176-178]; and, of course, physical activity processes regulate oganismal skeletal adapational responses.[179] Other epigenetic processes affecting bone tissue include local vascular factors.[180]

*Regulation of functional matrices.* Periosteal functional matrices are under closely similar epigenetic control. Mechanical loads regulate skeletal muscle (periosteal functional matrix) phenotype[181]; and chronic muscle stimulation can change its phenotype.[182-184] Numermous studies establish the neurotrophic role of neural innervation in muscle genome regulation.[185-188] It remains only to note the truism that, for muscle as for bone, mechanical epigenetic factors, broadly termed function (or ex-

ercise) significantly control musculoskeletal growth, development,[187,189,190] and maintenance of structural and physiological attributes.[191-193]

### 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,[11] and later amplified.[99] 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.[99]

It is acknowledged that the validity of this dialetic synthesis is significantly dependent on the validity of its epigenetic antithesis. In turn, a defensible epigenetic antithesis should convincingly suggest some process(es) 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 cyto(tissue)mechanics and molecular (nano)mechanics.[194-212]

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 (FEM) recently introduced into orthodontics and physical anthropology.[213-221]

CT provides compact, statistical descriptions of the collective growth behavior of such CAS continuity. 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.[10] 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 previously 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.[194,200] Such emergent self-organizing events can create phenotypic variability under constant genetic and other extraorganisaml epigenetic conditions.[222]

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."[113]

## CONCLUSIONS

Integration of pertinent advances in biomedical 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 fundamentally epigenetic thrust of the FMH. However, because the conceptual tension between hypotheses suggesting the regulatory primacy of either genomic (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 managerial phrase . . . "it is a win-win situation." Again, using a popular phrase, genomic and epigenetic processes are "apples and pears" More correctly, they are examples of totally differing types of causation genomic 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.

### REFERENCES

1. Jacob F. The logic of Life. New York: Pantheon Books, 1973.
2. Kessler DS, Melton DA. Vertebrate embryonic induction: mesodermal and neural patterning. Science 1994;266:596-604.
3. Moss ML, Young R. A functional approach to craniology. Am J Phys Anthrop 1960;18:281-92.
4. Moss ML. The functional matrix. In: Kraus B, Reidel R, editors. Vistas in orthodontics. Philadelphia: Lea & Febiger, 1962:85-98.
5. Moss ML. The primacy of functional matrices in orofacial growth. Trans Br Soc Study Orthod and Dent Pract 1968;19:65-73.
6. Moss ML. Twenty years of functional cranial analysis. Am J Orthod 1972;61:479-85.
7. Moss ML, Salentijn L. The primary role of functional matrices in facial growth. Am J Orthod 1969a;55:566-77.
8. Moss ML, Salentijn L. The capsular matrix. Am J Orthod 1969b;56:474-90.
9. Moss ML. The functional matrix hypothesis revisited. 1. The role of mechanotransduction. Am J Orthod Dentofac Orthop 1997;111:8-11.
10. Moss ML. The functional matrix hypothesis revisited. 2. The role of an osseous connected cellular network. Am J Orthod Dentofac Orthop 1997;112:221-6.
11. Moss ML. Genetics, epigenetics, and causation. Am J Orthod 1981;80:366-75.
12. Johnston MC, Bronsky PT. Prenatal craniofacial development: new insights on normal and abnormal mechanisms. Crit Rev Oral Biol Med 1995;6:368-427.
13. Dixon MJ. Positional cloning of a gene involved in the pathogenesis of Treacher Collins syndrome. Nature Genetics 1996;12:130-6.
14. Winter RM. Waats in a face? Nature Genetics 1996;12:24-9.
15. Herring SW. Epigenetic and functional influences on skull growth. In: Hanken J, Hall BK, editors. The skull. Chicago: University of Chicago Press 1993a.:153-206.
16. Dawkins R. The selfish gene. Oxford: Oxford University Press, 1976.
17. Harold FM. Ionic and electrical dimensions of hyphal growth. In: Wessels JG, Meinhardt F, editors. The Mycota. Berlin: Springer Verlag 1994;1:185-197.
18. Harold FM. From morphogenes to morphogenesis. Microbiol 1995;141:2765-78.
19. Herring SW. Formation of the vertebrate face: epigenetic and functional influences. Am Zool 1993b;33:472-89.
20. Hall BK. Epigenetic control in development and evolution. In: Goodwin BC, Holder N, Wylie CG, editors. Development and evolution. Cambridge: Cambridge University Press, 1983:353-79.
