

Mechanical Induction in Limb Morphogenesis: The Role of Growth-generated Strains and Pressures

J. H. HENDERSON^{1,2} and D. R. CARTER^{1,2}

¹Biomechanical Engineering Division, Mechanical Engineering Department, Stanford University, Stanford, CA, USA

²Rehabilitation R&D Center, VA Palo Alto Health Care System, Palo Alto, CA, USA

Morphogenesis is regulated by intrinsic factors within cells and by inductive signals transmitted through direct contact, diffusible molecules, and gap junctions. In addition, connected tissues growing at different rates necessarily generate complicated distributions of physical deformations (strains) and pressures. In this *Perspective* we present the hypothesis that growth-generated strains and pressures in developing tissues regulate morphogenesis throughout development. We propose that these local mechanical cues influence morphogenesis by: (1) modulating growth rates; (2) modulating tissue differentiation; (3) influencing the direction of growth; and (4) deforming tissues. It is in this context that we review concepts and experiments of cell signaling and gene expression in various mechanical environments. Tissue and organ culture experiments are interpreted in light of the developmental events associated with the growth of the limb buds and provide initial support for the presence and morphological importance of growth-generated strains and pressures. The concepts presented are used to suggest future lines of research that may give rise to a more integrated mechanobiological view of early embryonic musculoskeletal morphogenesis. (Bone 31:645–653; 2002) © 2002 by Elsevier Science Inc. All rights reserved.

Key Words: Growth-generated strain; Pressure; Stress; Induction; Limb morphogenesis; Mechanobiology; Skeletal development.

Introduction

In recent years there has been increased recognition and appreciation of the importance of physical factors in the morphogenesis of the musculoskeletal system. Muscle contractions begin in utero and in ovo in the late embryonic stages. The forces that are generated impose a time-dependent, distributed pattern of stresses (local force intensities) and strains (local deformations) throughout nearly all of the musculoskeletal tissues. These physical cues guide the growth and differentiation of mesenchymally derived cells throughout life and are critically involved in skeletal regeneration.⁶

Initial patterning and early embryonic morphogenesis of skeletal elements occur in the absence of muscle contractions. It is commonly assumed, therefore, that morphogenesis in the developing embryo is exclusively under the control of intrinsic genetic

regulation and the local chemical environment of the developing tissues. It is clear, however, that as different tissues form they begin to grow at different rates. Whenever two connected tissues grow at different rates, the more rapidly growing tissue is compressed, whereas the slower growing tissue is stretched in tension (**Figure 1**). During development complex configurations of connected tissues grow at different rates. Under these conditions, engineering principles predict the generation of time-varying, quasi-static stresses and strains throughout the developing cells and tissues. We propose that these patterns of mechanical cues are not unlike a complex field of morphogens that can directly influence cell and matrix biology and subsequent morphogenetic events. We use the terms *growth-generated strains* and *growth-generated stresses* to refer to the local deformations and corresponding internal force intensities that are created by differential growth in developing tissues. The sources of growth-generated strains and stresses are the same. Biological events at the tissue level are often related to hydrostatic pressure imposed on cells and tensile deformation of cells and local extracellular matrix (ECM).⁷ We will, therefore, emphasize growth-generated strain (particularly tensile strain) and pressure (hydrostatic compressive stress) when characterizing the growth-generated strain and stress environment present during early limb morphogenesis.

The objective of this *Perspective* is to present and examine the hypothesized regulatory role of growth-generated strains and pressures, using the limb as a model system. We review selected results on mechanotransduction and explore the concept that genetic control of limb morphogenesis is inherently carried out within a complex time history of growth-generated strains and pressures that strongly regulates early morphogenesis. Our perspective suggests that a richer appreciation of the events that control early skeletal patterning and development can be gained by understanding the relationships between growth-generated stress/strain and the local tissue, cell, and molecular biology.

Signals During Early Skeletal Morphogenesis

Morphogenetic Cell and Tissue Forces

Cells can actively produce both tensile forces and compressive pressures that are transmitted to connected cells and the extracellular environment (**Figure 2**). Previous research has considered the role of these *cell-generated stresses* during certain stages or events of early morphogenesis, including primary invagination,¹⁴ neurulation,⁴ and skeletal pattern formation.⁶³

Contractile proteins, such as members of the actin and myosin families, are the cellular components that produce cell-generated tensile forces or *tractions*. Muscle contraction is the most elaborate form of cell-generated traction but is not present in early

Address for correspondence and reprints: Dr. Dennis R. Carter, Biomechanical Engineering Division, Mechanical Engineering Department, Durand Building, Room 215, Stanford University, Stanford, CA 94305-4038. E-mail: dcarter@stanford.edu

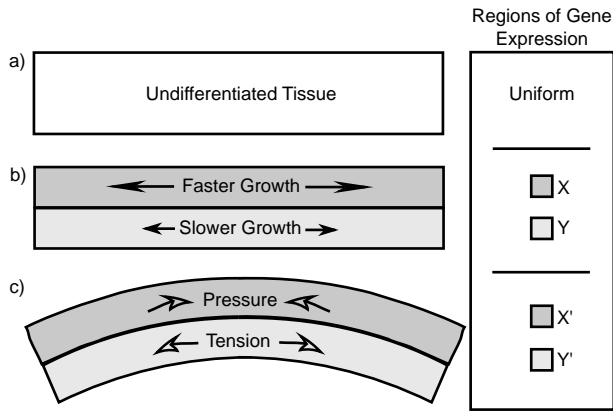


Figure 1. Differential growth of connected tissues generates pressure and tension. During development, regions of undifferentiated tissue (a) receive inductive signals that cause changes in gene expression (b). The different gene expression in regions X and Y can cause corresponding alterations in rates of growth (b), leading to more rapid growth in region X. The tissue that is growing faster (X) will undergo pressure (c) as the slower growing tissue (Y) acts as a constraint. Conversely, the slower growing tissue will undergo tension as the faster growing tissue stretches it. This basic rule of faster growing tissue in tension and slower in compression holds for any configuration of tissue. The tensions and pressures created may act as inductive signals and further alter gene expression, changing the X and Y expression to X' and Y'.

development. Another well-recognized example of cell-generated traction is associated with cell motility. Cell migration, such as neural crest cell migration, is critical to events of early development and is dependent on cell motility. To move, cells must exert a traction on the substratum (Figure 2). This cell-generated traction is visible when a fibroblast is cultured on a thin deformable sheet.³⁶ The basis of some biomechanical models of pattern formation is the experimentally observed effect of cell-generated traction on artificial substrates.⁸⁰

Growth is the dominant source of cell-generated pressures. The increase in volume associated with cell proliferation, ECM production, and cell hypertrophy exerts a radially directed pressure against the surrounding extracellular environment (Figure 2), as has been modeled for tumor growth.⁸ We hypothesize that when multiple cells in a tissue divide, mature, and hypertrophy the individually produced pressures combine over a region of tissue to form a significant source of cell-generated pressure.

