CONCISE REVIEW
Biological

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# Mechanobiology of Craniofacial Sutures

## ABSTRACT

Craniofacial sutures are soft connective-tissue joints between mineralized skull bones. Suture mechanobiology refers to the understanding of how mechanical stimuli modulate sutural growth. This review's hypothesis is that novel mechanical stimuli can effectively modulate sutural growth. Exogenous forces with static, sinusoidal, and square waveforms induce corresponding waveforms of sutural strain. Sutural growth is accelerated upon small doses of oscillatory strain, as few as 600 cycles delivered 10 min/day over 12 days. Interestingly, both oscillatory tensile and compressive strains induce anabolic sutural responses beyond natural growth. Mechanistically, oscillatory strain likely turns on genes and transcription factors that activate cellular machinery *via* mechanotransduction pathways. Thus, sutural growth is determined by hereditary and mechanical signals *via* the common pathway of genes. It is concluded that small doses of oscillatory mechanical stimuli have the potential to modulate sutural growth effectively: either accelerating it or initiating net sutural bone resorption for various therapeutic objectives.

**KEY WORDS:** mechanical, osteoblast, bone, fibroblasts, osteogenesis.

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A supplemental appendix to this article is published electronically only at http://www.dentalresearch.org.

## INTRODUCTION

Cranial and facial sutures are soft connective-tissue articulations between mineralized bones in the skull. Sutures exist only in craniofacial bones and have exceedingly complex forms. As joints, sutures absorb and transmit instantaneous mechanical stresses upon either natural activities such as mastication or exogenously applied forces such as orthopedic loading. Sutures facilitate the longitudinal growth of the majority of bones in the skull, for without them, skull bones can grow thicker but not longer. Due to these and other reasons, suture biology has fascinated not only scientists with diverse interests ranging from paleontological form to genetic patterning of sutural development, but also clinicians who attempt to correct myriad types of skeletal deformities by using orthopedic devices that exert mechanical forces to modulate sutural growth.

"Suture mechanobiology" is a term coined here to represent the field of determining (1) the nature of mechanical stimuli capable of engineering sutural growth, and (2) the mechanisms of transduction of mechanical signals into biological growth. Suture mechanobiology is an integral component of suture biology, as illustrated in Appendix Fig. 1 (www.dentalresearch.org). One cannot have a complete understanding of the biology of craniofacial sutures without understanding suture mechanobiology, for mechanical stresses undoubtedly play an essential role in the regulation of post-natal sutural growth. Great strides have been made, especially in the past decade, toward our improved understanding of suture mechanobiology. The present review was designed to accomplish three goals related to mechanical modulation of post-natal sutural growth: (1) to synthesize key knowledge on mechanobiology of craniofacial sutures, (2) to explore what constitutes optimal mechanical stimuli for engineering sutural growth, and (3) to probe a rarely discussed linkage between mechanical signal and sutural gene expression. The evolutionary, morphological, molecular, and genetic aspects of suture biology have been the subjects of recent careful reviews (Cohen, 2000; Herring, 2000; Opperman, 2000). Advances in sutural synostosis also have been comprehensively documented (Warren and Longaker, 2001; Wilkie and Morriss-Kay, 2001).

## MECHANICAL MODULATION OF SUTURAL GROWTH: SEARCH FOR OPTIMAL MECHANICAL STIMULI

Scientists and clinicians alike, particularly those who have applied mechanical forces to skeletal tissues in animal models or human patients, are quick to point out that forces are capable of affecting skeletal growth in not only the craniofacial skeleton, but also the appendicular skeleton in a variety of species (for reviews, see Wagemans *et al*., 1988; Kokich, 1992; Frost, 1996). However, widespread observations of force-mediated skeletal growth, though of vital importance to our current understanding of suture mechanobiology, fall short of addressing a fundamental question: What are the optimal mechanical stimuli for sutural growth? An ideal mechanical