21. Holliday R. DNA methylation and epigenetic mechanisms. Cell Biophys 1989;15:15-20.
22. Holliday R. Epigenetics: an overview. Dev Genet 1994;16:453-7.
23. Lewontin RC. The dream of the human genome. New York Review of Books 1992;39:31-40.
24. Lewontin RC. Biology as ideology. The doctrine of DNA. New York: Harper Collins, 1992.
25. Lewontin RC, Rose S, Kamin LJ. Not in our genes. New York: Pantheon Books, 1984.
26. Ho MW, Saunders PT. Beyond neo Darwinism an epigenetic approach to evolution. J Theor Biol 1979;78:573-91.
27. Lovtrup S. Epigenetics. A treatise on theoretical biology. London: John Wiley & Sons, 1974.
28. Bricn P. Le vivant: epigenese, evolution, epigenetique. Brussels: Ed. de l'Universite de Bruxelles, 1974.
29. Bleschschmidt E, Gasser RF. Biokinetics and biodynamics of human differentiation. Springfield: C. C. Thomas, 1978.
30. Corner MA. Reciprocity of structure-function relations in developing neural networks: the Odyssey of a self organizing brain through research fads, fallacies and prospects. Prog Brain Res 1994;102:3-31.
31. Jablonka E, Lamb MJ. Epigenetic inheritance and evolution. Oxford: Oxford University Press, 1995.
32. Holland JH. Hidden order. How adaptation builds complexity. Reading MA. Addison-Wesley, 1995.
33. Bonner JT. The evolution of complexity by means of natural selection. Princeton Princeton University Press, 1988.
34. Edelman GM. Topobiology: an introduction to molecular embryology. New York: Basic Books, 1988.
35. Gell-Mann M. The quark and the jaguar: adventures in the simple and the complex. New York: Freeman, 1994.
36. Holland JH. Adaptation in natural and artificial systems: an introductory analysis for adaptation in machine and nature. Amsterdam: North-Holland, 1992.
37. Waldrop MM. Complexity: the emerging science at the edge of order and chaos. New York: Simon & Schuster, 1992.
38. Bassingthwaighte JB, Liebovitch JS, West BJ. Fractal physiology. New York: Oxford University Press, 1994
39. Grcade L, Reuppel O. Interpreting the hierarchy of nature. San Diego: Academic Press, 1994.
40. Kauffman SA. The origins of order. New York: Oxford University Press, 1993.
41. Mitchell M. An introduction to genetic algorithms. Cambridge: MIT Press, 1996.
42. Langton CG, editor. Artificial life. An overview. Cambridge: MIT Press, 1995.
43. Salmivirata K, Ouellberg D, Hirsch E, Altruda F, Ekblom P. Integrin subunit expression associated with epithelial mesenchymal interactions during murine tooth development. Dev Dyn 1995;203:104-15.
44. Huysseune A. Phenotypic plasticity in the lower pharyngeal jaw dentition of *Astatotilapia ulindi* (Pisces: Cichlidae). Arch Oral Biol 1995;40:1069-74.
45. Meyer A. Ecological and evolutionary consequences of the trophic polymorphism in *Cichasoma citrinellum* (Pisces: Cichlidae) Biol J Linn Soc 1990;39:279-99.
46. Vandewalle P, Huysseune A, Aerts P, Verraes W. The pharyngeal apparatus in teleost feeding. In: Bels V, Chardon M, Vandewalle P, editors. Biomechanics of feeding invertebrates. Berlin: Springer Verlag, 1994:59-82.

47. Slavkin H, Editorial. Genetic and epigenetic challenges in tooth development. J Craniofac Genet Dev Biol 1988;8:193-8.
48. Lodish H, Baltimore D, Berk A, Zipursky SL, Matsudaira P, Darnel J. Molecular cell biology 3rd ed. New York: Sci Am Books, WH Freeman and Company, 1995.
49. Alberts B, Bray D, Lewis J, Raff M, Roberts, Watson JD. Molecular biology of the cell. 2nd ed. New York: Garland Pub., 1989.
50. Bishop JE, Waldholz M. The genome. The story of the most astonishing scientific adventure of our time. The attempt to map all the genes in the human body. New York: Simon & Schuster, 1992.
