REVIEW ARTICLE

# Growth and development: Hereditary and mechanical modulations

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Growth and development is the net result of environmental modulation of genetic inheritance. Mesenchymal cells differentiate into chondrogenic, osteogenic, and fibrogenic cells: the first 2 are chiefly responsible for endochondral ossification, and the last 2 for sutural growth. Cells are influenced by genes and environmental cues to migrate, proliferate, differentiate, and synthesize extracellular matrix in specific directions and magnitudes, ultimately resulting in macroscopic shapes such as the nose and the chin. Mechanical forces, the most studied environmental cues, readily modulate bone and cartilage growth. Recent experimental evidence demonstrates that cyclic forces evoke greater anabolic responses of not only craniofacial sutures, but also cranial base cartilage. Mechanical forces are transmitted as tissue-borne and cell-borne mechanical strain that in turn regulates gene expression, cell proliferation, differentiation, maturation, and matrix synthesis, the totality of which is growth and development. Thus, hereditary and mechanical modulations of growth and development share a common pathway via genes. Combined approaches using genetics, bioengineering, and quantitative biology are expected to bring new insight into growth and development, and might lead to innovative therapies for craniofacial skeletal dysplasia including malocclusion, dentofacial deformities, and craniofacial anomalies such as cleft palate and craniosynostosis, as well as disorders associated with the temporomandibular joint. (Am J Orthod Dentofacial Orthop 2004;125:676-89)

Growth and development is of tremendous interest to scientists, clinicians, and even the general public. Parents wonder whom their child resembles—the layperson's perception of craniofacial growth. For those who suffer from malocclusions, dentofacial deformities, and craniofacial anomalies such as cleft palate and craniosynostosis, the need to fully understand craniofacial growth is more than a scientific curiosity. In the past decade, there has been an increasingly rapid gain in the knowledge about prenatal and postnatal craniofacial growth by means of 2 general approaches. The first is observational studies at different levels, such as observing series of cephalometric films, microscopic sections of tissues, and the behavior of cells, extracellular matrix molecules, and genes. The second is manipulational studies such as modifying tissue growth by mechanical forces, chemical agents, recombinant tissue techniques, and the use of transgenic animal models. There is a benign lack of awareness of advances among related fields. For instance, key findings in craniofacial genetics might not be known to craniofacial orthopedics including orthodontics, and vice versa. Also lacking is a comprehensive synthesis of the necessary linkage between macroscopic growth and genes, matrix molecules, and cells that account for craniofacial growth.

Most craniofacial anomalies and dentofacial deformities result from inherited mutations and aberrant environmental modulation of multiple genes. Mechanical forces are the most studied environmental cues, and readily modulate bone and cartilage growth.[1-3] Current mechanotherapies for mandibular hypoplasia are vivid evidence of certain levels of clinical usefulness and the need for greater therapeutic effectiveness to stimulate mandibular growth.[4-6] Conversely, mechanotherapies for mandibular hyperplasia are aimed at restraining mandibular growth.[7-10] As discussed below, current mechanotherapies can most likely be improved when we understand more effective ways to communicate with bone and cartilage cells.

This review was designed to accomplish 3 goals

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**Table I.** Postnatal growth and development defined at various levels of understanding from genes to clinically visible growth; mandibular growth (eg, increases in mandibular length and height) results from changes at cellular, molecular, and genetic levels

| | *Growth (number and size)* | *Development (proliferation, apoptosis, differentiation, and maturation)* |
|---|---|---|
| Clinical | Increase in mandibular length | Change in the shape of mandibular condyle |
| Extracellular matrix | Increase in procollagen production and secretion by osteoblasts; increase in amount of collagen molecules in extracellular matrix | Formation of mineralization-competent matrix |
| Cellular | Increases in number of osteoblasts | Differentiation of osteoprogenitor cells into osteoblasts |
| Genetic | Gene regulation of osteoblast proliferation | Activation of differentiation marker genes |

related to hereditary and mechanical modulations of postnatal growth and development: (1) to clarify several key definitions and concepts that are the foundation for comprehensive understanding growth and development, (2) to synthesize current knowledge of bone and cartilage growth of the craniofacial skeletal lineage, and (3) to explore effective means of mechanical stresses to communicate with bone and cartilage cells.

Growth and development are progressive changes over time. *Growth* is defined as increases in number and size.[11-14] *Development* refers to a stage of growth and maturation encompassing morphogenesis, differentiation, and acquisition of functionality. As illustrated in Table I, growth and development so defined apply to different levels of biological organization, ie, genes, matrix molecules, and cells, as well as clinically visible changes in tissues, organs, and organisms.

Growth at the cellular and subcellular levels denotes net increases in the number or size of cells and in the mass of the extracellular matrix. At the macroscopic or clinical level, growth is exemplified by an increasing number of erupted teeth and the increasing size of the mandibular condyle. Growth in multicellular organisms is more frequently allometric (disproportional among adjacent structures) than isometric (proportional among structures).