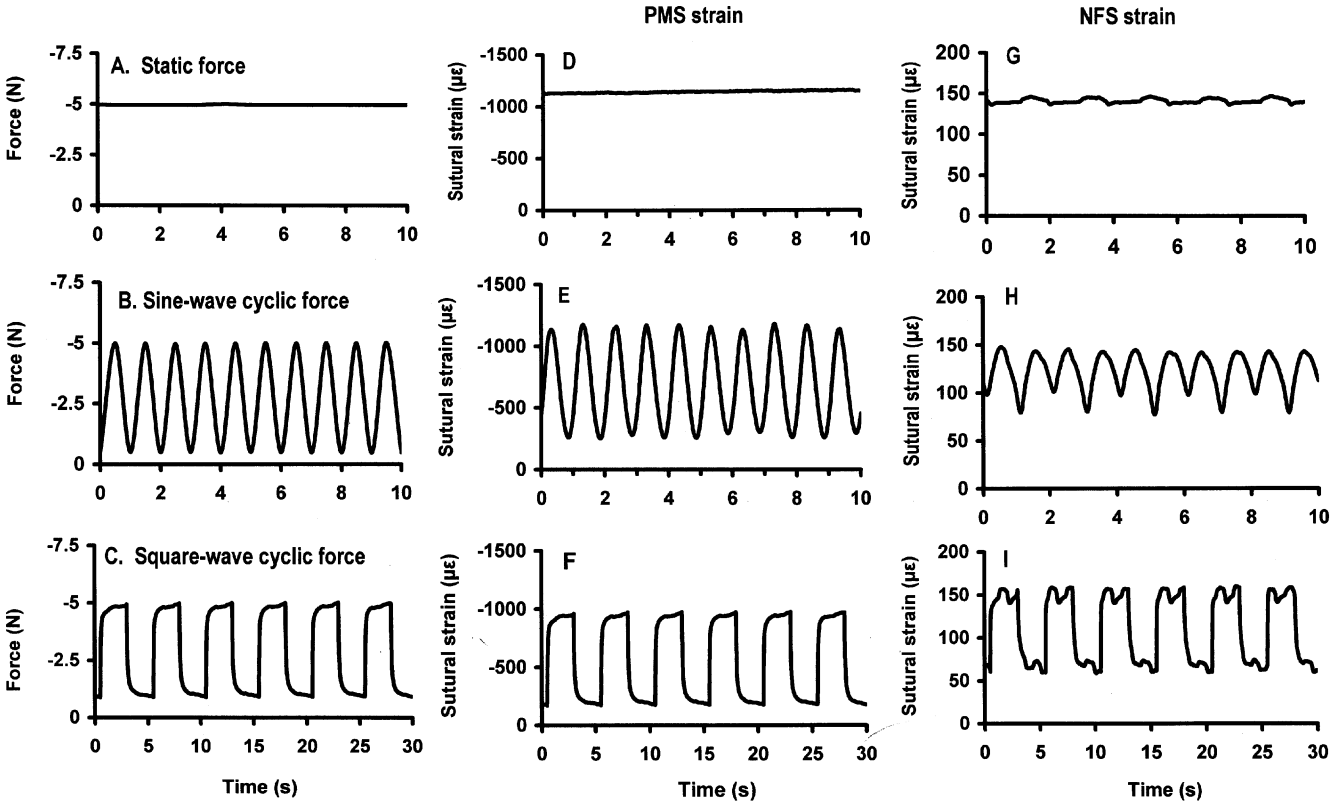


**Figure 1.** Representative waveforms and time courses of exogenous compressive forces (cf. Appendix Fig. 1: posteriorly directed horizontal arrow) at 5 Newtons applied to the maxillary incisors as measured by a load cell of a computerized servohydraulic system. In the left column, **(A)** static force, **(B)** sinusoidal cyclic force, and **(C)** square-wave cyclic force. Three plots in the center column **(D,F,G)** demonstrate the elicited waveforms of sutural strain of the pre-maxillomaxillary suture (PMS), whereas three plots in the right column **(H,I,J)** demonstrate representative waveforms of sutural strain of the nasofrontal suture (NFS). All strain traces were recorded with uni-axial strain gauges. The static force (A) and the resulting static sutural strains in the PMS **(D)** and NFS **(G)** lacked appreciable oscillation in force magnitude. Minor oscillation in G is attributable to rabbit breathing. Sinusoidal cyclic force **(B)** evoked corresponding sinusoidal sutural strains in the PMS **(E)** and NFS **(H)**. Square-wave cyclic force **(C)** evoked corresponding square-wave cyclic sutural strains in the PMS **(F)** and NFS **(1)**. Clearly, waveforms of PMS and NFS sutural strains are modulated by corresponding waveforms of exogenous forces.

stimulus has been considered as the minimum effective force that causes the maximum desirable skeletal growth in the shortest possible time (Frost, 1983).

Several long-term experiments performed in rhesus monkeys about two decades ago investigated the degree to which natural suture growth can be modified by sustained mechanical forces. Frequently, mechanical devices were fabricated based on inspiration from clinical orthopedic appliances. These devices were fixed to the skulls of experimental animals *in vivo*, and activated by calibrated springs to deliver static forces with isolated magnitudes. In separate studies, tensile or compressive forces were applied in either the anterior or posterior direction, respectively, in reference to the skull (*cf.* Appendix Fig. 2 in a rabbit model [www.dentalresearch.org]). Several conclusions can be drawn from these studies. The maxilla can be induced to grow anteriorly or posteriorly upon sustained application of anteriorly or posteriorly directed forces, respectively, over several months (for reviews, see Wagemans *et al.*, 1988; Kokich, 1992). Morphological bony changes can be visualized in bone adjacent to sutures. For example, the zygomatic arch is elongated with a slight depression near the zygomaticotemporal suture upon application of tensile (anterior) forces for up to 11 months (Jackson *et al.*, 1979). Sutural growth is up-regulated to the degree that the orientation of the entire maxilla changes in response to either anterior forces (Jackson *et al.*, 1979; Nanda and Hickory, 1984) or posterior forces (Tuenge and Elder, 1974).Sutures undergo anabolic changes such as increased sutural widths, angiogenesis, and bone apposition in response to anteriorly directed forces (Jackson *et al.*, 1979). Conversely, bone resorption takes place in the zygomaticotemporal and zygomaticomaxillary sutures in response to posteriorly directed forces (Tuenge and Elder, 1974).Despite the irreplaceable value of these data, the approach to the induction of bone adaptation by the application of continuous mechanical forces over several months is not time-efficient. Thus, sustained static mechanical forces are not the optimal stimulus for sutural growth.