In contrast to cell-generated stresses, imposed tensions and pressures originate outside of the affected cell or tissue (Figure

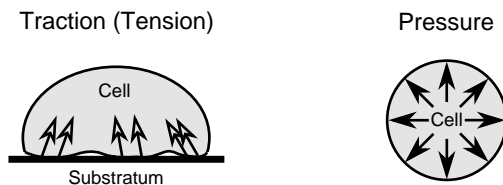


Figure 2. Cell-generated tractions (tensile forces) and pressures. Traction: cells must exert a traction on the substratum in order to move. The traction is generated by contractile proteins within the cell. Cell-generated tractions can only be transmitted to the surrounding environment through integrins, cadherins, and other physical connections. Pressure: increase in volume associated with cell proliferation, ECM production, and cell hypertrophy exerts an outwardly directed radial pressure against the surrounding environment.

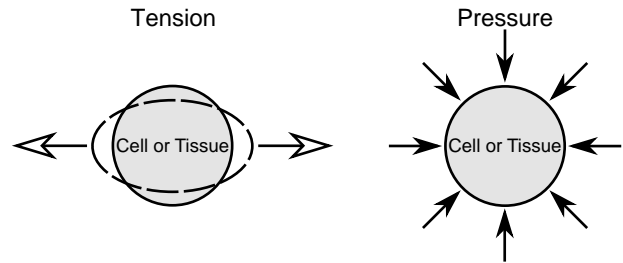


Figure 3. Imposed tensions and pressures have been shown to be very important in regulating cell and tissue function. Tension always causes a stretching (tensile strain) in some direction. The strains associated with uniform pressure are small, but directional and opposing pressures on either side of a cell tend to “sandwich” the cell and can produce a flattening similar to that due to tension. Shear stress, another component of the mechanical environment, always creates a tensile stretch in one direction. Strains are always accompanied by stresses. Situations exist, however, where developing tissues can undergo large strains with small stresses and vice versa. Compliant tissues or tissues that rapidly remodel in response to imposed strains, for example, can undergo significant stretching with only small corresponding stress. Conversely, tissues with high water content can be exposed to a large hydrostatic pressure (a specific kind of stress) with little associated strain.

3). During early development, sources of imposed stresses may include: (1) external forces applied to the embryo by the surrounding environment (e.g., the uterus); or (2) cumulative cell-generated tensions and pressures from neighboring cells (illustrated in Figure 1). Under normal developmental conditions, external forces are randomly applied to the embryo and are not thought to contribute significantly to morphogenesis. We believe, however, that during early development growth-generated stresses from neighboring cells are an important source of imposed tensions and pressures.

Cell Signaling and Mechanotransduction

During early embryonic morphogenesis, developing cells receive extrinsic signals that lead to particular changes in cell behavior—for instance, differentiation, migration, or proliferation. *Induction*, this process of an external signal directing a cell’s development, is commonly thought of in terms of a signal from one group of cells to a neighboring group of cells. Although the source of the inductive signal is often another cell, the source can also be acellular chemical or physical aspects of the extracellular environment. In our view of development, which includes the proposed mechanism of growth-generated strains and pressures, target cells receive inductive signals in four basic ways: (1) direct contact; (2) diffusible molecules; (3) gap junctions; and (4) imposed tensions and pressures (Figure 4).

A direct contact signal is produced when a plasma-membrane-bound receptor on the target cell surface binds to a ligand on another cell surface or in the ECM (Figure 4a). The bonds formed by these molecules are important for biochemical signal transmission and often for the physical connections that are established. Physical connections are necessary for both production of cell-generated tractions and pressures and for propagation of imposed tractions and pressures through tissues. The inductive signal can also be a diffusible molecule that binds to a receptor on or in the target cell (Figure 4b). Morphogens are diffusible molecules that elicit a qualitatively different cell response based on morphogen concentration. A great deal of interest has focused on the potential role of morphogens during limb patterning and

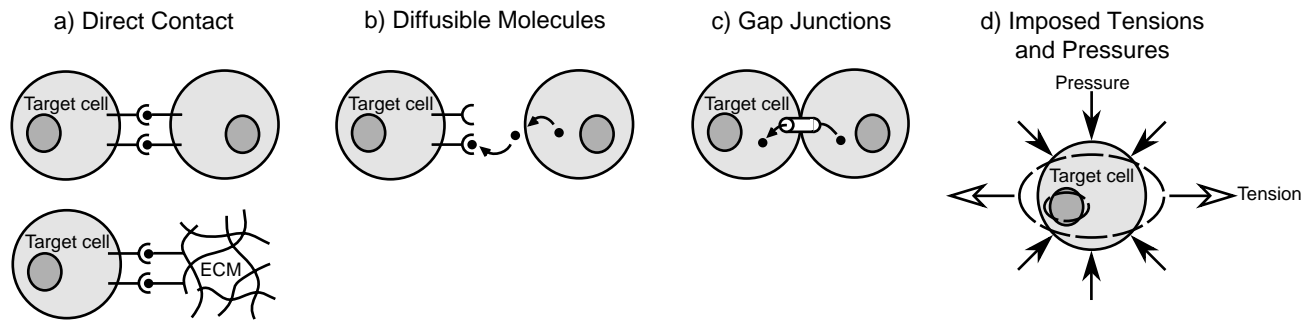


Figure 4. Primary modes of cell induction: the four basic ways in which inductive signals are transmitted to a target cell. (a) The signal can be a ligand present on a cell surface or in the extracellular matrix (ECM) that comes into direct contact with a receptor on the target cell surface. (b) The signal can be a diffusible molecule that binds with a receptor on the target cell surface (as shown) or with an intracellular receptor. (c) Gap junctions between adjacent cells allow the passage of small diffusible molecules that can act as signals. (d) We propose that imposed tensions and pressures, components of growth-generated strains and pressures from the mechanical environment, can act as inductive signals.

morphogenesis. Whether candidate molecules, such as sonic hedgehog (SHH), act directly as morphogens within the limb field remains unclear.⁴³ Gap junctions are the most abundant cell junctions and consist of channel-forming proteins that allow the passage of small molecules, such as ions (Figure 4c). If the inductive signal is a small molecule and gap junctions are present, the signal can pass directly from an adjacent cell to the target cell through the gap junctions.

When discussing the proposed inductive role of imposed tensions and pressures (Figure 4d), it is important to note a fundamental difference between these signals and signaling mechanisms such as direct contact, diffusible molecules, and gap junction. Both direct contact and gap junction signaling require close contact between the signaling cell or ECM and the target cell. Although diffusible molecules can act over a distance, the distance and rate of signal propagation are limited. In contrast, tensions and pressures created at one site of an organism may impose tensions and pressures at another site, simply due to the physical connections that are made between anatomical structures. In addition, the propagation of these signals could be virtually instantaneous. Therefore, differential growth in one portion of the developing limb has the potential to create growth-generated stresses over the entire limb and may influence development and morphogenesis at distant locations.

For an imposed tension or pressure to act as an inductive signal there must be a mechanism of mechanotransduction through which the cell translates the mechanical signal into a particular biological response. Although cell response to mechanical stimuli is recognized as a fundamental biological phenomenon,⁴⁵ the cellular pathways involved in mechanotransduction are poorly understood and have only recently received substantial attention.