Development at the cellular level can be described as differentiation and maturation of cell phenotypes from progenitor cells to terminally differentiated cells, such as from mesenchymal cells to mature osteoblasts or from proliferating chondrocytes to hypertrophic cells. Development can be exemplified at the subcellular level by self-assembly of immature collagen fibrils into mature and functional collagen fibers in the extracellular matrix or mineralization of the osteoid to form mature bone. At the clinical level, the increasing capacity of the maturing mandibular condyle to withstand mechanical stresses can be viewed as development.

*Force* is mass $\times$ acceleration. Counterintuitively, force is not a measurable property. One can only measure the effects of force such as *strain*, defined as changes in a structure's length over its original length. The definition of strain can only be satisfied by a change in the structure's length, which is inducible only repeatedly by a change in force magnitude, instead of a constant force. Multiple cycles of change in force magnitude are significant in that bone and cartilage cells respond more readily to rapid oscillation in force magnitude than to a constant force.[2,15-22] A force propagating through biological tissue is transduced as tissue-borne and cell-borne mechanical stresses, which in turn induce interstitial fluid flow.[23,24] Although fluid flow is a current focus of the mechanotransduction pathways, its anabolic effects appear to rely on evoking deformation of extracellular matrix molecules, transmembrane channels, cytoskeleton, and intranuclear structures, which by definition is strain.[2,25-27] Like force, *stress* is not measurable, but can be deduced from strain. Thus, mechanical strain becomes the common thread of all mechanical forces acting on tissues, cells, and genes.[2] Exogenous forces are transmitted in biological tissues as strain before cellular and genetic responses are elicited via a series of general mechanotransduction events as shown in Figure 1. An exogenous force must possess certain characteristics before it qualifies as a *mechanical stimulus*, defined as a mechanical signal capable of eliciting anabolic or catabolic growth response. All characteristics of mechanical signals, including magnitude and duration, have been examined in experiments and clinical practice of craniofacial orthopedics with the sole exception of force frequency.[2] Cyclic forces with sinusoidal waveforms induce accelerated growth of not only craniofacial sutures,[22,28] but also chondrogenesis of the cranial-base cartilage (the sphenooccipital synchondroses).[20,21]

Hereditary and mechanical modulations of growth

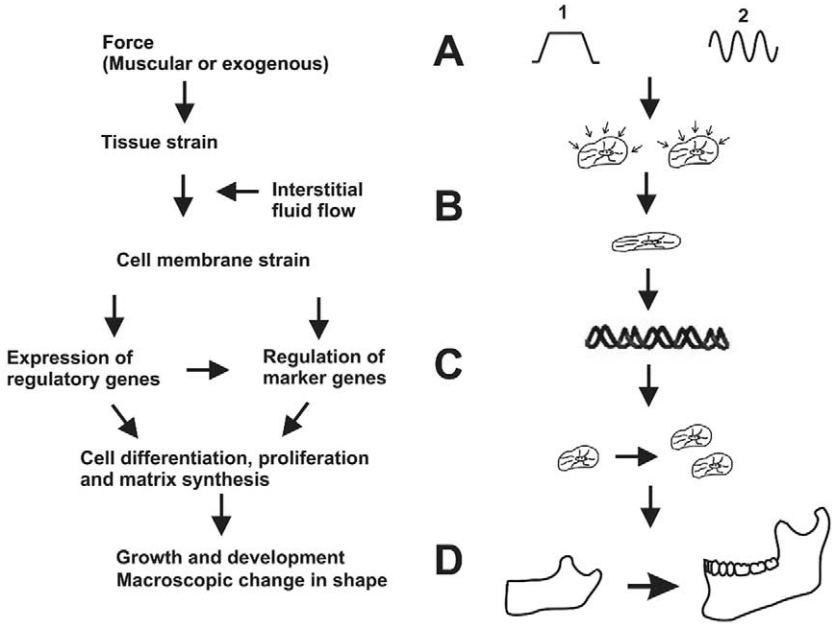


**Fig 1.** Mechanotransduction pathways describing how exogenous forces induce ultimate changes in macroscopic shape such as shape of mandible. Supporting evidence can be found in recent reviews.[2,23,24,27] **A**: Force can be induced on biological tissues from either muscular contraction or exogenous sources such as headgear or fixed orthodontic appliances. Force can be either static (*1*) or cyclic (*2*). **B**: Tissue strain and cell deformation result from application of endogenous or exogenous forces, leading to deformation of cell membrane and cytoskeleton. **C**: Genes are regulated via mechanotransduction pathways. **D**: Bone and cartilage cells proliferate (shown as 1 cell dividing into 2 daughter cells), differentiate, and produce extracellular matrix molecules; that is growth and development, macroscopically visible as changes in shape of mandible from newborn to adult.