Several recent experiments have attempted to determine whether small doses of oscillatory mechanical stimuli can expedite sutural growth. Given that static continuous forces are not the optimal stimulus for sutural growth, the first step was to explore whether exogenous forces with cyclic waveforms are expressed in craniofacial sutures. In two rabbit models, precise doses of tensile and compressive forces, shown as anteriorly and posteriorly directed arrows, respectively, in Appendix Fig. 2 (www.dentalresearch.org), were delivered to the rabbit maxilla for up to 20 min/day over

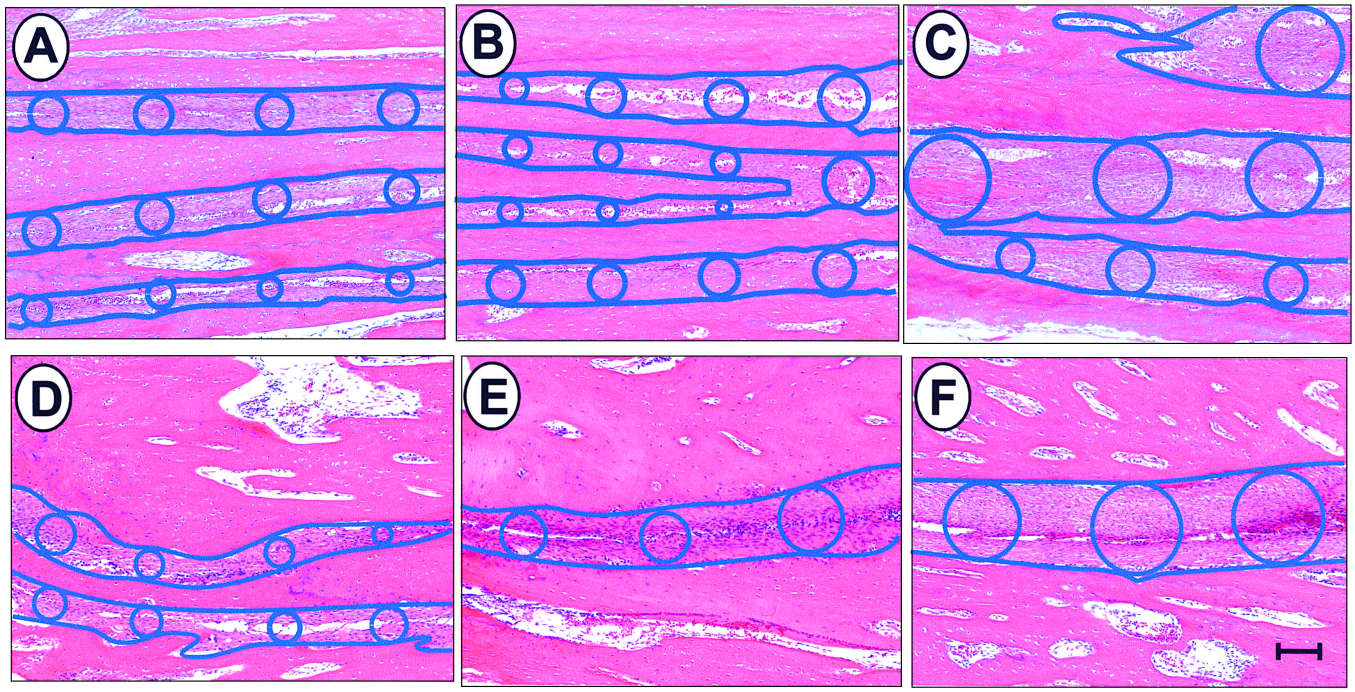


**Figure 2.** Representative photomicrographs of the pre-maxillomaxillary sutures (PMS) and nasofrontal sutures (NFS) in response to compressive strain in the PMS, and tensile strain in the NFS (*cf.* Fig. 1). The PMS treated with cyclic strain **(C)** showed wide sutural separation, in comparison with sham control **(A)** and static strain **(B)**. The same trend is true for the NFS: greater increase in sutural width by cyclic strain **(F)** than static strain **(E)** and natural growth **(D)**. Blue lines were manually drawn to indicate the sutural edge between fibrous connective tissue of the suture and mineralized sutural bone. For quantitative analysis, H&E-stained histological sections were subjected to computerized image analysis with constructed circles and grids overlaid under low power (4x). A circle is constructed in the grid's center with the diameter of the circle equal to the sutural width at a given location. The diameters of all circles *per* sutural specimen were averaged to indicate the mean sutural widths and subjected to ANOVA with Bonferroni tests. H&E stain; scale bar = 100 μm. Reproduced from a manuscript in the *J Bone Miner Res* (in press) with permission of the American Society for Bone and Mineral Research.