A number of gene families have been shown to have mechanosensitive members: ECM proteins (collagens, aggrecan, cartilage matrix protein, cartilage oligomeric matrix protein)^{9,75,85,89}; growth proteins regulating the cell cycle (cyclins, Cdk)⁶⁶; cytokines (IL-1, IL-6, IL-10)^{12,53}; growth factors (TGF- β , FGFs, BMP-2/4)^{12,68,72} vertebrate hedgehog family members (Indian hedgehog)⁸⁸; matrix metalloproteinases (MMPs)^{22,49}; and angiogenic and antiangiogenic factors (VEGFs, angiopoietin-2, METH-1).^{3,90} Stimulus-specific gene expression may be achieved by “mechanoresponsive” promoter elements. Stretch responsive elements have been found in the promoters of genes including tenascin-C and type X collagen.^{11,87,89}

Research is beginning to reveal the pathways through which cells transduce a mechanical signal into a biochemical signal. Tension applied to integrins results in internal cytoskeletal and nuclear scaffold realignment, and Ingber has suggested that the cytoskeletal framework of the focal adhesion complex may provide a major site for signal integration between growth factors and ECM-based signaling pathways.⁴² A number of other mechanisms of mechanotransduction have been proposed and investigated, including strain-related potentials, stretch-activated ion channels, membrane tension, and the primary cilium.⁴⁵ Transduced mechanical signals can lead to changes in transcriptional regulation through intracellular signaling. This signaling may occur through one or more second messenger systems, such as the adenylate cyclase/cAMP system, the inositoltrisphosphate (IP₃) system, or the Ca²⁺ system.²⁵ We predict that future investigation of growth-generated strains and pressures and continued elucidation of the biological mechanism of mechanotransduction will reveal a causal role for morphogenetic mechanical signals during development.

Table 1. Comparative developmental stages, with select events, in the chick and human^a

Chick	Age ^b (stage/time post conception)	Human	Age (days post conception)
Wing represented by thickened ridge	16/51–56 h	Appendicular ridges	30 d
Wing and leg buds first defined	17/52–64 h	Arm and leg buds	31 d
Digital plate defined in leg bud	24/4.5 d	Arm and leg buds fully formed	33–37 d
Three wing digits, four toes	28/5.5–6 d	Differentiation of handplate	38 d
Basic adult limb form achieved	36/10 d	Pentadactyl rudiments	42 d

^aFrom Hinchliffe and Johnson.⁴⁰

^bFrom Hamburger and Hamilton.³³

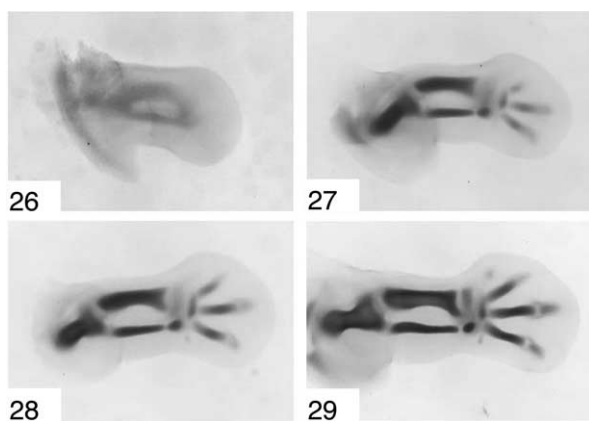


Figure 5. Cartilage condensation in the chick limb. During limb-bud elongation, skeletal rudiments of the limb begin to form and develop in a proximal-to-distal sequence. Stage 26–29; Alcian blue staining. Courtesy of Cliff Tabin.

Limb Growth and Development

Genes and Morphogens

The limb bud is the outgrowth of tissue that forms a future limb (Table 1). The vertebrate fore- and hindlimbs arise from two discrete areas of somatopleure along the embryonic flank.^{1,46} Before the limb buds appear, regions of cells along the ventrolateral sides of the trunk acquire the capacity to develop into a limb.⁷³ At this pre-bud stage, prior to visible structural changes, a great deal of signaling activity is already at work: forelimb/hindlimb identity is dependent on the action of members of the *Tbx* family of transcriptional regulators⁷⁴; fibroblast growth factor (FGF) family members are involved in organization of the pre-bud field; and retinoic acid (RA) activity is critical in the initiation of limb-bud outgrowth.⁷³ The cell biology of the developing limb has received a great deal of attention, and the signaling pathways between the apical ectodermal ridge (AER), progress zone, and zone of polarizing activity (ZPA) are well documented.⁷³

Morphology and Growth-generated Strains

During limb-bud elongation, the mesenchymal cells that initially formed the bud differentiate to form skeletal rudiments, tendons, and dermis. The skeletal rudiments begin to form and develop in a proximal-to-distal sequence (Figure 5). In vertebrates, limb muscles arise from a population of presumptive muscle cells of somitic origin that migrate into the limb-bud region (in the chick at approximately Hamburger and Hamilton³³ stage 12–14).⁵⁵ The muscle tissue joins with presumptive tendon tissue, which in turn connects to developing skeletal condensations, and the growing tissues undergo dependent and coordinated growth.⁸⁴

The bones of the limb arise in a sequential process of mesenchymal cell condensation, chondrogenesis, and endochondral ossification (Figure 6). Skeletal condensations in the chick limb begin to form at stage 22.⁸² These condensations establish the basic structure of the future skeletal elements and are initially continuous with the surrounding undifferentiated mesenchyme.¹⁹ A barrier between skeletal rudiment and adjacent tissue first appears at stage 27 and is composed of cells arranged circumferentially around the central region of the diaphyseal portion of

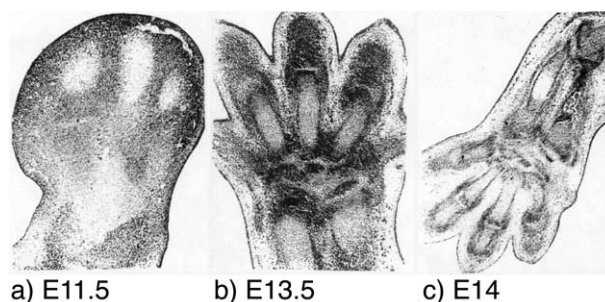


Figure 6. Coordinated growth and development of the musculoskeletal system in the mouse forelimb. The mouse forelimb at embryonic day (E) (a) E11.5, (b) E13.5, and (c) E14, which roughly corresponds to chick stages 22, 26, and 31, respectively. Skeletal condensations and associated soft tissues quickly form from the initially continuous mesenchyme of the limb bud. Note the appearance, between days E11.5 and E14, of skeletal tethering structures, such as ligament, tendon, and muscle condensations. Original magnifications: (a) $\times 87$; (b) $\times 80$; and (c) $\times 40$. Adapted from Dalglish.¹³

the rudiment.^{19,70} These peripheral cells are fibroblastic in nature and develop into a fibrous sheath surrounding the cartilage element.^{19,70} A true perichondrium forms at stage 30 when the cells in the barrier become elongated parallel to the rudiment long axis and overlap.^{19,70} Perichondrial consolidation continues and by stage 33 the perichondrium is bilayered with an outer layer of flattened, tangentially elongated, tightly bound fibroblasts, and an inner layer of osteoblasts.^{19,70} Osteogenesis commences as the osteoblasts begin depositing subperiosteal osteoid, which coincides with the development of the perichondrium into the periosteum.⁷⁰ Ossification continues until all nonarticular cartilage is replaced with bone.