and development share a common pathway via genes. As shown in Table II, genes involved in regulating bone and cartilage development can be divided into those encoding bone-matrix and cartilage-matrix proteins (generally considered marker genes) and those regulating cellular or other gene activities (regulatory genes). Regulatory genes include transcription factors and genes encoding growth factors/growth factor receptors. Transcription factors bind to regulatory DNA sequences and modulate the expression of target genes. Growth factors typically reside in the extracellular milieu and send mitogenic and differentiation signals to target cells via receptors on cell membrane. Mechanical forces are the most studied environmental cues and readily modulate bone and cartilage growth. Whereas genes obviously carry hereditary material, mechanical stimuli typically upregulate or downregulate genes before their elicited anabolic or catabolic responses are translated into macroscopic growth, such as an increase of mandibular length by 6 mm. Cells and matrix molecules in micrometer scale must ultimately add up to 6 mm. For instance, 120 osteoblasts lined up sequentially, with each producing 50 μm of collagen matrix, would lead to 6000 μm (= 6 mm) of osteoid. Subsequent mineralization of this collagen matrix would equate to 6 mm of bone. The key, therefore, is how to recruit 120 osteoblasts and line them up in sequence (120 osteogenic cell layers in reality). To date, our understanding of mechanical activation of bone and cartilage cells is incomplete.

## CARTILAGE GROWTH: HEREDITARY AND MECHANICAL MODULATIONS

The embryonic cranial base consists of primary cartilage, which is progressively replaced by bone. After birth, residual cartilaginous structures, known as synchondroses, persist between occipital and sphenoid bone as well as spheoid bone and ethmoid bones, serving as growth cartilage (Fig 2, *A*). Therefore, cartilage growth significantly contributes to overall growth of the embryonic cranial base and postnatal lengthening of the cranial base, as evidenced by mid-

**Table II.** Classes of selective genes involved in growth and development of cartilage and bone

| | *Genes* | *Functions* |
|---|---|---|
| Cartilage | | |
| Marker genes | Type II collagen | |
| | IIA isoform | Marker for chondroprogenitor cells |
| | IIB isoform | Marker for differentiated chondrocytes |
| | Type IX collagen | Interact with proteoglycans |
| | Type X collagen | Marker for hypertrophic chondrocytes |
| | Aggrecan | Cartilage-specific proteoglycan |
| Regulatory genes | | |
| Transcription factor 5 | | |
| | Sox 9 | Signals chondrocyte differentiation |
| Growth factor/receptors | | |
| | Indian hedgehog (Ihh) | Stimulates chondrocyte proliferation and PTHrP. |
| | Fibroblast growth factors/receptors (FGF/FGFR) | Inhibits chondrocyte proliferation and hypertrophy |
| | Transforming growth factors/receptors (TGFb/TGFbR) | Stimulates chondrocyte differentiation and hypertrophy |
| | Bone morphogenetic proteins/receptors (BMP/BMPR) | Stimulates chondrocyte hypertrophy |
| | Parathyroid hormone related peptide/receptors (PTHrP/PTHrPR) | Stimulates chondrocyte proliferation |
| | Retinoic acid receptors (RAR) | Stimulates chondrocyte hypertrophy |
| Bone | | |
| Marker genes | Alkaline phosphatase | Potential $Ca^{2+}$ carrier, hydrolyze inhibitors of mineral deposition such as pyrophosphates |
| | Type I collagen | Serves as scaffold of mineralization |
| | Bone sialoprotein | Nucleator of mineralization |
| | Osteopontin | Inhibits mineralization and promote bone resorption. |
| | Osteocalcin | Inhibits mineralization |
| | Osteonectin | May mediate deposition of hydroxyapatite |
| Regulatory genes | | |
| Transcription factors | Cbfa1/Runx2 | Required for osteogenic commitment and differentiation |
| | Osterix | Required for osteogenic differentiation |
| | Twist | Positive regulator of osteoblast differentiation |
| | Msx2 | Inhibits osteoblast differentiation |
| Growth factor/receptors | Fibroblast growth factors/receptors (FGF/FGFR) | Stimulates proliferation and differentiation. Generate survival signaling |
| | Transforming growth factors/receptors (TGFb/TGFbR) | Modulates bone remodeling |
| | Bone morphogenic proteins/receptors (BMP/BMPR) | Increases Cbfa1/Runx2 expression and stimulate differentiation |
| | Insulin like growth factor (IGF) | Stimulates cell proliferation, differentiation and matrix production |
| | Platelet derived growth factor (PDGF) | Signals cell proliferation and recruit progenitor cells by stimulating chemotactic migration |

face deficiency in various forms of chondrodysplasia.[29] The mandibular condylar cartilage is secondary cartilage (Fig 2, *B*),[30,31] but its significance in the contribution to mandibular growth has been a subject of considerable controversy.