12 days by a computerized servohydraulic system. Tensile and compressive forces were applied anteriorly at 2 N (Mao *et al*., 2003) and posteriorly at 5 N (Kopher and Mao, 2002) to the maxillary incisors, each with static, sinusoidal, and square waveforms (Figs. 1A, 1B, 1C). Sutural strain was measured with strain gauges and strain rosettes placed over the pre-maxillomaxillary suture (PMS) and nasofrontal suture (NFS) (*cf.* Appendix Fig. 2 [www.dentalresearch.org]). Indeed, static, sinusoidal, and square-wave exogenous forces (Figs. 1A, 1B, 1C) were expressed as corresponding sutural strain waveforms in not only the PMS adjacent to the exogenous load (Figs. 1D, 1E, 1F from Kopher and Mao, 2002), but also the NFS distant from exogenous load (Figs. 1G, 1H, 1I from Kopher and Mao, 2002). Exogenous compressive forces evoked compressive strain in the PMS, but tensile strain in the NFS (Figs. 1D, 1E, 1F vs. Figs. 1G, 1H, 1I from Kopher and Mao, 2002). Conversely, exogenous tensile forces evoked tensile strain in the PMS, but compressive strain in the NFS (Mao *et al*., 2003). These contrasting strain polarities likely are due to bending moments induced by either the tensile or compressive force. Sutural strain rate varied as a function of force frequencies from 0.2 Hz to 1 Hz in 0.2-Hz increments in both the PMS and NFS (Mao *et al*., 2003). Clearly, sutural strain waveforms and strain rates are modulated by waveforms and frequencies of exogenous forces.

Oscillatory mechanical strain, as characterized above, delivered in short doses as few as 1 Hz in 10 min/day over 12 days, engineers anabolic sutural responses (Kopher and Mao, 2002; Mao *et al*., 2003). We quantified sutural widths by constructing circles and grids over sutural histologic sections using computerized histomorphometric analysis. Significant increases in sutural width were observed upon either sinusoidal tensile strain (Mao *et al*., 2003) or compressive strain (Fig. 2 from Kopher and Mao, 2002) in either the PMS or NFS, in comparison with static sutural strain and natural suture growth. The numbers of sutural cells, quantified by means of standardized grids and computerized image analysis, were significantly higher in response to sinusoidal tension (Mao *et al*., 2003) or compression (Kopher and Mao, 2002) than corresponding static stimuli and natural growth. Fluorescence labeling of newly formed sutural bone demonstrates marked sutural osteogenesis stimulated by oscillatory strain in comparison with static strain and natural growth (Fig. 3 from Kopher and Mao, 2002). Taken together, the oscillatory component of sutural strain, rather than its peak amplitude, is anabolic stimuli for sutural growth. In other words, small doses of static strain without variation in amplitude induced by small doses of static forces are not an effective anabolic stimulus for sutural growth. Once oscillatory strain is introduced, strain rate becomes a new variable (absent in static strain), leading to infinite combinations of mechanical stimuli. Thus, our attempts to identify optimal mechanical stimulus for sutural growth are just the beginning.

By now, one might have noted that sutural growth is accelerated by both tension and compression (Kopher and Mao, 2002; Mao *et al*., 2003). In fact, our data go further by

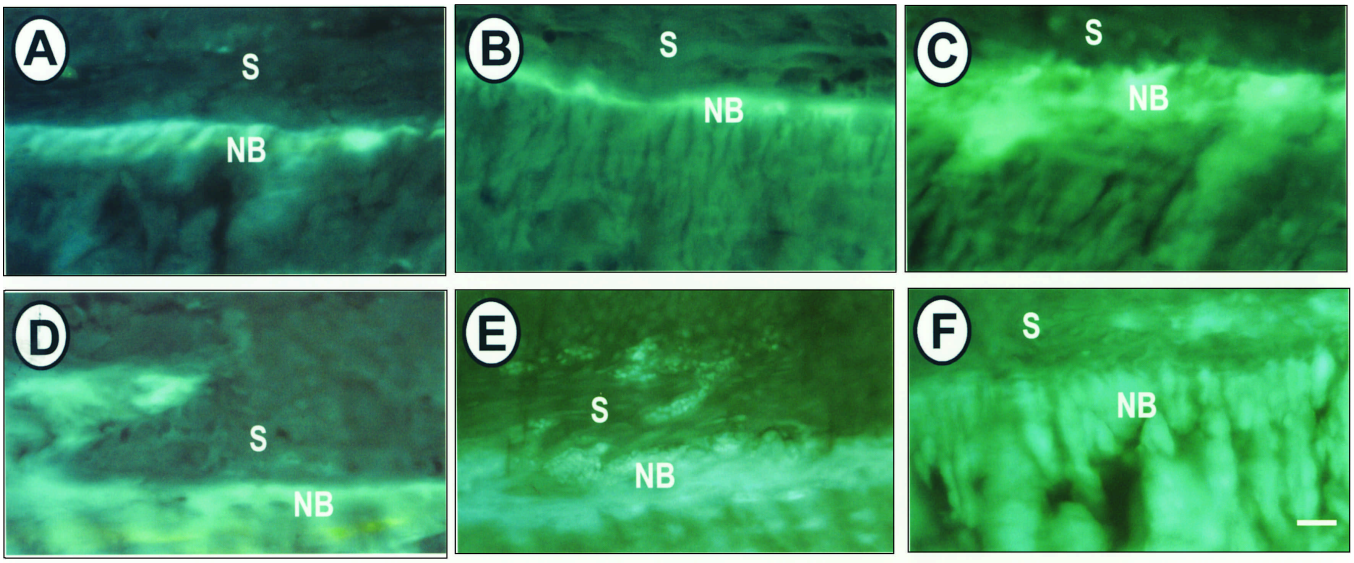


**Figure 3.** Representative photomicrographs demonstrating fluorescence-labeled new bone formation with fluorescent calcein green (arrows) in the pre-maxillomaxillary sutures (PMS) and nasofrontal sutures (NFS) in response to compressive strain in the PMS, but tensile strain in the NFS (*cf.* Fig. 1). **(A)** Sham control of the PMS under normal growth; **(B)** static loading of the PMS; **(C)** cyclic loading of the PMS; **(D)** sham control of the NFS under normal growth; **(E)** static loading of the NFS; and **(F)** cyclic loading of the NFS. Areas of newly mineralized bone are indicated by green fluorescence. S, suture; NB, new bone. The PMS and NFS specimens treated with cyclic loading demonstrated greater amounts of calcein uptake and therefore a great amount of bone apposition in comparison with sutures treated with static loading and sham control. Undemineralized section; scale bar, 10 μm. Reproduced from a manuscript in the *J Bone Miner Res* (in press) with permission of the American Society for Bone and Mineral Research.

