

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

Melvin L. Moss, DDS, PhD

New York, N.Y.

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?

Donald Enlow

**T**his 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).<sup>1</sup> This initial version, as augmented,<sup>2</sup> and stressing epigenetic primacy (as defined in Moss<sup>3</sup> and Herring<sup>4</sup>), 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.<sup>5,6</sup> And recent work on two topics, cellular transduction of informational signals and

From the Department of Anatomy and Cell Biology, College of Physicians and Surgeons, and School of Dental and Oral Surgery, Columbia University.

Reprint requests to: Prof. Emeritus Melvin L. Moss, Department of Anatomy and Cell Biology, Columbia University, 630 W. 168th St., New York, NY 10032. e-mail: moss@cucers1.civil.columbia.edu

Copyright © 1997 by the American Association of Orthodontists.

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

biologic cellular network theory, permit the presentation of this latest revision.<sup>7-10</sup>

### 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,<sup>1,11-18</sup> 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.<sup>3</sup> 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.<sup>16,17</sup> 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<sup>1,2</sup> 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)<sup>6,19-21</sup> and of the related macro and boundary element methods.<sup>9,22</sup>

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*.<sup>4,6,9</sup>

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<sup>23</sup>; 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,<sup>24-32</sup> by using the processes of mechanoreception and of mechanotransduction. The former transmits an extracellular physical stimulus into a receptor cell; the latter transduces or transforms the stimulus’s energetic and/or informational content into an *intracellular* signal. Mechanotransduction<sup>33</sup> is one type of cellular signal transduction.<sup>34-36</sup> There are several mechanotransductive processes, for example, mechano-electrical and mechano-chemical. Whichever are used, bone adaptation requires the subsequent intercellular transmission of the transduced signals.

### Osseous Mechanotransduction

Static<sup>37</sup> and dynamic<sup>38</sup> 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.<sup>39,40</sup>

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<sup>28,41</sup>; and, (4) the evoked bone adaptational responses are confined within each “bone organ” independently, e.g., within a femur, so there is no necessary “inter-bone” 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.<sup>10,42</sup>

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,<sup>43-46</sup> in many other cell types,<sup>25</sup> and significantly in fibroblasts.<sup>47</sup> When activated in strained osteocytes, they permit passage of a certain sized ion or set of ions, including  $K^+$ ,  $Ca^{2+}$ ,  $Na^+$ , and  $Cs^+$ .<sup>46,48-50</sup>

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.<sup>25,51</sup> Other bone cell mechanically stimulatory processes have been suggested.<sup>52</sup>

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

*Electrical processes.* These include several, non-exclusive mechanotransductive processes (e.g., electromechanical and electrokinetic), involving the plasma membrane and extracellular fluids. Electric field strength may also be a significant parameter.<sup>57</sup>

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.<sup>58,59,60-62</sup> It is also possible that such ionic flows generate osteocytic action potentials capable of transmission through gap junctions.<sup>63</sup>

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.<sup>42,64</sup> It is generally agreed that electrical effects in fluid-filled bone are not piezoelectric, but rather of electrokinetic, that is, streaming potential (SP) origin.<sup>42,65,66</sup> 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.<sup>66,67</sup>
3. *Electric field strength.* Bone responds to exogenous electrical fields.<sup>68</sup> Although the extrinsic electrical parameter is unclear, field strength may play an important role.<sup>69</sup> A significant parallel exists between the parameters of these exogenous electrical fields<sup>68,69</sup> and the endogenous fields produced by muscle activity. Bone responds to exogenous electrical fields in an effective range of 1 to 10  $\mu\text{V}/\text{cm}$ , strengths that are “. . .on the order of those endogenously produced in bone tissue during normal (muscle) activity”<sup>70</sup> (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.<sup>71</sup> 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 cytoskeletal 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.<sup>72-75</sup> (See Shapiro et al.,<sup>76</sup> for vimentin, and Green<sup>77</sup> 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,<sup>78</sup> possibly by first stimulating the activity of such components as the cfos genes.<sup>36,73,78-86</sup>

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.