### Genes involved in the regulation of cartilage growth

Cells in growth cartilage undergo a temporal and spatial sequence of proliferation, apoptosis, differentiation, and hypertrophy (Fig 2). Hypertrophic chondrocytes are then gradually replaced by osteoblasts along with angiogenesis[32] in a process known as endochondral ossification. Identification of various regulatory molecules involved in cartilage growth, listed in Table II, has been made more frequently from studies of the appendicular skeleton than the craniofacial skeleton. Briefly, parathyroid-hormone-related peptide (PTHrP) stimulates chondrocyte proliferation but inhibits chondrocyte hypertrophy,[33,34] as evidenced by dystrophic growth plate cartilage resulting from decreased cell proliferation and premature hypertrophy in PTHrP knock-out (targeted disruption of the gene) mice.[35,36] Indian hedgehog (Ihh) coordinates chondrocyte proliferation and hypertrophy through communication with PTHrP/parathyroid hormone receptor (PTH-R) signal-

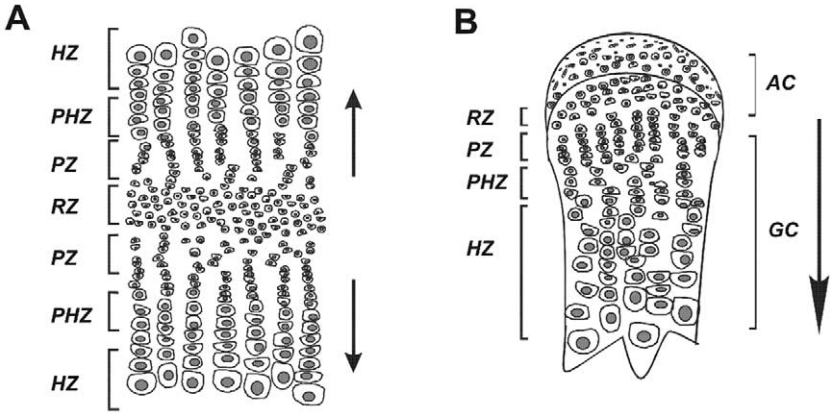


**Fig 2.** Schematic diagrams of typical cranial base synchondrosis (*A*), and both articular and growth cartilage components of mandibular condyle (*B*). **A**, Cranial base synchondrosis consists of 2 growth plates with their reserve zones (*RZ*) merged. Arranged in bipolar directions are proliferating zones (*PZ*), prehypertrophic zones (*PHZ*), and hypertrophic zones (*HZ*). Note that chondrocytes differentiate from reserve cells toward hypertrophic chondrocytes in typical columns and undergo apoptosis followed by replacement by subchondral bone. *Arrows* indicate direction of chondrocyte differentiation. **B**, Growing mandibular condyle consists of both articular cartilage (*AC*) and growth cartilage (*GC*). Articular cartilage of mandibular condyle consists of fibroblast-like cells immediately under articular surface and other chondrocyte-like cells. Growth cartilage component of mandibular condyle consists of differentiating chondrocytes that can be distinguished into RZ, PZ, PHZ, and HZ. *Downward arrow* indicates direction of chondrocyte differentiation.

ing pathways.[37] In contrast to Ihh/PTHrP signaling pathways, fibroblast growth factor (FGF)/fibroblast growth factor receptor (FGFR) signaling inhibits chondrocyte proliferation.[38-43] Transforming growth factor beta (TGFβ) induces chondrocyte differentiation from progenitor cells but inhibits chondrocyte proliferation, hypertrophy, and mineralization.[44-46] Bone morphogenetic proteins (BMPs) induce not only chondrogenic differentiation, but also hypertrophy and mineralization.[47-64] Both stimulatory and inhibitory pathways regulate chondrocyte cellular activities with apparent redundancy among various regulatory molecules, suggesting that cartilage growth and development is a complex process orchestrated by many genes. Some of these cartilage genes are expected to be target genes of mechanical stresses as discussed below.

### Mechanical modulation of cranial base growth

Growth cartilage of the cranial base is generally regarded as a "growth center" with its growth potential predetermined by genes and with little influence from environmental cues.[65,66] After the occipital bone adjacent to the spheno-occipital synchondrosis was found to experience mechanical strain upon simulated orthopedic forces,[67,68] we hypothesized that mechanical stimuli enhance the growth of the cranial base synchondrosis. To test this hypothesis, separate groups of young growing rabbits (litter mates) matched by age and sex were treated with 0-newton (N) exogenous forces (natural growth), 2-N static forces for 20 minutes per day over 12 days, or 2-N cyclic forces for the same duration. Upon harvest of the entire spheno-occipital synchondrosis including the subchondral bone, computerized histomorphometry was used to quantify the geometry of the spheno-occipital synchondrosis and its separate growth zones such as proliferating and hypertrophic zones. Bromodeoxyuridine (BrdU) was used to label chondrocytes undergoing mitosis.