demonstrating acceleration of sutural growth in the pre-maxillomaxillary and nasofrontal sutures upon either microscale tensile strain or compressive strain: Compressive forces induce compressive strain in the PMS, but tensile strain in the NFS (*cf.* Fig. 1 from Kopher and Mao, 2002), and *vice versa* (Mao *et al.*, 2003). There is ample evidence that exogenous compressive forces induce both periosteal and endocortical bone growth in long bones (Rubin and Lanyon, 1984; Turner *et al.*, 1995; Mosley and Lanyon, 1998). Yet, clinical dentistry subscribes to the notion that tension = bone formation, and compression = bone resorption. Orthodontists would readily point out from clinical experience that sustained static compression leads to bone resorption so that the tooth can move into the resorbed space, and at the same time the trailing space vacated by tooth movement is to be filled through bone formation. Accumulating literature in distraction osteogenesis demonstrates net bone formation upon static tensile distraction forces, and net bone resorption upon static compressive forces (contraction osteogenesis) (McCarthy *et al.*, 2001). Ironically, an opposite notion is recognized in clinical medicine: compression = formation, and tension = resorption (Frost, 1964; Burr and Martin, 1992). Orthopedic surgeons would testify that the normal curvature of a fractured and malformed bone is restored by bone resorption on the tensed convex surface, and concomitant bone formation on the compressed concave surface (Frost, 1964; Burr and Martin, 1992). So there remain the questions: Does tension = formation and compression = resorption? and *vice versa*?

Unfortunately, the answers to these clear-cut questions are complicated. The problem probably exists at several levels of mismatch of macro- and micro-mechanics, tissue-borne stresses, different architectural structures, and cellular responses. First, the clinician's notion of tension and compression refers to exogenous forces instead of tissue-borne mechanical microstrain. Exogenous forces are an imprecise determinant of biological growth, for the same force likely induces different growth responses of the rat maxilla and elephant maxilla due to scale. Any force applied to bone propagates as mechanical stresses through bone, measurable as sutural strain (Herring *et al.*, 1996; Hylander and Johnson, 1997; Kopher and Mao, 2002; Mao *et al.*, 2003). Cellular growth in bone likely is a function of certain parameters of mechanical stresses acting on cells *via* tissue-borne bone strain or its derivatives, such as fluid flow (Duncan and Turner, 1995; Burger and Klein-Nulend, 1999; Weinbaum *et al.*, 2001). Even if microscale tensile strain is successfully distinguished from microscale compression and delivered to a tissue (*e.g.*, suture or periodontal ligament), collagen fibers in a 3D mesh may become taut and thus compress the cells that reside within.

Second, convex and concave surfaces of long bones and cranial bones likely experience tensile and compressive microstrains, respectively, potentially accountable for their separate formation and resorption processes (Frost, 1964; Burr and Martin, 1992). However, Frost's flexural neutralization theory (*cf.* Frost, 1964) was not designed to account for mechanotransduction mechanisms for craniofacial sutures (and the periodontium). Sutural growth is likely modulated by microscale mechanical stresses that can be induced by either tensile or compressive force (Kopher and Mao, 2002; Mao *et al.*, 2003). Thus, architectural constraints may determine mechanotransduction patterns from exogenous forces to tissue-borne microscale strain, although there are likely common pathways at the cellular and subcellular levels.

Third, although force magnitude or, more precisely, strain amplitude likely plays a role (Frost, 1996), once above an anabolic threshold amplitude, bone growth appears to be determined by strain rate (Turner *et al.*, 1995; Martin *et al.*, 1998; Mosley and Lanyon, 1998). In other words, once above

the threshold, further increases in force or strain do not evoke more bone apposition (Rubin and Lanyon, 1987). Contrary to our original assumption that 5 N compressive forces would evoke net sutural bone resorption, sutural growth was accelerated (Kopher and Mao, 2002). It is likely that, given the appropriate parameters such as strain amplitude, rate, and dose, either tension or compression can evoke bone formation or resorption.

Fourth, sustained static tensile or compressive forces, as in orthodontics or following osteotomy in distraction osteogenesis, are likely to affect sutural cells and tissues in different ways from small doses of oscillatory mechanical strain. Both osteogenic and osteoclastic cells can likely be activated by a multitude of mechanical stimuli, including sustained stresses or transient oscillatory strain of, for instance, 600 cycles delivered for 10 min/day for over 12 days (Kopher and Mao, 2002). Mechanical strain can inhibit osteoclastogenesis *in vitro* (Rubin *et al*., 1999).