51. WT's C. Exons, introns, and talking genes: the science behind the human genome project. New York: Basic Books, 1992.
52. Cotran RS, Kumar V, Robbins SL. Pathologic basis of disease. Philadelphia: WB Saunders, 1994.
53. Darnell J, Lodish H, Baltimore D. Molecular cell biology. 2nd ed. New York: Sci Am Books, 1990.
54. Morgan TH. The theory of the gene. New Haven: Yale University Press, 1926.
55. Sinnot EW, Dunn LC. The principles of genetics. New York; McGraw-Hill Book Company, 1939.
56. Kevles DJ. In the name of eugenics: genetics and the uses of human heredity. New York: Knopf, 1985.
57. Gould S. The mismeasure of man. New York: WW Norton, 1981.
58. Mayr E, Provine WB. The evolutionary synthesis. Cambridge: Harvard University Press, 1981.
59. Davis J. Mapping the code: the human genome project and the choices of modern science. New York: John Wiley, 1992.
60. Olson MV. A time to sequence. Science 1995;270:394-6
61. Crystal RG. Transfer of genes to humans: early lessons and obstacles to success. Science 1995;270:404-9.
62. Mann CC. Behavioral genetics in transition. Science 1994;264:1686-9.
63. Bouchard TJ Jr. Genes, environment and personality. Science 1994;264:1700-1.
64. Crabbe JC, Belknap JK, Buck KJ. Genetic animal models of alcohol and drug abuse. Science 1994;264:1715-23.
65. Myers MP, Wager-Smith K, Wesley CS, Young WY, Segal A. Positional cloning and sequence analysis of the Drosophila clock gene, timeless . . . Science 1995; 270:805-8.
66. Blum K, Cull JG, Braverman ER, Comings DE. Reward deficiency syndrome. Am Sci 1996;84:132-45.
67. Herrnstein RJ, Murray C. The Bell curve. New York: The Free Press, 1994.
68. Reddi AH. Cartilage morphogenesis: the role of bone and cartilage morphogenetic proteins, homeobox genes and extracellular matrix. Matrix Biol 1994;14:599-606.
69. Atchley WR. Genetic and developmental aspects of variability in the mammalian mandible. 1993. In: Hanken J, Hall BK, editors. The skull. Chicago: University of Chicago Press, 1993:1-207-47.
70. Ignelzi MA, Lee Y-H, Maxson RE Jr, Sneed ML. Genetically engineered mice: tools to understand craniofacial development. Crit Rev Oral Biol Med 1995;6: 181-201.
71. Ishiguro K. Genetic study of mandibular prognathism by multivariate analysis. Jpn J Human Genet 1972;38:68-72.
72. Lovell DP, Johnson FM. Quantitative genetic variation in the skeleton of the mouse. I. Variation between inbred strains. Genet Res Camb 1983;42:169-82.
73. Festing M. A multivariate analysis of sibline divergence in the shape of the mandible in C57BL/Gr mice. Genet Res Camb 1973;21:121-32.
74. Bailey DW. Genes that affect the shape of the murine mandible. Congenic strain analysis. J Hered 1985;76:107-14.
75. Bailey DW. Genes that affect the shape of the murine mandible. Recombinant inbred strain analysis. J Hered 1986a;77:17-25.
76. Bailey DW. Mandibular morphogenesis gene linked to the H-2 complex in mice. J Craniofac Genet Dev Biol 1986b;2 (Suppl):533-9.
77. Lacombe D. Clinical dysmorphology beyond developmental genetics: recent advances in some human developmental genes. Int Rev Genet 1995;38:137-44.
78. Balme DM. 1992 Aristotle. De Partibus Animalium I and De Generatione Animalium I. Oxford: Clarendon Aristotle Series, Clarendon Press, 1992.
79. Boyd E. Origins of the study of human growth. Portland, OR: University of Oregon, School of Dentistry, 1980.
80. Ross WD, Smith JA, editors. Works of Aristotle. Oxford: Oxford University Press, 1908-12.
81. Singer C. A short history of anatomy and physiology from the Greeks to Harvey. 2nd ed. New York: Dover Publ, 1957.
82. Vogl C, Atchley WR, Cowley D, Cranshaw P, Murphy JD, Pomp D. The epigenetic influence of growth hormone on skeletal development. Growth Dev Aging 1993;57:163-82.
83. Scandalios JG, ed. Genomic responses to environmental stress. San Diego: Academic Press, 1990.
84. Bernard C. An introduction to the study of experimental medicine. London: Macmillan, 1865 (translation 1927).
85. Coulter SJ. Archives for developmental mechanics. W. Roux, editor (1894-1924). Roux's Arch Dev Biol 1994;204:99-107.
86. Churchill EB, Chabry, Roux and the experimental method in nineteenth century embryology. In: Giere RN, Westfall RS, editors. Foundations of the scientific method: the nineteenth century. Bloomington, Indiana University Press, 1973.
87. Kauffman S. At home in the universe. The search for the laws of self-organization and complexity. Oxford: Oxford University Press, 1995.
88. Kauffman S. At home in the universe. The search for the laws of self-organization and complexity. Oxford: Oxford University Press, 1995.