Cranial base cartilage treated with cyclic forces had a significant increase in the overall length and area (Fig 3). By contrast, cranial base cartilage treated with static forces of matching peak load and duration underwent marginal increases in chondrogenesis over natural chondral growth.[20] Cell kinetics experiments showed that BrdU-labeled chondrocytes treated with cyclic forces had significant increases in the proliferating zone of spheno-occipital synchondrosis samples treated with cyclic forces in comparison with natural chondral growth and those treated with static forces.[21] These data are remarkable for several reasons. First, mechanical stimuli were applied for only about 1% of total daily time over a total duration of 12 days. Thus, the anabolic responses are most likely elicited by mechanically activated cell proliferation, differentiation, and

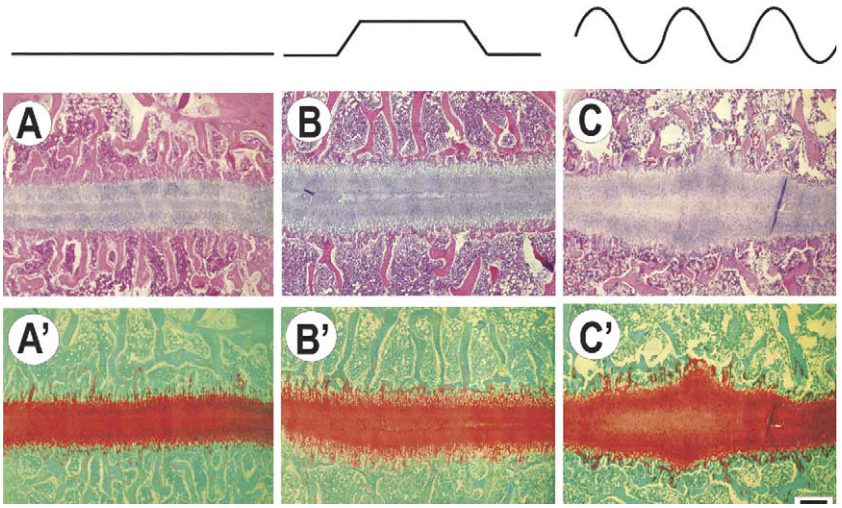


**Fig 3.** Chondrogenesis of rabbit cranial base cartilage is enhanced by cyclic mechanical forces. Three waveforms at top indicate treatments of corresponding 3 histological specimens in same columns. More chondrogenesis was evoked by cyclic forces (*C* and *C′*) than sham control (*A* and *A′*) and static forces (*B* and *B′*). Sham control: no force application; static (*middle*): ramp force followed by continuous force of constant magnitude; cyclic force (*right*): ramp force followed by rapid oscillation in force magnitude. Histological characteristics of cranial-base growth cartilage in sham control (*A, A′*), static (*B, B′*) and dynamic (*C, C′*) specimens stained with hematoxylin and eosin (*A, B, C*) and safranin O/fast green (*A′, B′, C′*). Computer-assisted histomorphometry was used to quantify geometry of chondrogenesis; scale bar: 500 μm. Used with permission of Wang and Mao.[20]

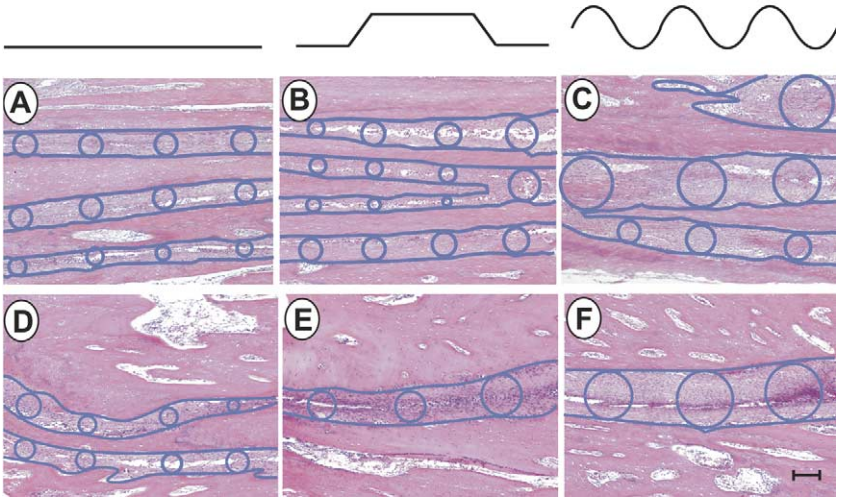


**Fig 4.** Suture growth is enhanced by cyclic mechanical forces. Representative photomicrographs of geometric widths of both premaxillomaxillary suture (PMS) and nasofrontal suture (NFS) in rabbit. **A**, sham control of PMS under normal growth; **B**, static loading of PMS; **C**, cyclic loading of PMS; **D**, sham control of NFS under normal growth; **E**, static loading of NFS; **F**, cyclic loading of NFS. *Blue lines* were manually drawn to indicate sutural edge between fibrous connective tissue of suture and mineralized sutural bone. *Blue circles* were manually drawn to represent sutural geometry with diameter of each circle equal to width of the suture in center of each standardized grid block (not shown). Hematoxylin and eosin stain; scale bar, 100 μm. Used with permission of Kopher and Mao.[22]