We structure our analysis of limb morphogenesis using the following postulate: Growth-generated strains and pressures from the developing mechanical environment influence morphogenesis in one or more of four ways: (1) modulating growth rates; (2) modulating tissue differentiation; (3) influencing the direction of growth; (4) and deforming tissues.

Growth rate. Skeletal tissue growth is accomplished through cell mitosis, cell hypertrophy, and ECM production. Mechanical modulation of growth rate occurs when a cell receives a signal in the form of an imposed strain or pressure from the mechanical environment and the signal is transduced into an alteration of the cell's rate of hypertrophy, mitotic rate, or rate of ECM production. A number of gene families that have been shown to have mechanosensitive members are important to growth: ECM proteins; growth proteins regulating the cell cycle; and growth factors. Increasing levels of static compression loading of newborn-calf articular cartilage cause upregulation of extracellular signal-regulated kinase-1/2 phosphorylation¹⁸ and a decrease in matrix synthesis.⁶⁷ There is a stretch-responsive element in the promoter of collagen X,⁸⁷ and it is likely that other genes involved in regulation of growth rate have mechanoresponsive promoter elements.

Previous experiments provide evidence that *perichondrial constraint* modifies rates of tissue growth. Developing skeletal rudiments grow faster than the perichondria that surround them.⁵¹ Based on a number of experimental observations from chick and other experiments, Wolpert^{83,84} proposed that the perichondrium constrains cartilage growth in the radial direction, while allowing growth to proceed in the longitudinal direction. This concept of "directed dilation" is consistent with Carey's⁵ observation that the porcine femur increases more in width than

length until a recognizable perichondrium forms, after which longitudinal growth far exceeds radial expansion. In addition, both mutant³⁹ and in vitro^{52,70} experimentation have shown that improper formation or removal of the perichondrium results in altered morphology consistent with Wolpert's theory.

Wolpert's concept of perichondrial constraint is based on the idea that cartilage rudiment growth creates pressure in the rudiment and tension in the surrounding perichondrium.⁸³ This imposed tension and pressure may have multiple mechanobiological effects. As Wolpert recognized, the forces will physically deform the growing tissue and create preferential elongation in the longitudinal direction. Perhaps more importantly, these growth-generated stresses may act as inductive signals, causing the cartilage and perichondrium to alter the rate of growth, direction of growth, or pathway of differentiation. A number of molecular signals to and from the perichondrium are known to be associated with controlling cartilage growth and differentiation, including: the parathyroid hormone-related peptide (PTHrP) and Indian hedgehog (Ihh) signaling pathway³⁷; tenascin-C (TN-C) and syndecan-3⁴⁸; bone morphogenetic protein (BMP)-4¹⁷; and *Wnt-4* and *Wnt-5a*.³⁷ These molecules are expressed in time-dependent, localized expression patterns in the growing cartilage elements and perichondria.

The similarity between the areas of tissue predicted to be under tension and/or pressure and the observed molecular expression domains raises the possibility that growth-generated strains and pressures are responsible for regulating the observed expression patterns of the molecular signals. TN-C, for example, is expressed in the developing skeleton and presumptive perichondria⁴⁸ and regulates osteoblast differentiation in culture.⁵⁴ Statically applied tension upregulates collagen XII and TN-C in fibroblasts cultured on a collagen matrix when compared with cells cultured on a relaxed collagen matrix.^{10,11} Cyclic stretching of chondrocytes induces the expression of *Ihh*, which upregulates BMP-2/4 and type X collagen transcription through BMP-responsive elements in the type X collagen gene.^{86–88}

Rooney and Archer⁷⁰ removed the entire perichondrium from a stage 32 chick ulna and cultured the rudiment overnight. A simple computer analysis of the growth-generated stresses in perichondrial constraint predicts that experimental removal of the perichondrium severely reduces the magnitude of growth-generated pressures in the rudiment (unpublished data). During culture following perichondrium removal, Rooney and Archer observed an average overall increase in rudiment length of 16% greater than controls.⁷⁰ This increase in rudiment growth may be a response to the removal of rudiment pressure that is normally generated by the presence of the perichondrium. Transection of the perichondrium, which would be predicted to reduce growth-generated pressures while maintaining rudiment-perichondrium interactions, also resulted in an increase (11%) in average rudiment length.⁷⁰

Analysis of the results from musculoskeletal development experiments suggests that growth-generated strains due to *musculotendon tethering* also modulate growth rates during limb development. Developing skeletal rudiments grow faster than the musculotendon units (MTUs) that connect them, and it has been commonly assumed that the difference in growth rates creates tension throughout the MTUs.^{26,77–79} Using a simple, static finite element computer analysis of an MTU-tethered skeletal rudiment, we have demonstrated that the difference in growth rates creates tension in the rudiment at the sites of tendon attachment and pressure in much of the remaining rudiment.³⁸ A large body of experimental research, making use of limb-tissue transplantation techniques, muscular dysgenesis mutants, and induced paralysis models, support the hypothesis that growth-generated

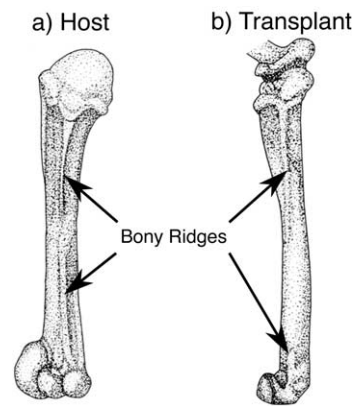


Figure 7. Limb tissue transplantation. (a) Right humerus of host. (b) Nerveless right humerus of transplant developed on the umbilical cord. Note the presence, with reduction in size, of bony ridges at sites of muscle attachment. Adapted from Hamburger.³⁴

strains of this type influence morphogenesis of the growing skeletal rudiments and associated MTUs.

The beginning of motility in the chick fore- and hindlimbs occurs at stage 29 (~6.5 days).³² Once muscle contractions commence it is difficult to separate the morphogenetic influence of growth-generated strains from the cyclic strains imposed by muscle contraction. A number of now classic limb-tissue transplantation experiments^{30,31,34,60–62} allowed observation of the developing limb with the effective elimination of muscle contraction. The experiments showed that, in the absence of muscle function, bony ridges still formed at tendon insertion sites. We suggest that growth-generated static strain of muscle is the morphogenetic determinant responsible for this finding. Ridges formed under transplantation conditions are smaller than those produced during normal development, which is consistent with growth-generated strains in transplants yielding a reduced effect when compared with the normal combination of growth-generated strains and muscle contraction as seen in host animals (Figure 7).³⁴

Muscular dysgenesis (*mdg*), an autosomal-recessive trait in the mouse, produces a severe generalized deficiency of skeletal musculature and complete lack of muscle contraction.^{2,64,65} The appearance of muscular abnormality in *mdg* mice occurs *prior* to the onset of muscle contraction. Over time, the number of muscular abnormalities increases and the skeletal muscles of the mutant are smaller, less well organized, less compact, and less firm than wild-type.⁶⁵ Just as transplantation experiments should provide a model of skeletal morphogenesis under the influence of growth-generated strains and pressures of normal magnitude, the *mdg* mouse should provide a model of development with reduced or absent MTU-associated growth-generated strains and pressures. Limb transplants exhibit ridges at the sites of tendon insertion, whereas the *mdg* homozygote presents with a consistent absence of similar ridges.⁶⁴ Although further experimentation is required to verify the role of growth-generated strains in ridge formation, we present these results as an initial indication that, in the absence of muscle contraction, growth-generated strains may not only be present and sufficient for ridge formation but also necessary.

