The skeleton is a complex multifunctional system. In addition to its mechanical/structural support function, it contains the marrow in which blood cells are made and, therefore, is a critical part of the circulatory and immune systems. Also, in that it is the major reservoir for the essential element calcium, a critical component of intracellular signaling pathways, the skeleton is an integral component of the endocrine system. Furthermore, the skeleton is a dynamic system that is subject to modification (remodeling) throughout life under the influence of both intrinsic (chemical) signals and extrinsic (mechanical) signals. Therefore, it is axiomatic that properly regulated crosstalk between the biochemistry and physiology of the skeleton and the chemical biology of the organism is of critical importance both in the complex processes of development and the maintenance of physiologic homeostasis. Thus, gaining insight in the nature and regulation of these interactions is of considerable interest to researchers and clinicians in a broad spectrum of biomedical disciplines.

Bone is formed during embryonic life and grows (formation exceeds resorption) rapidly through childhood. In humans, growth peaks around 20 yr of age. Thereafter, the skeleton enters a prolonged period (lasting approx 40 yr) when bone mass remains relatively stable. During this period, resorption and reformation (remodeling) of both cortical and trabecular bone occur continuously and contemporaneously, resulting in an annual turnover of approx 10% of the adult skeleton with essentially no net effect on bone mass. The maintenance of skeletal mass is regulated through a balance between the activity of cells that resorb bone (osteoclasts) and those that form bone (osteoblasts). Unfortunately, the balance between resorption and formation degenerates with age and, if uncompensated, can have debilitating consequences. For women, the balance terminates at menopause. Bone loss also occurs in men, but usually later in life. Clinical disorders in which bone resorption exceeds formation are common and include osteoporosis, Paget’s disease of bone, and bone wasting secondary to such cancers as myeloma and metastatic breast cancer. Osteoporosis is the most common bone resorption disorder. It affects one in three women after the fifth decade of life. The pathophysiology of this condition includes genetic predisposition and alteration of systemic and local hormone levels coupled with environmental influences. Treatment is based on drugs that inhibit bone resorption either directly or indirectly: bisphosphonates, calcitonin, estrogens, and synthetic estrogen-related compounds (SERMs—selective estrogen receptor modulators).

The search for more effective anti-osteoporosis drugs with fewer side effects continues. In this regard, it is of both great interest and potentially enormous import to note that recent evidence indicates that low bone mineral density (BMD) appears to protect women over the age of 65 from primary breast cancer. It was reported that women in the highest BMD quartile have approximately three times the risk of developing bone cancer than those in the lowest quartile. Also, those with the highest BMD, obtained from measurements of the wrist, forearm and heel, have almost six times the risk of advanced disease.

Less prevalent than disorders of bone loss are clinical disorders of reduced bone resorption, such as osteopetrosis, and pycnodysostosis (owing to cathepsin K deficiency), that are the consequence of genetic defects. Unfortunately, progress in the search for effective treatments for these orphan diseases often is stymied by lack of support.
Significant insight into many aspects of vertebrate skeletal development has been obtained through molecular and genetic studies of animal models and humans with inherited disorders of skeletal morphogenesis, organogenesis, and growth. Morphogenesis, the developmental process of pattern formation and the establishment of the body plan that is the template for the architecture of the adult form, is an exquisitely complicated program. Our understanding of it contains many gaps. The information for the pattern and form of the vertebrate skeleton emanates from mesenchymal cells during embryonic development. Morphogenesis requires three key ingredients: inductive signals, responding stem cells and a supportive extracellular matrix. Within the vertebrate morphogenetic program, skeletal development is controlled by sequence-dependent activation/inactivation of specific genes that results in the distribution of cells from cranial neural crest, sclerotomes, and lateral plate mesoderm into a pattern of mesenchymal condensations at sites in which skeletal elements will develop. Condensation is the earliest stage of organ formation at which tissue-specific genes are upregulated. It is generated through interactions between molecules in the extracellular matrix such as the cell adhesion molecules fibronectin, N-CAM and N-cadherin. Cell adhesion also is mediated, albeit indirectly, via activation of particular CAM genes by the products of the Hox genes, Hoxa-2 and Hoxd-13. Cells proliferate and differentiate, under the control of transcription factors, into chondrocytes or osteoblasts forming, respectively, cartilage or bone. Proliferation within the condensations is mediated through the activation of cell surface receptors such as syndecan-3, a receptor for fibroblast growth factor 2 (FGF-2), the antiadhesive matrix component, tenasin-C, a ligand for the epidermal growth factor (EGF) receptor (EGFR), the Hox genes, Hoxd-11-13 and transcription factors such as CFPKH-1, MFH-1, and osf-2. Growth of condensations is regulated by BMPs, which activate a number of genes including Pax-2, Hoxa-2, and Hoxd-11. Conversely, growth is blocked via inhibition of BMP signaling by the BMP antagonist, Noggin. Defects in the formation of specific bones and joints can occur through mutation of genes involved in the control of bone and joint development. Information derived from ongoing and future research focused on the identification of the genes/gene targets involved in skeletal development and maintenance should open new avenues for the development of therapeutic measures for treating defects resulting either from mutation or trauma.