associated matrix synthesis, instead of pure mechanical stretch. Second, Frost's chondral modeling theory states that chondrogenesis should be upregulated by a range of exogenous static forces.[69] Here we demonstrated that the oscillatory components of cyclic force, instead of constant force magnitude, are more effective for stimulating chondrogenesis.[20,21] Third, little is known about the mechanical modulation of genes in the cranial base, although gene expression in the appendicular growth plate upon mechanical stimulation has been studied. Cartilage genes are upregulated by mechanical stimuli to induce chondrocyte proliferation and differentiation.[15,70-72] For instance, the expression of Ihh is upregulated by cyclic mechanical stresses applied to embryonic chick sternum chondrocytes in 3-dimensional culture.[73] The remaining tasks are along several fronts: to determine the optimal mechanical stimuli for chondrogenesis and subchondral osteogenesis; to investigate mechanotransduction pathways, including mechanosensitive genes, of chondrocytes in synchondrosis; and to quantify subepiphyseal osteogenesis to determine the contribution of synchondroseal growth to facial bones.

### Contribution of condylar cartilage to mandibular growth

In long bones that develop by endochondral ossification, secondary ossification centers divide the cartilaginous epiphysis into articular cartilage and growth plate shortly before or after birth.[74] Then, articular cartilage and growth plate cartilage evolve differently during postnatal development, especially in their responses to mechanical forces.[69] After the pubertal growth spurt, the appendicular growth plate is completely replaced by bone, whereas articular cartilage is normally present for the rest of life (Fig 2, *B*). By contrast, secondary ossification centers have not been reported in the mandibular condyle. Thus, the mandibular condyle in growing subjects consists of both articular and growth components,[30,31,75] which together are called fibrocartilage because of the presence of both type I and type II collagens.[76,77] The articular surface layer of the neonatal mandibular condyle has been found to change from homogeneous distribution of mechanical properties[78] to a gradient distribution of mechanical properties in the adult mandibular condyle.[79] These data suggest that articular cartilage of the mandibular condyle increases its ability to withstand mechanical stresses during growth, most likely regulated by cells immediately under the articular surface.[76,80] The cells under the articular zone of the mandibular condyle proliferate and further differentiate into hypertrophic chondrocytes.[76,81,82] Nearly all mitotic cells in the condylar cartilage of growing pigs are located in the prechondroblastic zone.[83] Various proteoglycans such as aggrecan, versican, biglycan, and decorin have been found in condylar cartilage.[59,84-87] For instance, versican is located in the articular component of the mandibular condyle, instead of growth cartilage.[84,85] Many aspects of subchondral osteogenesis, such as bone formation rate and mineral apposition rate, are not well understood; recent encouraging data have described several bone histomorphometry parameters such as cells per bone surface area and bone turnover rates in ovarectomized rats.[88-90] Quantitative biological approaches are vitally important to enhance our understanding of mandibular growth.

### Mechanical modulation of chondral growth of the mandibular condyle

In contrast to recent experimental data on mechanical modulation of chondral growth of the cranial base,[20,21] experiments of mechanical modulation of the mandibular condyle cartilage have been performed for decades. Yet, controversy exists regarding to what extent mechanical stimuli accelerate or retard mandibular growth. In organ culture of the mandibular condyle explants of 7-day-old mice, simulated articular function promoted chondral proliferation and differentiation.[91,92] When transplanted to nonarticulating environments such as the cerebral hemisphere, the cartilaginous component of the condyle was replaced by bone.[93-95] These experiments, however, were not designed to demonstrate the precise characteristics of the mechanical stimuli, eg, constant stress vs intermittent stress. Constant and intermittent mechanical stresses have different effects on cell proliferation and matrix synthesis in mandibular condyle cartilage of 4-day-old rats in organ culture.[96] There remains the possibility that chondrocytes in the articular portion of condylar cartilage might respond to mechanical stimuli differently than chondrocytes in the growth portion of condylar cartilage.