Tissue differentiation. Mechanical modulation of tissue differentiation occurs when an imposed strain from the mechanical environment is transduced into an alteration of a cell's pathway of differentiation. Formation of bony ridges may involve growth-

generated strain-modulated tissue differentiation: Growth-generated strains and muscle contraction would prompt chondrocytes at the tendon attachment site on the developing rudiment to hypertrophy, precipitating osteogenesis and ridge formation.

Type X collagen is expressed by hypertrophic chondrocytes during the process of endochondral bone formation.⁸⁶ Wu et al.⁸⁷ showed that the type X collagen gene is a mechanoresponsive gene under cyclic loading conditions. Subsequent mapping of the mechanoresponsive element in the gene promoter showed overlap with BMP-responsive elements.⁸⁶ Based on this finding Wu et al. suggested that mechanical loading stimulates type X collagen transcription through upregulation of BMPs.⁸⁶ Further experimentation indicated that BMP-2/4 are located downstream of *Ihh* in biomechanical regulation of type X collagen gene transcription in hypertrophic chondrocytes.⁸⁶ A static stretch-responsive element of the collagen type X gene, or a related gene, may be responsible for growth-generated strain modification of tissue differentiation during bony ridge formation.

Injection of neuromuscular blocking agents into chick embryos is another widely used technique for the study of immobilization during limb development.^{16,28,29,41,56} As with limb-transplantation experiments, paralysis experiments have the potential to shed light on the role of growth-generated strains in limb morphogenesis by removing the confounding effects of muscle contraction. Movement in the chick embryo begins at 3.5 days (~stages 21–23), but motility in the wings and legs does not occur until 6.5 days (~stage 30).³² Paralyzing agents are commonly injected at days 6–7 and induce paralysis prior to the onset of muscle contraction in the limb. As with limb-tissue transplants, paralyzed embryos show normal musculoskeletal differentiation and joint formation prior to the stage of joint cavitation, but muscle activity is necessary for joint cavitation and musculoskeletal maintenance.^{16,57,59} Ridges at tendon insertions develop but are reduced in size,^{29,41} further supporting the limb-transplantation findings.

Paralyzed embryos exhibit the presence of patellas with reduced size.^{16,41} Sesamoid bones, such as the patella, are thought to arise from a combination of mechanical and biological factors.^{24,50,71} Passive stretch of tendons around bony protuberances may create growth-generated pressures in the tendon of sufficient magnitude to induce sesamoid formation even in the absence of muscle contraction. Further experimentation that separately examines tendon development without loading, with growth-generated pressures, and with muscle activity, is needed to differentiate between this and other possible explanations.

Stress due to growth is quasi-static, but stress due to muscular activity is cyclic. Although cyclic stresses have received substantial attention,^{15,68,69,75,81} few investigations have examined the role of static stresses in tissue differentiation. Hall et al.²⁷ showed that pressures in the “physiological” range, applied for 20 sec, 5 min, or 2 h, stimulated matrix synthesis rates in articular cartilage. Carter et al.⁷ used a computer model to predict quasi-static stress and strain patterns in tissues during distraction osteogenesis. The model predicted tissue differentiation patterns consistent with a histological study of distraction osteogenesis in a mouse model. Kanzaki et al.⁴⁷ examined the effect of static mechanical stress on osteoclastogenesis-supporting activity of periodontal ligament (PDL) cells. PDL cells were compressed continuously and then cocultured with peripheral blood mononuclear cells (PBMCs). Under static mechanical stress the PDL cells upregulated osteoclastogenesis from PBMCs by receptor activator of nuclear factor- κ B ligand (RANKL) upregulation via prostaglandin E₂ synthesis.

Direction of growth. The mechanical environment can also influence the *direction* of tissue growth. Regulation of growth rate and regulation of tissue differentiation involve significant

changes in cellular gene expression. Mechanical regulation of direction of growth, however, can primarily be a physical directing of growth processes through reorganization of cytoskeletal structure without a major change in gene expression. Over time, a cell's cytoskeleton can adapt to the primary strain direction,⁴⁵ strains in the ECM can induce directional motion of cells,⁶³ and growth of developing skeletal rudiments often becomes highly directional.^{70,84}

During the endochondral growth and ossification of long bones, the chondrocytes of the physis, or growth plate, are oriented into chondrocyte columns arranged so that cartilage growth and ossification is directed toward the bone end.⁶ In a similar process earlier in development, flattened diaphyseal chondrocytes of developing cartilage rudiments orient themselves so that hypertrophy creates growth almost entirely along the long axis of the rudiment.⁷⁰ Rooney and Archer⁷⁰ found alterations in direction of growth during culture following perichondrium removal. With resection of the perichondrium and, thus, removal of the predicted constraining and directing growth-generated strains, rudiment morphology changed as the previously organized directional growth was lost. Stage 32 ulna rudiments produced an S-shaped element in >90% of cases.

Deformation. Mechanical modulation of cell growth rate, tissue differentiation, or direction of growth involves an active cellular response to an external mechanical signal. In contrast, tensions and pressures can also directly cause macroscopic deformation of entire tissues. This mode of mechanical influence changes the shape of the tissue without the need for an active cellular response. A simple example arises from folding of undifferentiated epithelium; that is, in contrast to invagination or evagination, which are believed to involve active cellular deformation, epithelial folding can result from externally applied forces.²¹

Tissue deformation occurs without an active cellular response but can lead to critical morphogenetic tissue interactions, especially during early development. An interesting example is found outside of our limb model system. During cranial development, tissue deformation plays an important role in formation of the positional relationships between skull and brain tissues. By embryonic day 10.5 (E10.5) the mouse telencephalon has begun to expand to form the cerebral hemispheres. During the next 3 days, as the cerebral hemispheres continue to expand, they take with them a thin layer of neural crest-derived cells, which later form the meninges.⁴⁴ Exposure of embryos to RA at E10.0 reduces the meningeal neural crest and inhibits parietal ossification, suggesting that intramembranous ossification of the mesodermal parietal bones requires interaction with neural crest-derived meninges.⁴⁴ These findings illustrate the important role growth-generated tissue deformations can play by facilitating necessary tissue rearrangements and interactions.

In an experiment relevant to our discussion of limb morphogenesis, Glücksmann²³ grew a cultured rudiment into “barrier rudiments” and observed the resulting deformation of the growing rudiment. Barriers were placed in the direction of expansion of a rudiment. The rudiment “thus had to grow against a resistance,”²³ and growth-generated stresses were created. The growth-generated stresses, which in this experiment amounted to an axial force and “buckling,” resulted in the rudiment bending or even breaking.