Fifth, given the complexity of the craniofacial skeleton, exogenous compressive and tensile forces likely are expressed as shear stresses in craniofacial sutures. Sixth, osteoblasts and osteoclasts do not work in isolation, in that osteoclast activation requires the presence of several factors released by osteoblasts (Teitelbaum, 2000). At this time, the short answer to the questions of whether tension = formation and compression = resorption, and *vice versa,* seems to be that these paradigms are an oversimplification of the mechanical modulation of skeletal tissues. One must specify tension or compression at what strain amplitude, rate, and in what dose.

## MECHANICAL STIMULI "COMMUNICATE" WITH SUTURAL CELLS AND GENES: MECHANOTRANSDUCTION

Cells must be at work before biological growth takes place, for growth is defined as increases in number and mass (**Gray's Anatomy**). Number refers to cells, whereas mass refers to the volume of the extracellular matrix. Clinically detectable growth cannot occur unless cell proliferation, differentiation, maturation, and subsequent synthesis of the extracellular matrix take place. Exogenous forces do not directly induce sutural growth, because they do not directly "communicate" with cells. Any exogenous force applied to bone is transmitted as mechanical stresses in bone, measurable as bone strain on the cortical surface or in craniofacial sutures. The field of identifying cellular, molecular, and genetic pathways responsible for mechanical modulation of skeletal tissues is known as mechanotransduction. Although the precise mechanisms of mechanotransduction are not clearly understood at this time, certainly myriad steps and pathways are involved (Duncan and Turner, 1995; McLeod *et al*., 1998; Gillespie and Walker, 2001). Although a detailed description of mechanotransduction is beyond the scope of the present article, one needs to have a reasonable appreciation of mechanotransduction to understand suture mechanobiology.

Oscillatory mechanical stimuli up-regulate sutural cell proliferation *in vivo*. We have observed increased numbers of sutural cells, quantified by computerized cell counting, in both the pre-maxillomaxillary and nasofrontal sutures upon small doses of oscillatory strain (Kopher and Mao, 2002; Mao *et al*., 2003). This is true for both compressive and tensile microstrains, and in parallel with increased sutural width, indicating coordinated sutural growth rather than a unilateral increase in either cell proliferation or increased matrix synthesis (Kopher and Mao, 2002; Mao *et al*., 2003). Application of sustained static tensile stresses up-regulates sutural cell proliferation in a popular model of the rat interparietal suture. In explant culture, cell proliferation increases upon tensile strain for 24 hrs (Hickory and Nanda, 1987). Despite the knowledge that has been gained from these studies on sutural cell proliferation in response to different types of mechanical stimuli (tension *vs*. compression) or oscillatory *vs.* static strain, and different magnitudes of mechanical stresses, one common shortcoming is that sutural cells are not clearly distinguished between fibroblastic and osteoblastic populations. Historically, mesenchymally derived cells of osteogenic and fibroblastic lineages were given distinct names as osteoblasts and fibroblasts. Each fibrogenic and osteogenic cell lineage likely consists of an array of differentiating cells toward the final cell type of fibroblasts or osteoblasts. Distinguishing these cell populations at various stages of differentiation in response to mechanical stimulation would likely advance our understanding of sutural growth. In addition, sutural strain must be normalized against sutural cross-sectional area to obtain precise stresses experienced by sutural cells.

Increasing numbers of genes and transcription factors have been found to be expressed in sutural growth (Rice *et al*., 2000; Wilkie and Morriss-Kay, 2001). Several genes that are involved in sutural development have been found to participate in mechanotransduction. FGF-2 is up-regulated upon about 600-mN tensile stresses applied to the rat coronal suture (Yu *et al*., 2001). Upon mechanically induced rat tooth movement, the osteocalcin gene is up-regulated along with collagen I and alkaline phosphatase genes (Pavlin *et al*., 2001). A short dose of mechanical stretch applied to cultured calvarial osteoblasts up-regulates an early response gene, Egr-1 mRNA (Dolce *et al*., 1996). Tensile stresses induce sustained up-regulation of BMP-4 gene expression, followed by increasing expression of Cbfa1/Osf-2, an osteoblast-specific transcription factor (Ikegame *et al*., 2001).

Up-regulation of genes and transcription factors in sutures is often accompanied by increased protein synthesis. Type III collagen synthesis increases significantly with application of static mechanical stresses to explant sutures (Meikle *et al*., 1984; Yen *et al*., 1990; Tanaka *et al*., 2000). Also, in the interparietal suture model, 600-mN forces have been shown to increase alkaline phosphatase activity (Miyawaki and Forbes, 1987). In Rawlinson *et al*. (1995), small explants of the rat parietal and ulnar bones were cultured and subjected to different *in vitro* mechanical stresses. Cellular glucose 6-phosphate dehydrogenase (G6PD) activities in osteoblasts were significantly higher after the ulna explant underwent small doses of mechanical stresses (600 cycles @ 1 Hz) than the control ulnar explant (without loading). In contrast, there was no significant difference in the G6PD activity between the parietal bone explants with or without loading. These data comparing craniofacial and appendicular osteoblasts, despite unequal stimulation paradigms and removal of sutures from the calvarial bone, may motivate other investigators to compare osteogenic responses of different skeletal lineages.