For most of the skeleton, bones develop from cartilage models comprised of assemblies of chondrocytes in an extracellular collagen-containing matrix that they secrete. The replacement of cartilage by bone is the result of a genetic master program that controls and coordinates chondrocyte differentiation, matrix alteration and mineralization. During the conversion of the cartilage model into bone, the composition of the matrix, including collagen types, is modified, ultimately becoming mineralized through a process termed endochondral ossification and populated by osteocytes. Disruption of the rate, timing, or duration of chondrocyte proliferation and differentiation results in shortened, misshapen skeletal elements. In the majority of such disruptions, vascularization also is perturbed. It has been proposed that vascularization plays a key role in the synchronization of the processes involved in endochondral ossification. Bone formation also occurs via intramembranous ossification, in which bone cells arise directly from mesenchyme without an intermediate cartilage anlage. Data indicate that this process is the result both of a positive selection for osteogenic differentiation and a negative selection against the progressive growth of chondrogenic cells in the absence of a permissive
or inductive environment. In any case, through the processes of bone growth and remodeling, an adult skeleton is shaped and molded and continually remodeled in response to environmental alterations. In effect, the adult skeleton is not a static entity. Bone is metabolically active throughout life and, under the influence of mechanical stress, nutrition, and hormones, bone remodeling occurs continually. However, bone remodeling is compromised as a function of both post-menopausal hormonal changes and aging, resulting in health problems of increasing magnitude as the proportion of the aged in the global population increases.

Mutations in genes encoding structural proteins of the extracellular matrix can perturb the coordination of events necessary for normal skeletal development. The magnitude of the disruption of the process of ordered skeletal development is dependent on both the role of the mutated gene product in the developmental process and the degree of its functional perturbation. The range of mutational consequences is broad, including disruption of ossification/mineralization and linear growth and the structural integrity and stability of articular cartilage. Evidence indicates that osteochondrodysplasias resulting from defects in structural proteins are inherited in an autosomal dominant manner and that a spectrum of related clinical phenotypes can be produced by different mutations in the same gene. In addition, as might be expected, haploinsufficiency of a gene product usually produces a milder clinical phenotype than do mutations resulting in the synthesis of highly structurally abnormal proteins. The synthesis of structurally abnormal protein can produce a dominant-negative effect that is the primary determinant of phenotype. Thus, inherited defects that interfere with post-translational modification of matrix proteins such as hydroxylation, sulfation and/or proteolytic cleavage, can result in distinct osteochondrodysplasias. In the future, it may be possible to identify genes and pathways that can maintain, repair, or stimulate the regeneration of bone and joint structures at post patterning stages of development.

In this regard, it is to be noted that metabolites of vitamin A, including retinoic acid (RA), comprise a class of molecules that are of critical importance in development and homeostasis. Retinoic acid functions through a class of nuclear hormone receptors, the RA receptors (RARs), to regulate gene transcription. Retinoic acid receptor-mediated signaling plays a fundamental role in skeletogenesis. In the developing mammalian limb, RA induces the differentiation of a number of cell lineages including chondrocytes. However, excess RA is a potent teratogen that induces characteristic skeletal defects in a stage- and dose-dependent manner. Genetic analyses have shown that RAR deficiency results both in severe deficiency of cartilage formation in certain anatomical sites and the promotion of ectopic cartilage formation in other sites. In the developing limbs of transgenic mice expressing either dominant-negative or weakly constitutively active RARs, chondrogenesis is perturbed, resulting in a spectrum of skeletal malformations. Recently, RA was reported to bind two circadian clock proteins, Clock and Mop4, and may play a role in regulating circadian rhythms. Thus, it may be possible to utilize these interactions to manipulate the body’s response to therapeutic drugs, which is entrained in the circadian flow.