89. Waldrop MM. Complexity. The emerging science at the edge of order and chaos. New York: Simon & Schuster, 1992.
90. Lewin R. Complexity. Life at the edge of chaos. New York. Macmillan, 1992.
91. Holland JH. Hidden Order. How adaptation builds complexity. Reading MA: Addison-Wesley, 1995.
92. Teasta B, Kier LB. Complex systems in drug research. 1. The chemical levels. Complexity 1996;2(4):29-36.
93. Rose G, Siebler M. Cooperative effects of neuronal assemblies. Exp Brain Res 1995;106:109-16.
94. Hopfield JJ. Neural networks and physical systems with emergent computational abilities. Proc Natl Acad Sci USA 1982;79:2254-8.
95. Segel LA Grappling with complexity. Complexity 1995;1(2):18-25.
96. Wallace WA. Causality and scientific explanation. Ann Arbor: University of Michigan Press, 1974.
97. Muragaki Y, Mundlos S, Upton J, Olsen BR. Altered growth and branching patterns in synpolydactyly caused by mutations in HOXD13. Science 1996;272: 548-51.
98. Corner MA. Reciprocity of structure function relations in developing neural networks: the Odyssey of a self-organizing brain through research fads, fallacies and prospects. Prog Brain Res 1994;102:3-21.
99. Moss ML. The functional matrix hypothesis revisited. 3. The genomic thesis. Am J Orthod Dentofac Orthop 1992;102:538-42.
100. Gove PB, editor, Webster's seventh new collegiate dictionary. Springfield MA: G&C Merriam Co, 1970.
101. Hodgkin J. Epigenetics and the maintenance of gene activity states in *Caenorhabditis elegans*. Dev Genet 1994;15:471-77.
102. Rodolfsen J, Klein-Nulend J, Burger EH. Mechanical stimulation by intermittent hydrostatic compression promotes bone-specific gene expression in vitro. J Biomech 1995;28:1493-503.
103. Kim Y-J, Grodzinsky AJ, Plaas AHK. Compression of cartilage results in differential effects on biosynthetic pathways for aggrecan, link protein and hyaluronan. Arch Biochem Biophys 1996;328:331-40.
104. Civitelli R. Cell cell communication in bone. Calcif Tissue Int 1995;56(Suppl 1):S29-S31.
105. Duncan RL, Turner CH. Mechanotransduction and the functional response of bone to mechanical strain. Calcif Tissue Int 1995;57:344-58.
106. Meier AH, editor. A is for . . . gene. Sci Med 1996;3:72.
107. Vernon RB, Gage PH. Between molecules and morphology. Extracellular matrix and the creation of vascular form. Am J Pathol 1995;147:873-83.
108. Sasahara M, Tanizawa H, Kaneko Y, Fuse A, Sato K. Expression patterns of Raf-1 suggest multiple roles in tooth development. Calcif Tissue Int 1996;58:60-4.
109. Noble D, Boyd CAR. The challenge of integrative physiology. In: Noble D, Boyd CAR, editors. The logic of life. The challenge of integrative physiology. Oxford: Oxford University Press, 1993:1-15.
110. Green DG. Emergent behavior in biological systems. In: Green DG, Bossomaier TJ, editors. From biology to computation. Amsterdam: IOS Press, 1993:424-35.
111. Raff CB, Trinkhaus E, Walker A, Larsen CS. Posteranial robusticity in *Homo*. I. Temporal trends, mechanical interpretation. Am J Phys Anthropol 1995;91:21-53.
112. Sasaki Y, Nonaka K, Nakata M. The effects of four strains of recipients on the intrauterine growth of the mandible in mouse fetuses. J Craniofac Genet Dev Biol 1994;14:113-23.
113. Latham KE, McGrath J, Solter D. Mechanistic and developmental aspects of genetic imprinting in mammals. Int Rev Cytol 1995;160:53-98.
114. Jorgensen R. Developmental significance of epigenetic repositions on the plant genome: a paragenetic function for chromosomes. Dev Genet 1994;15:523-30.
115. Moehrle A, Paro R. Spreading the silence: epigenetic transcriptional regulation during *Drosophila* development. Dev Genet 1994;15:478-84.
116. Meinhardt H. Pattern formation and the activation of particular genes. In: Goldbeter A, editor. Cell cell signaling: from experiment to theoretical models. London: Academic Press, 1989.
117. Davies PF, Brand RA, editors. Cell mechanics. 1995. J Biomech 1995;28:1421-559.
118. Herring SW. Development of functional interactions between skeletal and muscular systems. In: Hall BK, editor. Bone. Boca Raton: Academic Press 1994;9:165-91.