## SUTURE MECHANOBIOLOGY AND CRANIOFACIAL ORTHOPEDICS: THE NEXT DECADE

Sutures are designed primarily for the longitudinal growth of craniofacial bones, for without sutures most skull bones can grow thicker but not longer. Like appendicular growth plates that are completely replaced by bone at the conclusion of longitudinal growth, loss of osteogenic ability of the suture with aging in many species is a vivid indication of its primary function to allow for longitudinal growth. Sutures are composed of soft connective tissue and experience mechanical stresses. Certainly true in mechanics and perhaps also true in connective tissue biology, to connect means to withstand forces. Thus, the suture's ability to withstand, absorb, and transmit mechanical stresses is also intrinsic, and likely related to its primary function of growth, for the appropriate mechanical stimuli readily mediate sutural growth. Craniofacial sutures thus are a unique model for investigating the mechanical modulation of biological growth. Suture's uniqueness can be described as consisting of mesenchymally derived cells and their matrices in a confined environment ready to be loaded with tension, shear, and compression, thus unparalleled by other skeletal systems such as the periosteum, tendons, and ligaments. It is arguably the most convenient model for studying interactions between fibroblastic and osteoblastic lineages. Sutural cells are likely activated by microscale cell-borne strain resulting either from bone strain or its mechanical derivatives downstream from bone strain, such as fluid flow. Although fluid flow appears to be the current favorite of inducing cellular responses, it needs to evoke deformation of cell membrane and cytoskeleton, which by definition is strain. The net effect is what we call sutural growth visible by quantitative means. The key to "communicate" with sutural cells appears to be oscillatory strain, instead of static strain lacking oscillation in amplitude. Taken together, the next decade of suture biology and craniofacial orthopedics likely will witness

- continuing identification of genes and transcription factors that are expressed in sutures during both natural growth and upon mechanical stimulation;
- improved comprehension of mechanotransduction pathways related to sutural cells, molecules, and genes;
- enhanced understanding of sutural growth from studies that demonstrate the expression of genes, synthesis of extracellular matrix molecules, and the behavior of sutural cells, of both osteogenic and fibrogenic lineages, in natural and mechanically modulated growth; and
- increasingly clearer demonstration of optimal mechanical stimuli capable of engineering sutural growth or net sutural bone resorption for different therapeutic goals.

Upon completion of the human genome project and exponential increases of research output in fields such as mechanobiology, biomedical engineering, functional genomics, and proteomics, the stage is set for upcoming shifts in the paradigms of suture biology and craniofacial orthopedics. There are reasons to believe that the next decade of suture mechanobiology research and orthopedic practice (including orthodontics) may witness exciting new advances as a result of effective utilization of genetic and engineering tools.

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**CONCISE REVIEW**
Biological

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# Mechanobiology of Craniofacial Sutures

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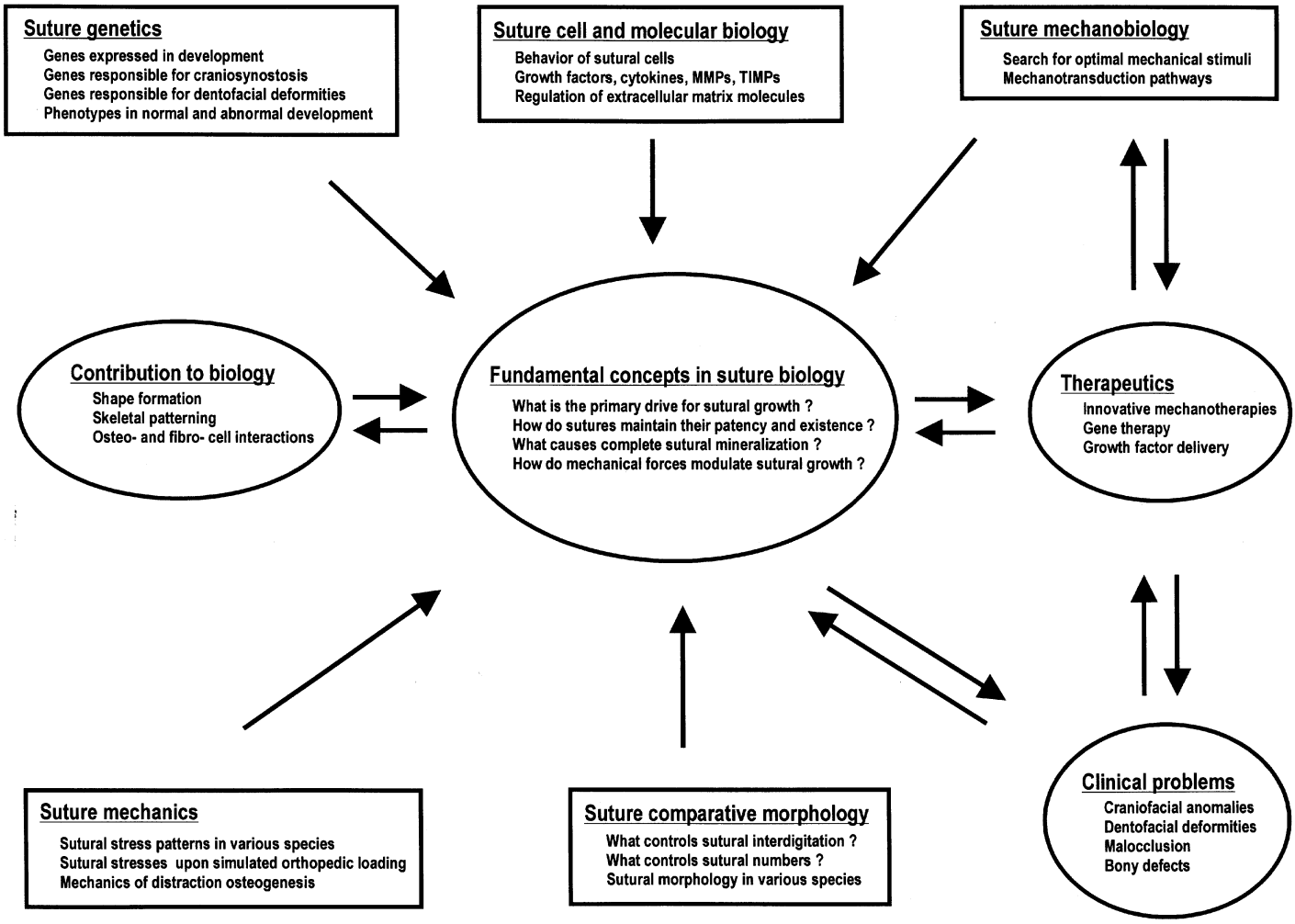
## APPENDIX