A number of growth factors interact with osteoblasts or their precursors during bone development, remodeling or repair. Traditionally, morphogenetic signals have been studied in embryos. However, it was observed that implantation of demineralized adult bone matrix into subcutaneous sites in a variety of species resulted in local bone induction. Not
only did this model system mimic the process of limb morphogenesis, it also permitted the isolation of bone morphogenetic proteins (BMPs). The BMPs constitute a large family of morphogenetic proteins within the transforming growth factor-β (TGF-β) superfamily. It is to be emphasized that these morphogens and related cartilage-derived morphogenetic proteins (CDMPs) that initiate, promote, and maintain chondrogenesis, have actions on systems other than bone. Indeed, bone morphogenetic proteins are multifunctional growth factors involved in many aspects of tissue development and morphogenesis, including, for example, regulation of FSH action in the ovary. The mechanism underlying the phenomenon of bone matrix-induced bone induction is under intense investigation by biomedical engineers and orthopedic researchers.

Growth/differentiation factor-5 (GDF-5), a BMP family member, has been shown to be essential for normal appendicular skeletal and joint development in humans and mice. It has been reported that GDF-5 promotes the initial stages of chondrogenesis by promoting cell adhesion and increased cell proliferation. In the mouse GDF-5 gene mutant brachypod, the defect is manifested early in chondrogenesis (embryonic day [E]12.5) as a reduction in the size of the cartilage blastema. The defect is associated with a decrease in the expression of cell surface molecules resulting in a decrease in cell adhesiveness and, consequently, perturbation of cartilage model competence. Another member of the family, BMP-6, has been shown to be overexpressed in prostate cancer and appears to be associated with bone-forming skeletal metastases. In the United States, prostate cancer became the number one cancer among white males in the mid-1980s and has increased dramatically since then. A study of benign and malignant prostate lesions by in situ hybridization showed that BMP-6 expression was high at both primary and secondary sites in cases of advanced cancer with metastases. Does upregulation of BMP-6 promote metastasis or is it involved in the body’s defense armamentarium? Is it a target for therapeutics? Such questions are under active investigation by cancer researchers.

Two families of growth factors, the TGF-β superfamily and the insulin-like growth factors (IGF) superfamily, appear to be the principal proximal regulators of osteogenesis. However, these growth factors are not specific for cells of the osteoblast lineage. The mechanism by which skeletal tissue is specifically induced and maintained involves both complex interactions among circulating hormones, growth factors, and regulators of the activity of specific genes. For example, nuclear transcription factors such as core binding factor a1 (Cbfa1), a transcription factor essential for osteoblast differentiation and bone formation, and CCAAT/enhancer binding protein b (C/EBPb), that function as regulators of the expression/activity of specific bone growth factors and receptors, are activated in response to glucocorticoids, sex steroids, parathyroid hormone (PTH), and prostaglandin E2 (PGE2). Many environmentally available chemicals, both natural and man-made, have either sex steroid or anti-sex steroid activity. Evidence suggests that such chemicals have negatively impacted fish populations and other animals by interfering with the mechanism of action of reproductive hormones. However, their impact on other mechanisms such as growth have not been thoroughly investigated.

Members of the tumor necrosis factor (TNF) family of ligands and receptors have been identified as critical regulators of osteoclastogenesis. Osteoprotegerin (OPG), a member of the TNF receptor family, plays a key role in the physiological regulation of osteoclastic bone resorption. OPG, a secreted decoy receptor produced by osteoblasts and marrow stromal cells, acts by binding to its natural ligand, OPGL (also known as RANKL [recep-
tor activator of NF-κB ligand), thereby preventing OPGL from activating its cognate receptor RANK, the osteoclast receptor vital for osteoclast differentiation, activation and survival. In vitro studies have suggested that estrogen stimulates OPG expression whereas parathyroid hormone (PTH) inhibits its expression and stimulates the expression of RANKL. This construct provides a molecular mechanism for the regulation of the osteoclastic bone resorption and osteoblastic bone formation couple and basis for the bone loss of postmenopausal osteoporosis, aging and pathologic skeletal changes (e.g., osteopetrosis, glucocorticoid-induced osteoporosis, periodontal disease, bone metastases, Paget’s disease, hyperparathyroidism, and rheumatoid arthritis). Environmental toxicants and endocrine disruptors also may perturb the normal balance between osteoclastic and osteoblastic activity by interfering with homeostasis and/or accelerating aging processes. With regard to endocrine disruption, OPG has been linked to vascular disease, particularly arterial calcification in estrogen-deficient individuals, the aged, and those afflicted with immunological deficits.