Available experimental evidence demonstrates that mandibular condyle cartilage in growing animals accelerates growth upon application of mechanical stresses by increased cell proliferation.[82,96-98] Increasing chondrocyte mitosis is associated with increasing synthesis of type II collagen and various types of proteoglycans in cartilage matrix.[85,87,99,100-105] In general, reduced mechanical stimuli are associated with reduced cell mitosis and matrix synthesis.[106-108] At present, the amount of growth modulated by mechanical stimuli is not known, probably as a result of a lack of quantitative measures of mandibular growth and a lack of knowledge of the precise magnitude and frequency of me-

chanical stimuli used to modulate mandibular growth. Without applying quantitative biology methods such as cell labeling and computerized histomorphometry,[20-22,83,109] it is impossible to know the amount of mandibular growth. Without knowledge of the magnitudes and frequencies of both the applied forces and the induced tissue strain, it is difficult to formulate the amount of mandibular growth as a function of mechanical stimuli. With tools now available to measure the precise characteristics of mechanical stimuli and to quantify the amount of biological growth, it is possible to know how many millinewtons or millipascals of mechanical stimuli over a given time equate to how much condylar growth.

Current controversy about clinical effectiveness in modulating mandibular growth by using mechanical appliances should not be regarded as evidence that mandibular growth cannot be regulated effectively by mechanical stimuli.[110] It is probable that effective ways to communicate with various cell populations of condylar cartilage by many mechanical stimuli remain to be learned. It is also possible that multiple cell lineages in the mandibular condyle, eg, fibroblastic, chondrogenic, and osteoblastic, respond more effectively to mechanical stimuli that are not commonly experienced in normal function. Even mature mandibular condylar cartilage demonstrates anabolic responses to growth hormones,[39,111-114] indicating progenitor cells in the adult mandibular condyle.

## SUTURE GROWTH: HEREDITARY AND MECHANICAL MODULATIONS

Like growth plates and cranial base synchondroses, sutures exist primarily to enable longitudinal growth. A typical craniofacial suture consists of fibroblast-like cells residing in unmineralized matrices that are sandwiched between 2 osteogenesis fronts. The presence of unmineralized type I collagen fibers between advancing osteogenic fronts in the suture's center indicates that suture mesenchyme consists of fibrogenic cells, in addition to the likely presence of mesenchymal cells capable of differentiating into fibrogenic, chondrogenic, and osteogenic lineages.[115-119] The existence of unmineralized suture mesenchyme is essential for the continuous growth of the adjacent bones. The fact that premature suture fusion (synostosis) occurs only in approximately 1 of every 2500 live human births[120] suggests that the differentiation of sutural fibroblastic and osteoblastic cells and the production of their respective matrices are delicately balanced by a high degree of regulation during normal development.[121] As somewhat theatrically articulated by Pruzansky,[122] the suture's survival is at the mercy of constant competition between fibroblastic and osteoblastic cells, with the latter constantly threatening to overpower the former.[123] However, a similar statement can probably be made about the relationship between growth cartilage and subchondral bone.

### Genes involved in suture growth

Sutural bone growth is achieved through a series of cellular activities, including recruitment of mesenchymal cells to the osteogenic cell lineage, stimulation of committed osteogenic cell proliferation, and differentiation followed by matrix mineralization at the advancing osteogenic front. Meanwhile, progression of osteogenesis needs to be tightly controlled at the advancing osteogenic front to preserve sutural existence. Apoptosis (programmed cell death) might be a critical mechanism to control the number of osteoblasts and thus sutural osteogenesis.[124] Much of our knowledge of regulatory genes involved in sutural growth is acquired from genetic studies that link gene mutations to inherited skeletal dysplasia.[125-129] The list of these genes includes transcription factors, such as Cbfa1/Runx2, Msx-2, and Twist, and a recently identified secreted factor, Nell-1, as well as growth factor/receptors, such as FGFs, FGFRs, BMPs, and TGFβs.[130-143] Cbfa-1/Runx2 is required for osteoblast commitment and differentiation, as evidenced by the absence of bone formation upon targeted disruption of the Cbfa-1/Runx 2 gene in mice[144,145] and craniocleidodysostosis syndrome caused by a heterozygous null mutation in humans. Activating (gain of function) mutations of Msx-2 and FGF/FGFR leads to accelerated bone formation by stimulating cell proliferation and differentiation in the suture and premature sutural ossification known as craniosynostosis in humans.[131,138] By contrast, a null (loss of function) mutation of Twist, a transcription factor negatively regulating the FGFR gene expression, also causes craniosynostosis in humans. Although TGFβ-3 mutations have not been linked to craniosynostosis, TGFβ-3 has been shown to play an important role in preventing the cranial suture from premature ossification or synostosis by promoting the apoptosis of sutural osteoblasts.[135,146,147] NELL-1 overexpression induces accelerated osteoblast differentiation in synostosed suture phenotype.[143] Sutural growth is orchestrated by genes that have been shown to regulate several cell lineages such as mesenchymal cells, fibroblast-like cells in suture mesenchyme and osteoblasts.[2] Though similar to the development of long bones in many aspects,[148-150] genetic regulation of sutural bone growth has some specific features.[2] As demonstrated below, mechanical stresses readily increase anabolic rates of sutural cells and thus are

expected to modify activities of many of the genes mentioned above, although only limited information is available at this time.