119. Sandy JR, Farndale RW, Meikle MC. Recent advances in understanding mechanically induced bone remodeling and their relevance to orthodontic theory and practice. Am J Orthod Dentofac Orthop 1995;108:221-22.
120. Nebe B, Rychly J, Knopp A, Bober W. Mechanical induction of (beta)1 integrin-mediated calcium signaling in a hepatocyte cell line. Exp Cell Res 1995;218:479-84.
121. Visser NA, Vankampen GPJ, Dekoning MJMT, Vandenkroot JK. The effects of

loading on the synthesis of biglycan and decorin in intact mature articular cartilage in vitro. Connect Tissue Res 1994;30:241-50.
122. van't Veen SJ, Hagen JW, van Ginkel FC, Prahl-Andersen P, Burger EH. Intermittent compression stimulates cartilage mineralization. Bone 1995;17:461-5.
123. Raab-Cullen DM, Thiede DN, Petersen DN, Kimmel DB, Recker RR. Mechanical loading stimulates rapid changes in periosteal gene expression. Calcif Tissue Int 1994;55:473-8.
124. Stanford CM, Stevens JW, Brand RA. Cellular deformation reversibly depresses matrix detectable levels of bone-related mRNA. J Biomech 1995;28:1419-27.
125. Guignandon A, Vico L, Alexandre C, Lafage-Proust M-H. Shape changes of osteoblastic cells under gravitational variations during parabolic flight—relationship with $PGE_2$ synthesis. Cell Struct Funct 1995;20:369-75.
126. Rodan GA, Roder SB. The cells of bone. In: Riggs BL, Melton LJ, editors. Osteoporosis: etiology, diagnosis and management. 2nd ed. Philadelphia: Lippincott Raven Publ;1995:1-39.
127. Golombiek T, Dajee DR, Bezwada WR. Extracellular matrix interactions: I. Production of extracellular matrix with attachment and growth-sustaining functions by UWO2 ovarian cancer cells growing in protein-free conditions in vitro. Cell Dev Biol 1995;31:387-95.
128. Yamada KM, Miyamoto S. Integrin transmembrane signaling and cytoskeletal control. Curr Opin Cell Biol 1995;7:681-9.
129. Ekblom P. Extracellular matrix in animal development—an introduction. Experientia 1995;51:851-2.
130. Gumbiner BM. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell 1996;84:345-57.
131. Roskelley CD, Bissel MJ. Dynamic reciprocity revisited: a continuous, bidirectional flow of information between cells and the extracellular matrix regulates mammary epithelial cell function. Biochem Cell Biol. 1995;73:391-7.
132. Carvalho RS, Scott JE, Suga DM, Yen EHK. Stimulation of signal transduction pathways in osteoblasts by mechanical strain potentiated by parathyroid hormone. J Bone Miner Res 1994;9:999-1011.
133. Wang N, Ingber DE. Control of cytoskeletal mechanics by extracellular matrix, cell shape and mechanical tension. Biophys J 1994;66:2181-9.
134. Ivig M, Ceshek B, Muller A, Grainer M. Growth regulation by cell shape alteration and organization of the cytoskeleton. Europ J Cell Biol 1995;67:147-57.
135. Gordon R. Mechanical engineering of the cytoskeleton in developmental biology. Int Rev Cytol 1994;150.
136. Ingber DE, Dike L, Karp S, Lilley H. Cellular tensegrity: exploring how the mechanical changes in the cytoskeleton regulate cell growth, migration and tissue pattern during morphogenesis. Int Rev Cytol. 1994;150:173-228.
137. Belousov LV, Savaliev SV, Naumidi II, Novoselov VV. Mechanical stresses in embryonic tissues: patterns, morphogenetic role and involvement in regulatory feedback. Int Rev Cytol 1994;150:1-33.
138. Goodwin BC. What causes morphogenesis? Bioessays 1985;3:35-6.
139. Wright M, Jobanputra PM, Bavington C, Sauer DM, Nuki G. Effects of intermittent pressure-induced strain on the electrophysiology of cultured human chondrocytes: evidence for the presence of stretch activated membrane ion channels. Clin Sci 1996;90:61-71.
140. Matyas S, Edwards P, Minford A, Shrive N, Wilson J, Bray R, Frank C. Ligament tension affects nuclear shape in situ: an in vitro study. Connect Tissue Res 1994;31:45-53.
141. Duncan RL, Hruska KA. Chronic, intermittent loading alters mechanosensitive channel characteristics in osteoblast-like cells. Am J Physiol 1994;267:C1499-1n.
142. Gullick F, Ratcliffe A, Mow VC. Chondrocyte deformation and local tissue strain in articular cartilage: a confocal microscopy study. J Orthop Res 1995;13:410-21.