As Glücksmann further noted, once bending occurred the convex side of the deformed rudiment was “subjected to tension and the concave to pressure.”²³ In addition to causing deformation, these growth-generated stresses could further act as regulatory signals. Glücksmann observed that the pressure and tension stresses exerted on the cartilage led to “disintegration of the hyaline ground substance and its replacement by a fibrillar

system,” an example of the mechanical environment creating both deformation and tissue differentiation. Similarly, perichondrial constraint involves modulation of growth magnitude and growth direction. As these two examples illustrate, the mechanical environment may often influence morphogenesis through a combination of mechanisms.

Research Challenges

Although we have focused on skeletal morphogenesis in the limb, the principles of growth-generated strains and pressures discussed are widely applicable to development as a whole. Human clinical observations and animal studies^{35,58} have shown that normal rapid growth of the brain and associated changes in intracranial pressure and volume play a critical role in cranial morphogenesis. Growth of the brain generates an outward pressure on the internal surface of developing calvariae and creates tensile strains within the calvariae and across fibrous sutures.⁷⁶ Although the importance of pressures and tensile strains during cranial development is well established, research is only now beginning to reveal the connection between these mechanical cues and the genes expressed in the cranium and dura mater.²⁰ Investigation of growth-generated strains in cranial morphogenesis should provide significant insight into skeletal development, normal cranial development, and conditions such as hydrocephalus, microcephalus, and craniosynostosis. Knowledge gained in the limb and axial skeleton can be used to explore the role of growth-generated strains and pressures in other organ systems.

Many fundamental questions related to growth-generated strains and pressures remain. Although investigation of growth-generated strains in the axial skeleton will provide many interesting opportunities, limb morphogenesis alone presents a number of immediate research possibilities and challenges. The material properties of tissues during early development are largely unknown and must be measured to facilitate accurate and meaningful computational modeling of growth-generated strains. The measurement of these properties will not be trivial, as the sample size available for testing will challenge the limits of current techniques of macro- and micromaterial property measurement. The magnitudes and distributions of growth-generated strains in the developing limb are also unknown and methods of imaging and measuring these deformations must be developed. Measurement of these magnitudes will improve our general understanding of growth-generated strains and will provide concrete patterns and values with which to test the accuracy of computational models. Once refined, computational models of growth-generated strains can be combined with genetic assays, such as visualization of gene expression, to probe the connection between the mechanical aspects of growth-generated strains and the biological response. The results of these investigations will likely provide a new tool for exploring mechanisms of mechano-transduction.

Acknowledgments: This work was supported by a Fannie and John Hertz Foundation Fellowship and a Burt and Deedee McMurtry Stanford Graduate Fellowship to J.H.H.

References

1. Amprino, R. The development of the vertebrate limb. *Clin Orthop* 188:263–284; 1984.
2. Banker, B. Q. Muscular dysgenesis in the mouse (mdg/mdg). I. Ultrastructural study of skeletal and cardiac muscle. *J Neuropathol Exp Neurol* 36:100–127; 1977.
3. Bongrazio, M., Baumann, C., Zakrzewicz, A., Pries, A. R., and Gaehtgens, P. Evidence for modulation of genes involved in vascular adaptation by prolonged exposure of endothelial cells to shear stress. *Cardiovasc Res* 47:384–393; 2000.
4. Brodland, G. W. and Clausi, D. A. Embryonic tissue morphogenesis modeled by FEM. *J Biomech Eng* 116:146–155; 1994.
5. Carey, E. J. Direct observations on the transformation of the mesenchyme in the thigh of the pig embryo, with special reference to the genesis of the thigh muscles of the knee and hip joints, and of the primary bone of the femur. *J Morphol Physiol* 37:1–77; 1922.
6. Carter, D. and Beaupre, G. *Skeletal Function and Form: Mechanobiology of Skeletal Development, Aging, and Regeneration*. Cambridge, UK: Cambridge University; 2001.
7. Carter, D. R., Beaupre, G. S., Giori, N.J., and Helms, J. A. Mechanobiology of skeletal regeneration. *Clin Orthop* 355 (Suppl.): S41–S55, 1998.
8. Chen, C. Y., Byrne, H. M., and King, J. R. The influence of growth-induced stress from the surrounding medium on the development of multicell spheroids. *J Math Biol* 43:191–220; 2001.
9. Chi, S., Chung, M., Hulme, P., Doege, K., Duncan, N., and Matyas, J. Mechanical compression stimulates the aggrecan promoter of chondrocytes embedded in agarose gel. *Trans Ortho Res Soc* 27:32; 2002.
10. Chiquet, M., Matthisson, M., Koch, M., Tannheimer, M., and Chiquet-Ehrismann, R. Regulation of extracellular matrix synthesis by mechanical stress. *Biochem Cell Biol* 74:737–744; 1996.
11. Chiquet-Ehrismann, R., Tannheimer, M., Koch, M., Brunner, A., Spring, J., Martin, D., Baumgartner, S., and Chiquet, M. Tenascin-C expression by fibroblasts is elevated in stressed collagen gels. *J Cell Biol* 127:2093–2101; 1994.
12. Cillo, J. E. Jr., Gassner, R., Koepsel, R. R., and Buckley, M. J. Growth factor and cytokine gene expression in mechanically strained human osteoblast-like cells: Implications for distraction osteogenesis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 90:147–154; 2000.
13. Dalgleish, A. E. *Development of the Limbs of the Mouse*. Ph.D. Thesis. Stanford, CA: Stanford University; 1964.
14. Davidson, L. A., Koehl, M. A., Keller, R., and Oster, G. F. How do sea urchins invaginate? Using biomechanics to distinguish between mechanisms of primary invagination. *Development* 121:2005–2018; 1995.
15. Djurasovic, M., Aldridge, J. W., Grumbles, R., Rosenwasser, M. P., Howell, D., and Ratcliffe, A. Knee joint immobilization decreases aggrecan gene expression in the meniscus. *Am J Sports Med* 26:460–466; 1998.
16. Drachman, D. B. and Sokoloff, L. The role of movement in embryonic joint development. *Dev Biol* 14:401–420; 1966.
17. Duprez, D., Bell, E. J., Richardson, M. K., Archer, C. W., Wolpert, L., Brickell, P. M., and Francis-West, P. H. Overexpression of BMP-2 and BMP-4 alters the size and shape of developing skeletal elements in the chick limb. *Mech Dev* 57:145–157; 1996.
18. Fanning, P., Emkey, G., Smith, R., Grodzinsky, A., and Trippel, S. Response of cartilage to mechanical loading is correlated with sustained ERK1/2 activation. *Trans Ortho Res Soc* 26:172; 2001.
19. Fell, H. B. The histogenesis of cartilage and bone in the long bones of the embryonic fowl. *J Morphol Physiol* 40:417–459; 1925.
20. Fong, K. D., Warren, S. M., Loba, E. G., Henderson, J. H., Fang, T. D., Cowan, C. M., Carter, D. R., and Longaker, M. T. Mechanical strain affects dura mater biology: Implications for immature calvarial healing. *Plast Reconstr Surg*. In press.
21. Fristrom, D. The cellular basis of epithelial morphogenesis. A review. *Tissue Cell* 20:645–690; 1988.
22. Fujisawa, T., Hattori, T., Takahashi, K., Kuboki, T., Yamashita, A., and Takigawa, M. Cyclic mechanical stress induces extracellular matrix degradation in cultured chondrocytes via gene expression of matrix metalloproteinases and interleukin-1. *J Biochem (Tokyo)* 125:966–975; 1999.
23. Glücksmann, A. The role of mechanical stresses in bone formation in vitro. *J Anat* 76:231–239; 1942.
24. Goldberg, I. and Nathan, H. Anatomy and pathology of the sesamoid bones. The hand compared to the foot. *Int Orthop* 11:141–147; 1987.
25. Guilak, F., Sah, R., and Setton, L. A. Physical regulation of cartilage metabolism. In: Mow, V. C. and W. C. Hayes, Eds. *Basic Orthopaedic Biomechanics*. New York: Raven 1997.
26. Haines, R. W. The laws of muscle and tendon growth. *J Anat* 66:578–585; 1932.
27. Hall, A. C., Urban, J. P., and Gehl, K. A. The effects of hydrostatic pressure on matrix synthesis in articular cartilage. *J Orthop Res* 9:1–10; 1991.