During skeletogenesis, cartilage matures either into permanent cartilage that persists as such throughout the organism’s life or transient cartilage that ultimately is replaced by bone. How cartilage phenotype is specified is not clear. In vitro studies have shown that Cbfa1 is involved in induction of chondrocyte maturation. In this regard, it is of interest to note that transgenic mice overexpressing either Cbfa1 or a dominant-negative (DN)-Cbfa1 in chondrocytes exhibit dwarfism and skeletal malformations. These phenotypes are mediated through opposing mechanisms. In the former case, Cbfa1 overexpression accelerates endochondral ossification resulting from precocious chondrocyte maturation whereas in the latter, DN-Cbfa1 overexpression suppresses maturation and delays endochondral ossification. In addition, mice overexpressing Cbfa1 fail to form most of their joints and what would be permanent cartilage in normal mice enters the endochondral pathway of ossification. In contrast, in DN-Cbfa1 transgenic mice, most chondrocytes exhibit a marker for permanent cartilage. It may be concluded from these observations that proper temporal and spatial expression of chondrocyte Cbfa1 is required for normal skeletogenesis, including formation of joints, permanent cartilage, and endochondral bone.

Both gain-of-function and loss-of-function mutations in fibroblast growth factor receptor 3 (FGFR3) have revealed unique roles for this receptor during skeletal development. Loss-of-function alleles of FGFR3 lead to an increase in the size of the hypertrophic zone, delayed closure of the growth plate and the subsequent overgrowth of long bones. Gain-of-function mutations in FGFR3 have been linked genetically to autosomal dominant dwarfing chondrodysplasia syndromes in which both the size and architecture of the epiphyseal growth plate are altered. Analysis of these phenotypes and the biochemical consequences of the mutations in FGFR3 demonstrate that FGFR3-mediated signaling is an essential negative regulator of endochondral ossification.

Thorough understanding of bone physiology and how it is modified throughout all stages of life, from in utero development to advanced age, is of great current interest for its potential application to the establishment of criteria for the achievement and maintenance of bone health and the reestablishment of bone health following trauma and disease. Other clinical applications include:

- Establishment of criteria for the achievement of optimal bone strength throughout life, its maintenance in such long-term microgravity situations as space travel, and the
facilitation of readjustment to normogravity upon return to earth. This will require establish-
ment of rapid and precise methods for distinguishing mechanically competent bone from incompetent bone.
• Establishment of optimal conditions for the healing of fractures, osteotomies, and arthrodoses.
• Understanding the mechanics of induction by falling of metaphyseal and diaphyseal fractures of the radius in children, but primarily metaphyseal fractures in the aged.
• Improvement of the endurance of load-bearing implants.
• Understanding the mechanism(s) of osteopenia and osteoporosis and how and why, during menopause, healthy women lose only bone adjacent to marrow.

Furthermore, because of the multifunctionality and interactions of the skeletal system, biomedical researchers and practitioners of almost every clinical discipline have great interest in bone biology. Even a cursory review of the bone biology literature will reveal the depth of interest in the field. Publications emanate from a broad spectrum of biomedical areas that include: adolescent medicine, anatomy, anthropology, biochemistry, biomechanics, biomedical engineering, biophysics, cardiology, cell and molecular biology, clinical nutrition research, dentistry, developmental biology, endocrinology, enzymology, epidemiology, food science, genetics, genetic counseling, gerontology, hematology, histology, human nutrition, internal medicine, medicinal chemistry, metabolism, microbiology, neurology, oncology, orthopedics, pediatric medicine, pharmacology and therapeutics, physical and rehabilitation medicine, physiology, plastic surgery, public health, radiology and imaging research, space and sports medicine, trace/essential element research, vascular biology, vitaminology and cofactor research, women’s health, teratology, and toxicology.

Bone biology is a diverse field, and our goal in developing The Skeleton: Biochemical, Genetic, and Molecular Interactions in Development and Homeostasis was to provide researchers and students with an overview of selected topics of current interest in bone biology and to stimulate their interest in this fascinating and diverse field.

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