143. Norton LA, Andersen KL, Arenholt Bindslev D, Andersen L, Melsen B. A methodical study of shape change in human oral cells perturbed by a simulated orthodontic strain in vitro. Arch Oral Biol 1995;40:863-72.
144. Banes AJ, Tsuzaki M, Yamamoto J, Fischer T, Brigman B, Brown T, Miller L. Mechanoreception at the cellular level: the detection, interpretation, and diversity of responses to mechanical signals. Biochem Cell Biol 1995;73:349-65.
145. Holtrvall K, Camper L, Juhlinsson S, Kimura JH, Lundgren-Akerlund E. Chondrocyte and chondrosarcoma cell integrins with affinity for collagen type II and their response to mechanical stress. Exp Cell Res 1995;221:496-503.
146. Buck TT, Krystosek A. Role of the cytoskeleton in genomic regulation and cancer. Int Rev Cytol 1992;132:75-108.
147. Wilson E, Sudhir K, Ives HE. Mechanical strain of rat vascular smooth muscle cells is sensed by specific extracellular matrix/integrin interactions. J Clin Invest 1995;96:2364-72.
148. Cruchman JR, Woods A. Transmembrane signaling generated by cell-extracellular matrix interactions. Kidney Int 1995;47(Suppl 49):58-12.
149. Ingber DE. The riddle of morphogenesis: a question of solution chemistry molecular cell engineering? Cell 1993;75:1249-52.
150. Haskin C, Cameron I. Physiological levels of hydrostatic pressure alter morphology and organization of cytoskeletal and adhesion proteins in MG 63 osteosarcoma cells. Biochem Cell Biol 1993;71:27-35.
151. Roskelley CD, Srebrow A, Bissell MJ. A hierarchy of ECM mediated signalling regulates tissue-specific gene expression. Curr Opin Cell Bio. 1995;7:736-47.
152. Holliday R. Mechanisms for the control of gene activity during development. Biol. Rev 1990;65:431-71.
153. Holliday R. DNA methylation, epigenetic inheritance. Philos Trans R Soc Lond B Bio. Sci 1990;326:329-38.
154. Jablonka E, Lamb MJ. Epigenetic inheritance and evolution. Oxford: Oxford University Press, 1995.
155. Janousek B, Siroky J, Vyskot B. Epigenetic control of sexual phenotype in a dioecious plant, *Melandrium album*. Mol Gen Genet 1996;250:483-90.
156. Jathnin KE, Strain-specific differences in mouse oocytes and their contribution to epigenetic inheritance. Development 1994;120:3419-26.
157. Bestor TH, Chandler VL, Feinberg AP. Epigenetic effects in eukaryotic gene expression. Dev Genet 1994;15:458-62.
158. Wolpert L. Positional information and pattern formation in development. Dev Genet 1994;15:485-90.
159. MacLeod VC. A possible role in chemical carcinogenesis for epigenetic, heritable changes in gene expression. Mol Carcinog 1996;15:241-50.
160. Jorgensen R. Developmental significance of epigenetic impositions on the plant genome: a paragenetic function for chromosomes. Dev Genet 1994;15:523-32.
161. Mikula BC. Environmental programming of heritable epigenetic changes in paramutant r-gene expression using temperature and light at a specific stage of early development in maize seedlings. Genetics 1995;140:1379-87.
162. Clifton KH. Comments on the evidence in support of the epigenetic nature of radiogenic initiation. Mutat Res 1996;358:77-80.
163. McClintock B. The control of gene action in maize. Brookhaven Symp Biol 1966;18:162-84.
164. Biewener AA, Bertram JEA. Mechanical loading and bone growth in vivo. In: Hall BK, editor. Bone. Bone growth-B. Boca Raton: CRC Press, 1993;7:1-36.
165. Burger EH, Veldhuijzen JP. Influence of mechanical factors on bone formation, resorption and growth in vitro. In: Hall BK, editor. Bone. Bone growth B. Boca Raton: CRC Press, 1993;7:37-56.
166. Schaffer JL, Rizen M, L Italien G, Benbrahim A, Mcgerman J, Device for the application of a dynamic biaxially uniform and isotropic strain to a flexible cell culture membrane. J Orthop Res 1994;12:709-19.
167. Skedros JG, Mason MW, Bloebaum RD. Differences in osteonal micromorphology between tensile and compressive cortices of a bending skeletal system: indications of potential strain-specific differences in bone microstructure. Anat Rec 1994;239:405-13.
168. McMahon JM, Boyde A, Bromage TG. Pattern of collagen fiber orientation in ovine calcaneal shaft and its relation to locomotor-induced strain. Anat Rec 1995;242:147-58.