28. Hall, B. K. A simple, single-injection method for inducing long-term paralysis in embryonic chicks, and preliminary observations on growth of the tibia. *Anat Rec* 181:767–777; 1975.
29. Hall, B. K. and Herring, S. W. Paralysis and growth of the musculoskeletal system in the embryonic chick. *J Morphol* 206:45–56; 1990.
30. Hamburger, V. The development and innervation of transplanted limb primordia of chick embryos. *J Exp Zool* 80:347–389; 1939.
31. Hamburger, V. Morphogenetic and axial self-differentiation of transplanted limb primordia of 2-day chick embryos. *J Exp Zool* 77:379–399; 1938.
32. Hamburger, V. and Balaban, M. Observations and experiments on spontaneous rhythmical behavior in the chick embryo. *Dev Biol* 7:533–545; 1963.
33. Hamburger, V. and Hamilton, H. L. A series of normal stages in the development of the chick embryo. *J Morphol* 88:49–92; 1951.
34. Hamburger, V. and Waugh, M. The primary development of the skeleton in nerveless and poorly innervated limb transplants of chick embryos. *Physiol Zool* 13:367–384; 1940.
35. Hanlo, P. W., Gooskens, R. J., van Schooneveld, M., Tulleken, C. A., van der Knaap, M. S., Faber, J. A., and Willems, J. The effect of intracranial pressure on myelination and the relationship with neurodevelopment in infantile hydrocephalus. *Dev Med Child Neurol* 39:286–291; 1997.
36. Harris, A. K., Wild, P., and Stopak, D. Silicone rubber substrata: A new wrinkle in the study of cell locomotion. *Science* 208:177–179; 1980.
37. Hartmann, C. and Tabin, C. J. Dual roles of Wnt signaling during chondrogenesis in the chicken limb. *Development* 127:3141–3159; 2000.
38. Henderson, J. H. and Carter, D. R. The role of growth-generated stresses in limb morphogenesis. Proceedings of the West Coast Regional Developmental Biology Meeting. The Society for Developmental Biology. Bodega Bay, CA, 2002. Abstract #12.
39. Hinchliffe, J. R. and Ede, D. A. Abnormalities in bone and cartilage development in the talpid mutant of the fowl. *J Embryol Exp Morphol* 19:327–339; 1966.
40. Hinchliffe, J. R. and Johnson, D. R. *The Development of the Vertebrate Limb*. New York: Oxford University; 1980.
41. Hosseini, A. and Hogg, D. A. The effects of paralysis on skeletal development in the chick embryo. II. Effects on histogenesis of the tibia. *J Anat* 177:169–178; 1991.
42. Ingber, D. E. Tensegrity: The architectural basis of cellular mechanotransduction. *Annu Rev Physiol* 59:575–599; 1997.
43. Ingham, P. W. and McMahon, A. P. Hedgehog signaling in animal development: Paradigms and principles. *Genes Dev* 15:3059–3087; 2001.
44. Jiang, X., Iseki, S., Maxson, R. E., Sucov, H. M., and Morriss-Kay, G. M. Tissue origins and interactions in the mammalian skull vault. *Dev Biol* 241:106–116; 2002.
45. Jones, D., Leivseth, G., and Tenbosch, J. Mechano-reception in osteoblast-like cells. *Biochem Cell Biol* 73:525–534; 1995.
46. Jurand, A. Ultrastructural aspects of early development of the fore-limb buds in the chick and the mouse. *Royal Soc London B Biol Sci* 162:387–405; 1964.
47. Kanzaki, H., Chiba, M., Shimizu, Y., and Mitani, H. Periodontal ligament cells under mechanical stress induce osteoclastogenesis by receptor activator of nuclear factor kappaB ligand up-regulation via prostaglandin E2 synthesis. *J Bone Miner Res* 17:210–220; 2002.
48. Koyama, E., Shimazu, A., Leatherman, J. L., Golden, E. B., Nah, H. D., and Pacifici, M. Expression of syndecan-3 and tenascin-C: Possible involvement in periosteum development. *J Orthop Res* 14:403–412; 1996.
49. Lambert, C. A., Colige, A. C., Munaut, C., Lapiere, C. M., and Nusgens, B. V. Distinct pathways in the over-expression of matrix metalloproteinases in human fibroblasts by relaxation of mechanical tension. *Matrix Biol* 20:397–408; 2001.
50. Le Minor, J. M. Comparative anatomy and significance of the sesamoid bone of the peroneus longus muscle (os peroneum). *J Anat* 151:85–99; 1987.
51. Lewis, J. Growth and determination in the developing limb. In: Ede, D. A., Hinchliffe, J. R. and Balls, M., Eds. *Vertebrate Limb and Somite Morphogenesis: The Third Symposium of the British Society for Developmental Biology*. New York: Cambridge University 1977.
52. Long, F. and Linsenmayer, T. F. Regulation of growth region cartilage proliferation and differentiation by perichondrium. *Development* 125:1067–1073; 1998.
53. Long, P., Hu, J., Piesco, N., Buckley, M., and Agarwal, S. Low magnitude of tensile strain inhibits IL-1beta-dependent induction of pro-inflammatory cytokines and induces synthesis of IL-10 in human periodontal ligament cells in vitro. *J Dent Res* 80:1416–1420; 2001.
54. Mackie, E. J. and Ramsey, S. Modulation of osteoblast behaviour by tenascin. *J Cell Sci* 109:1597–1604; 1996.
55. McLachlan, J. and Wolpert, L. The spatial pattern of muscle development in skeletal limb. In: Goldspink, D. F., Ed. *Development and Specialization of Skeletal Muscle*. New York: Cambridge University 1980.
56. Mikic, B., Johnson, T. L., Chhabra, A. B., Schalet, B. J., Wong, M., and Hunziker, E. B. Differential effects of embryonic immobilization on the development of fibrocartilaginous skeletal elements. *J Rehabil Res Dev* 37:127–133; 2000.
57. Mitrovic, D. Development of the articular cavity in paralyzed chick embryos and in chick embryo limb buds cultured on chorioallantoic membranes. *Acta Anat* 113:313–324; 1982.
58. Mooney, M. P., Siegel, M. I., Burrows, A. M., Smith, T. D., Losken, H. W., Dechant, J., Cooper, G., Fellows-Mayle, W., Kapucu, M. R., and Kapucu, L. O. A rabbit model of human familial, nonsyndromic unicoronal suture synostosis. II. Intracranial contents, intracranial volume, and intracranial pressure. *Child's Nerv Syst* 14:247–255; 1998.
59. Murray, P. D. and Drachman, D. B. The role of movement in the development of joints and related structures: The head and neck in the chick embryo. *J Embryol Exp Morphol* 22:349–371; 1969.
60. Murray, P. D. F. Corio-allantoic grafts of the two-day chick, with special reference to the development of the limbs, intestine and skin. *Aust J Exp Biol Med Sci* 5:237–256; 1928.
61. Murray, P. D. F. An experimental study of the development of the limbs of the chick. *Proc Linn Soc NS Wales* 58:187–263; 1926.
62. Murray, P. D. F. and Huxley, J. S. Self-differentiation in the grafted limb-bud of the chick. *J Anat* 59:379–384; 1925.
63. Oster, G. F., Murray, J. D., and Harris, A. K. Mechanical aspects of mesenchymal morphogenesis. *J Embryol Exp Morphol* 78:83–125; 1983.
64. Pai, A. C. Developmental genetics of a lethal mutation, muscular dysgenesis (mdg) in the mouse: I. Genetic analysis and gross morphology. *Dev Biol* 11:82–92; 1965.
65. Pai, A. C. Developmental genetics of a lethal mutation, muscular dysgenesis (mdg) in the mouse: II. Developmental analysis. *Dev Biol* 11:93–109; 1965.
66. Petermann, A. T., Hiromura, K., Blonski, M., Pippin, J., Monkawa, T., Durvasula, R., Couser, W. G., and Shankland, S. J. Mechanical stress reduces podocyte proliferation in vitro. *Kidney Int* 61:40–50; 2002.
67. Ragan, P. M., Badger, A. M., Cook, M., Chin, V. I., Gowen, M., Grodzinsky, A. J., and Lark, M. W. Down-regulation of chondrocyte aggrecan and type-II collagen gene expression correlates with increases in static compression magnitude and duration. *J Orthop Res* 17:836–842; 1999.
68. Robbins, J. R., Evanko, S. P., and Vogel, K. G. Mechanical loading and TGF-beta regulate proteoglycan synthesis in tendon. *Arch Biochem Biophys* 342:203–211; 1997.
69. Robbins, J. R. and Vogel, K. G. Regional expression of mRNA for proteoglycans and collagen in tendon. *Eur J Cell Biol* 64:264–270; 1994.
70. Rooney, P. and Archer, C. W. The development of the perichondrium in the avian ulna. *J Anat* 181:393–401; 1992.
71. Sarin, V. K. and Carter, D. R. Mechanobiology and joint conformity regulate endochondral ossification of sesamoids. *J Orthop Res* 18:706–712; 2000.
72. Sato, M., Ochi, T., Nakase, T., Hirota, S., Kitamura, Y., Nomura, S., and Yasui, N. Mechanical tension-stress induces expression of bone morphogenetic protein (BMP)-2 and BMP-4, but not BMP-6, BMP-7, and GDF-5 mRNA, during distraction osteogenesis. *J Bone Miner Res* 14:1084–1095; 1999.
73. Schaller, S. A., Li, S., Ngo-Muller, V., Han, M. J., Omi, M., Anderson, R., and Muneoka, K. Cell biology of limb patterning. *Int Rev Cytol* 203:483–517; 2001.
74. Simon, H. T-box genes and the formation of vertebrate forelimb- and hindlimb specific pattern. *Cell Tissue Res* 296:57–66; 1999.
75. Smith, R. L., Rusk, S. F., Ellison, B. E., Wessells, P., Tsuchiya, K., Carter, D. R., Caler, W. E., Sandell, L. J., and Schurman, D. J. In vitro stimulation of articular chondrocyte mRNA and extracellular matrix synthesis by hydrostatic pressure. *J Orthop Res* 14:53–60; 1996.
76. Sperber, G. H. *Craniofacial Development*. New York: Decker; 2001.
77. Stewart, D. M. Effect of age on the response of four muscles of the rat to denervation. *Am J Physiol* 214:1139–1146; 1968.
78. Stewart, D. M. The role of tension in muscle growth. In: Goss, R. J. Ed. *Regulation of Organ and Tissue Growth*. New York: Academic 1972.
79. Taber, L. A. Biomechanical growth laws for muscle tissue. *J Theor Biol* 193:201–213; 1998.
80. Taber, L. A. Biomechanics of growth, remodeling, and morphogenesis. *Appl Mech Rev* 48:487–545; 1995.
81. Valhmu, W. B., Stazzone, E. J., Bachrach, N. M., Saed-Nejad, F., Fischer, S. G., Mow, V. C., and Ratcliffe, A. Load-controlled compression of articular