### Mechanical modulation of suture growth

To test the hypothesis that suture fibrogenesis and osteogenesis can both be modulated by mechanical forces in compression and tension, we subjected separate groups of young growing rabbits (litter mates) matched by age and sexto 2-N tensile forces or 5-N compressive forces against the maxillary incisors for up to 20 minutes per day over 12 days. Force waveforms were either static or cyclic (Fig 4). Sham controls consisted of young growing rabbits (litter mates) matched by age and sex. Cyclic forces with sinusoidal waveforms in both tension and compression induced anabolic suture growth responses.[22,28] Suture widths were quantified by constructing circles and grids over microscopic sections by means of computerized histomorphometric analysis. Significant increases in suture width were observed on application of either sinusoidal tensile forces[28] or compressive forces[22] (Fig 4) over static forces and natural suture growth. The numbers of sutural cells, quantified by using standardized grids and computerized image analysis, were significantly higher in response to sinusoidal tension[28] or compression[22] than corresponding static forces and natural growth. Fluorescence labeling of newly formed sutural bone shows marked sutural osteogenesis stimulated by cyclic forces in comparison with static forces and natural growth.[2,22] Thus, there were parallel increases in both suture width and sutural osteogenesis, suggesting escalated synthesis of extracellular matrices of both fibrogenic and osteogenic cells. An increasing number of genes and transcription factors, some of which are expressed in normal suture development,[126-128] are found to participate in mechanotransduction of sutural cells. Among these, FGF-2 is upregulated at about 600 mN tensile stresses applied to the rat coronal suture.[151] A short dose of mechanical stretch applied to cultured calvarial osteoblasts upregulates an early response gene, Egr-1 mRNA.[152] Tensile stresses applied to mouse calvarial sutures induce sustained upregulation of BMP-4 gene expression, followed by increasing expression of Cbfa1/Osf-2, an osteoblast-specific transcription factor.[153] It is also possible that mechanical stimuli upregulate genes that are not typically expressed in normal suture development.

## CONVERGING THOUGHTS AND CLINICAL IMPLICATIONS

The complexity of craniofacial growth and development can never be underestimated; it offers tremendous intellectual challenges to those who attempt to comprehend it. Three cell lineages primarily involved in craniofacial growth and development—osteogenic, chondrogenic, and fibrogenic—derive from a common progenitor of mesenchymal cells. The behavior of all these cells including commitment, proliferation, apoptosis, differentiation, and matrix synthesis is controlled by genes. Genes can be regulated by environmental cues including myriad types of mechanical stimuli. Genetics, bioengineering, and quantitative biology approaches have already revealed considerable insights into craniofacial growth and development. For instance, a transgenic mouse model demonstrates that intramembranous ossification of the parietal bone requires interaction with neural crest-derived meninges, whereas ossification of the neural crest-derived frontal bone is autonomous.[154] However, neither cells nor genes alone sufficiently account for the development and maintenance of pattern.[155] In contrast to a quantum increase in our knowledge of genetic regulation of craniofacial growth, much less is known about how environmental cues such as mechanical forces regulate genes involved in skeletal growth. As much as one cannot correctly comprehend growth and development without a thorough understanding of cell condensation, proliferation, apoptosis, and differentiation, all of which are controlled by genes, one might face substantial difficulty in understanding growth and development without thorough knowledge of mechanical modulation of chondrogenesis and osteogenesis. Although many skeletal disorders are increasingly well explained by gene mutations, many others probably result from aberrant gene-environment interactions.[156] The genetic involvement in other skeletal disorders such as fractures and osteoarthritis and temporomandibular disorders might be a predisposition at most. The missing link between hereditary regulation of development and clinically observable craniofacial growth most likely includes mechanical stimulus. The emerging field of mechanobiology will further enhance our comprehensive understanding of growth and development.[1-3] Thus, combined approaches of genetics, bioengineering, and quantitative biology are often necessary to deal with the complexity of growth and development.

Previous synthetic accounts of craniofacial growth and development have used a "top-down" approach—examining changes in the length of the mandible over time and attributing addition or subtraction of length at different locations to bone formation or resorption.[110,157-160] Although this approach is valid and has been partially adopted in this review, it is not sufficient and might benefit from a complementary "bottom-up" approach to examine how cellular growth contributes to

addition and subtraction of, for instance, the increase in mandibular length under the influence of genes and environmental cues.[2,161,162] Growth and development can only be understood correctly and comprehensively by a combination of both approaches.

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