169. Ruspanti M, Guizzardi S, Strocchi R, Ruggeri A. Different fibrillar architectures coexisting in Haversian bone. Ital J Anat Embryol 1995;100(Supp. 1):103-12.
170. Petrtyl M, Hert J, Fiala P. Spatial organization of the haversian bone in man. J Biomech 1995;29:161-9.
171. Soltz J-K, Li Z, Wen L-Y. Dynamic behavior of a biphasic cartilage model under cyclic compressive loading. J Biomech 1995;28:357-64.
172. Kantomaa T, Tuominen M, Pirttiniemi P. Effect of mechanical forces on chondrocyte maturation and differentiation in the mandibular condyle of the rat. J Dent Res 1994;73:1750-5.
173. Roening O. Cartilage, function and craniofacial morphogenesis. Proc Finn Dent Soc 1991;87:231-7.
174. Visser NA, Vanlsamper GPJ, Dekonong MH, Vanderkorst JK. The effects of loading on the synthesis of biglycan and decorin in intact mature articular cartilage in vitro. Connt Tissue Res 1994;30:241-50.
175. Garcia AM, Black AC, Gray ML. Effects of physicochemical factors on the growth of the mandibular condyle in vitro. Calcif Tissue Int 1994;54:499-504.
176. Persson M. The role of movements in the development of sutural and diarthrodial joints tested by long term paralysis of chick embryos. J Anat 1983;137:591-9.
177. Barnanabane M. Whether mobility influences osteocentric features at the articular ends of the metacarpal bones. Acta Morphol Neerl Scand 1982;20:111-5.
178. Engelsma SO, Janssen HWB, Duterloo HS. An in-vivo transplantation study of the growth of the mandibular condyle in a functional position in the rat. Arch Oral Biol 1980;25:305-11.
179. Price JS, Jackson B, Pastell R, Russell RG, Lanyon LE. The response of the skeleton to physical training: a biochemical study in horses. Bone 1995;17:221-7.
180. Critchlow MA, Bland YS, Ashhurst DF. The effects of age on the response of rabbit periosteal osteoprogenitor cells to exogenous transforming growth factor-beta2. J Cell Sci 1994;107:499-516.
181. Voytik SL, Przyborski M, Badlak SF, Konieczny S. Differential expression of muscle regulatory factor genes in normal and denervated adult rat hindlimb muscles. Dev Dyn 1993;198:214-24.
182. Jarvis JC, Sutherland H, Mayne CN, Gilroy SJ, Salmons S. Induction of fast-oxidative phenotype by chronic muscle stimulation: mechanical and biochemical studies. Am J Physiol 1996;270:C300-12.
183. Mayne CN, Sutherland H, Jarvis JC, Gilroy SJ, Chavan AJ, Salmons S. Induction of a fast oxidative phenotype by chronic muscle stimulation: histochemical and metabolic studies. Am J Physiol 1996;270:C313-20.
184. Renaud T, Fontaine-Perus J, Garfinhaut MF. Seasonal variation in the phenotype of the adult ferret cremaster muscle. Experientia 1996;52:184-7.

185. Moss ML. An introduction to the neurobiology of orofacial growth. Acta Biotheor 1973;22:256-59.
186. Moss ML. Neurotrophic regulation of craniofacial growth. In: McNamara JA, editor. Control mechanisms of craniofacial growth. Ann Arbor: University of Michigan Press, 1975: Monogr 3: 75-90.
187. Walker DW, Luff AR. Functional development of fetal limb muscles: a review of the roles of activity, nerves and hormones. Reprod Fertil Dev 1995;7:391-8.
188. Grinnell AD. Dynamics of nerve-muscle interaction in developing and mature neuromuscular junctions. Physiol Rev 1995;75:789-834.
189. Cooper DM. Evidence for the mechanisms of exercise modulation of growth—an overview. Med Sci Sports Exerc 1994;26:733-40.
190. Rafuse VF, Landmesser LT. Contractile activity regulates isoform expression and polysialylation of NCAM in cultured myotubes: involvement of $Ca^{2+}$ and protein kinase C. J Cell Biol 1996;132:969-83.
191. Kawakami Y, Abe T, Kuno S Y, Fukunaga T. Training-induced changes in muscle architecture and specific tension. Eur J Appl Physiol 1995;72:37-43.
192. Duchateau J. Bed rest induces neural and contractile adaptations in triceps surae. Med Sci Sports Exerc 1995;27:1581-9.