- cartilage induces a transient stimulation of aggrecan gene expression. *Arch Biochem Biophys* 353:29–36; 1998.
82. Wezeman, F. H. Morphological foundations of precartilage development in mesenchyme. *Microsc Res Techn* 43:91–101; 1998.
 83. Wolpert, L. Cartilage morphogenesis in the limb. In: Abercrombie, M., Bel-lairs, R., Curtis, A. S. G., and Dunn, G., Eds. *Cell Behaviour: A Tribute to Michael Abercrombie*. New York: Cambridge University; 1982.
 84. Wolpert, L. Cellular basis of skeletal growth during development. *Br Med Bull* 37:215–219; 1981.
 85. Wong, M., Siegrist, M., Goodwin, K., and Park, Y. Hydrostatic pressure, tension and unconfined compression differentially regulate expression of cartilage matrix proteins. *Trans Ortho Res Soc* 27:33; 2002.
 86. Wu, Q., Long, F., Linsenmayer, T. F., and Chen, Q. Biomechanical regulation of type X gene expression by indian hedgehog/bone morphogenetic protein pathway. *Trans Ortho Res Soc* 27:34; 2002.
 87. Wu, Q., Long, F., Linsenmayer, T. F., and Chen, Q. Identification of a mechanoresponsive region in the promoter of type X collagen gene. *Trans Ortho Res Soc* 26:79; 2001.
 88. Wu, Q., Zhang, Y., and Chen, Q. Indian hedgehog is an essential component of mechanotransduction complex to stimulate chondrocyte proliferation. *J Biol Chem* 276:35290–35296; 2001.
 89. Wu, Q. Q. and Chen, Q. Mechanoregulation of chondrocyte proliferation, maturation, and hypertrophy: Ion-channel dependent transduction of matrix deformation signals. *Exp Cell Res* 256:383–391; 2000.
 90. Zheng, W., Seftor, E. A., Meininger, C. J., Hendrix, M. J., and Tomanek, R. J. Mechanisms of coronary angiogenesis in response to stretch: Role of VEGF and TGF-beta. *Am J Physiol Heart Circ Physiol* 280:H909–H917; 2001.
-

Date Received: May 3, 2002

Date Revised: August 9, 2002

Date Accepted: September 9, 2002