**Appendix Figure 1.** Suture biology can be divided into various fields of investigation, indicated by rectangular boxes. Outlines within each box represent important areas or questions to be addressed in the author's opinion. All these fields contribute to the understanding of several fundamental questions, exemplified in the oval in the center. Suture biology contributes to several pending problems in biology, such as shape formation, skeletal patterning, and interactions between osteogenic and fibrogenic lineages. Suture mechanobiology is an integral component of suture biology. One cannot have a complete understanding of the biology of craniofacial sutures without understanding suture mechanobiology, for mechanical stresses undoubtedly play an essential role in regulating post-natal sutural growth. Attempts to improve our understanding of fundamental concepts would facilitate instrumentation of innovative mechanotherapies, potential gene therapies, and growth factor delivery for several orthopedic disorders, such as craniofacial anomalies, dentofacial deformities, malocclusion, and bony defects.

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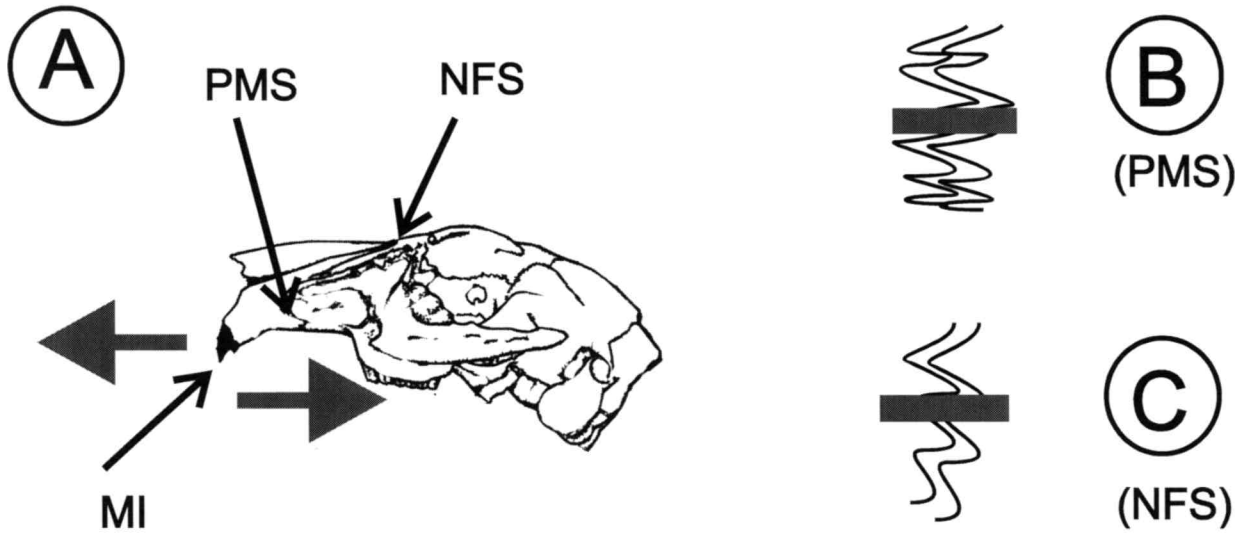


**Appendix Figure 2.** Delivery of exogenous forces and orientation of craniofacial sutures for bone-strain measurements. **(A)** Schematic diagram illustrating the left side of a rabbit skull, a segment of the pre-maxillomaxillary suture (PMS), and the location of the nasofrontal suture (NFS). The anteriorly directed horizontal arrow indicates the direction of tensile forces applied to the maxillary incisors (MI). The posteriorly directed horizontal arrow indicates the direction of compressive forces applied to the maxillary incisors (MI). **(B)** The PMS has a wavy, complex course, extending from the oral cavity between the premaxilla and maxilla rostrally toward the nasal bone. The strain gauge/rosette was placed in the intra-oral portion of the PMS. The PMS has a high degree of sutural interdigitation at its inferior end. The dark rectangle indicates the location of the strain gauge parallel to the direction of exogenous forces. **(C)** The NFS has an intermediate degree of sutural interdigitation among all rabbit craniofacial sutures. The dark rectangle indicates the location of the strain gauge perpendicular to the suture's longitudinal course.