193. Kannus P, Sievanen H, Vuori I. Physical loading, exercise and bone. Bone. 1996;18(Suppl):1S-3S.
194. Hess B, Mikhailov A. Self-organization in living cells. Science 1994;264:223-4.
195. Boyd CAR, Noble D, editors. The logic of life. The challenge of integrative physiology. Oxford: Oxford University Press, 1993
196. Bak P, Paczuski M. Complexity, contingency and criticality. Proc Natl Acad Sci USA 1995;92:6689-96.
197. Guastiar R, Shapiro B, Wagner P. Complex systems, complexity measures, grammimers and model inferring. Chaos Solitons and Fractals, 1996;5:635-51.
198. Shea MC. Complexity and evolution: what everybody knows. Bio. Phil 1991;6: 302-24.
199. Cast. JL. Connectivity, complexity and catastrophy. New York: John Wiley, 1979.
200. Stein AA. Self organization in biological systems as a result of interaction between active and passive mechanical stresses. mathematical model. In: Akkas N, editor. Biomechanics of active movement and division of cells. Berlin: Springer Verlag, 1994: NATO ASI Series 484;459-64.
201. Mendel JM. Fuzzy logic systems for engineering: a tutorial. Proc IEEE 1995;83: 345-77.
202. Corbit JD, Garbary DJ. Fractal dimension as a quantitative measure of complexity in plant development. Proc R Soc Lond B Biol Sci 1995;262:1-6.
203. Yates FE. Order and complexity in dynamical systems—homodynamics as a generalised mechanics for biology. Math Comput Model 1994;19:49-74.
204. Crutchfield JP. Observing complexity and the complexity of observation. In: Atmanspracher H, editor. Inside versus outside. Berlin: Springer Verlag, 1993:235-72.
205. Haken H. Information and self-organization: a macroscopic approach to complex systems. Berlin: Springer Verlag, 1988.
206. Badii R. Complexity and unpredictable scaling of hierarchical structures. In: Bountis T, editor. Chaotic dynamics, theory and practice. New York: Plenum Press, 1994.
207. Grassberger P. Information and complexity measures in dynamical systems. In: Atmanspracher H, Scheingraber H, editors. Information dynamics. New York: Plenum Press, 1991.
208. Gell-Mann M. The Quark and the Jaguar—adventures in the simple and the complex. London: Little, Brown & Co. 1994.
209. Bonner JT. The evolution of complexity. Princeton: Princeton University Press, 1988.
210. Crutchfield JP. Is anything ever new? Considering emergence. In: Cowan G, Pines D, Melzner D, editors. Complexity: metaphors, models and reality. Redwood City: Addison-Wesley, 1994:479-97.
211. Mitchell M, Hraber PT, Crutchfield JP. Revisiting the edge of chaos: evolving cellular automata to perform computations. Complex Systems 1993;7:89-130.
212. Sipper M. Studying artificial life using a simple, general cellular model. Artif Life J 1995;2:1-35.
213. Moss ML. Finite element method comparison of murine mandibular form differences. J Craniofac Genet Dev Biol 1988;8:3-20.
214. Moss, ML, Skalak R, Patel H, Sen K, Moss-Salentijn L, Shinozuka M, Vilmann H. Finite element modeling of craniofacial growth. Am J Orthod 1985;87:453-72.
215. Cheverud JM, Hartman SE, Richtsmeier JT, Atchley WR. A quantitative genetic analysis of localized morphology in mandibles of inbred mice using finite element scaling analysis. J Craniofac Genet Dev Biol 1991;11:122-37.
216. McGuinness N, Wilson AN, Jones M, Middleton J, Robertson NR. Stresses induced by edgewise appliances in the periodontal ligament—a finite element study. Angle Orthod 1992;62:15-22.
217. Hart RT, Hennebel VV, Thongpreda N, VanBuskirk WC, Anderson RC. Modeling the biomechanics of the mandible: a three dimensional finite element study. J Biomech 1992;25:261-86.
218. Lele S, Richtsmeier JT. On comparing biological shapes: detection of influential landmarks. Am J Phys Anthropol 1992;87:49-65.
219. Fine MB, Lavelle CLB. Diagnosis of skeletal form on the lateral cephalogram with a finite element based expert system. Am J Orthod Dentofac Orthop 1992;101:318-29.
220. Richtsmeier JT. Comparative study of frontal, Crozon and Apert craniofacial morphology using finite element scaling analysis. Am J Phys Anthropol 1987;74: 473-93.
221. Korioth TWP, Romilly DP, Hannam AG. Three dimensional finite element stress analysis of the dentate human mandible. Am J Phys Anthropol 1992;88:69-96.
222. Molenaar PCM, Boomsma DI, Dolan CV. A third source of developmental differences. Behav Genet 1993;23:519-24.