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Review

The life cycle of chondrocytes in the developing skeleton

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Abstract

Cartilage serves multiple functions in the developing embryo and in postnatal life. Genetic mutations affecting cartilage development are relatively common and lead to skeletal malformations, dysfunction or increased susceptibility to disease or injury. Characterization of these mutations and investigation of the molecular pathways in which these genes function have contributed to an understanding of the mechanisms regulating skeletal patterning, chondrogenesis, endochondral ossification and joint formation. Extracellular growth and differentiation factors including bone morphogenetic proteins, fibroblast growth factors, parathyroid hormone-related peptide, extracellular matrix components, and members of the hedgehog and Wnt families provide important signals for the regulation of cell proliferation, differentiation and apoptosis. Transduction of these signals within the developing mesenchymal cells and chondrocytes results in changes in gene expression mediated by transcription factors including Smads, Msx2, Sox9, signal transducer and activator of transcription (STAT), and core-binding factor alpha 1. Further investigation of the interactions of these signaling pathways will contribute to an understanding of cartilage growth and development, and will allow for the development of strategies for the early detection, prevention and treatment of diseases and disorders affecting the skeleton.

Keywords: cartilage, chondrogenesis, endochondral ossification, limb bud, neural crest cells

Introduction

Cartilage is a connective tissue of diverse embryonic origin, that serves multiple prenatal and postnatal functions. Cartilage provides structural support for the early embryo, forms a template for developing endochondral bones, provides for rapid postnatal growth of the skeleton, cushions the joints, allows for flexible facial structure, and repairs fractured bones. Chondrocytes exhibit a life cycle of proliferation, differentiation, maturation, and apoptosis; the rate of each of these processes is dependent on temporal and spatial cues within the body. Identifying and characterizing these cues will reveal the molecular basis of cartilage form and function. Mutations or deregulation of these determinants of chondrogenesis and cartilage development can lead to skeletal malformation, limited skeletal function, or predisposition to injury. Congenital skeletal malformations are common and can be caused by a number of factors: inherited individual or multiple gene mutations; or acquired gene–environment interactions. Recent progress in linkage analysis and positional cloning...

BMP = bone morphogenetic protein; bp = brachypodism; Cbfa1 = core-binding factor alpha 1; CDMP1 = cartilage-derived morphogenetic protein 1; CNCC = cranial neural crest cell; EO = endochondral ossification; FGF = fibroblast growth factor; FGFR = fibroblast growth factor receptor; HMG = high-mobility group; Ihh = Indian hedgehog; PTHrP = parathyroid hormone-related peptide; VEGF = vascular endothelial growth factor.
has identified many genetic mutations associated with human skeletal syndromes or predispositions to certain skeletal diseases (Table 1). Sequencing the human genome also allows for the identification of the genetic loci of many bone- and cartilage-associated genes that serve as candidate links with additional skeletal disorders. Because of genetic background or gene–environment interactions, single gene mutations can cause different disorders. Furthermore, the same phenotype can be the result of mutations in different genes [1]. Complex diseases that are subject to multifactorial influences, such as osteoarthritis [2,3] provide the greatest challenges ahead. An understanding of the mechanisms that regulate chondrogenesis and cartilage development will, therefore, contribute to early gene-based detection of diseases and disorders that affect cartilage, and will provide the necessary foundation for novel prevention and treatment strategies, such as gene therapy and tissue engineering.

**Genetic and biomechanical determinants of chondrogenesis**

Although chondrocytes appear to be a uniform cell type comprising the majority of the cells in cartilage, the origins and elaborations of the cartilage lineage are diverse. Chondrocytes arise from cranial neural crest cells (CNCCs) of the neural ectoderm, cephalic mesoderm, sclerotome of the paraxial mesoderm, or somatopleure of the lateral plate mesoderm. Terminal differentiation of chondrocytes results in different types of cartilage: hyaline; elastic; and fibrous. Chondrocyte differentiation, therefore, provides unique opportunities for the study of ‘what, when and how’ a repertoire of morphogenetic signals are integrated into the developmental program. A number of molecules have been shown to function in cartilage formation. These include classes of extracellular ligands and their cognate receptors and cytoplasmic transducers [4], nuclear receptors [5], transcription factors or DNA-binding proteins [6], matrix proteins [7], matrix modifiers including matrix metalloproteinases [8], adhesion molecules [9] and the cytoskeleton [10]. The functions of these molecules have been reviewed in the literature cited and references therein. Although much is known about the gene products that characterize the cartilage phenotype, very little is known about the combinations of gene products that reflect the genesis of the cartilage cell lineage. Furthermore, the growth and development of the skeleton are particularly susceptible to the influence of biomechanical forces [11]. Mechanical loading regulates the shape, repair, regeneration, and senescence of the skeleton. Mechanical signals are transduced through the extracellular matrices, modify cell–matrix and cell–cell interactions, and impact on transcriptional responses. The interplay, therefore, between genetic and biomechanical determinants controls the integrity of cartilage produced both in vivo and in vitro [12].

**Mesenchymal cell condensation**

Chondroprogenitor mesenchymal cells aggregate into chondrogenic nodules as a necessary step in chondrocyte differentiation. This condensation process is dependent on signals initiated by cell–matrix and cell–cell adhesion, and these signals are modified by the cell’s response to growth and differentiation factors in the extracellular environment. Condensation is hallmarked by changes in cell adhesion and cytoskeletal architecture [9,13]. The roles of N-cadherin, fibronectin, syndecans, tenascins, thrombospondins, neural cell adhesion molecule, focal adhesion kinase and paxillin in chondrogenic condensation have been reported. These molecules are expressed in restricted temporal and spatial patterns that correlate with chondroprogenitor cell condensation. Perturbations of the functions of these molecules leads to disruption in cell aggregation and inhibition of normal cartilage formation. Cell–cell and cell–matrix interactions activate cytoplasmic kinases, phosphatases and GTPases that can, in turn, be modulated by signaling from growth and differentiation factors such as the bone morphogenetic proteins (BMPs) and Wnts [14–16]. Although chondrogenesis is regulated by combinatorial signaling of a large number of factors, cell condensation can be regarded as the major event of the cell’s commitment to the cartilage lineage, after which tissue-specific transcription factors and structural proteins begin to accumulate.

**Bone morphogenetic proteins**

BMPs are a pleiotropic group of extracellular ligands, first coined due to the ability of demineralized bone matrix (containing BMPs) to induce bone formation when injected into muscular compartments of animals [17]. Since then, a large number of molecules of the BMP family, and its superfamily, the transforming growth factor-betas (TGF-βs) have been cloned and identified, with mammalian BMP2 and BMP4 being the prototypes of the Drosophila homologue, decapentaplegic [18,19]. BMPs signal through transmembrane serine threonine kinase receptors [20]. There are two types of receptors, type I and type II, each having a number of subtypes and varying affinities to the different BMPs. Downstream from the receptors are various cytoplasmic and nuclear transducers, both positive and negative [21]. Over the past three decades since the discovery of BMPs, their ability to induce ectopic bone and cartilage formation and the mechanism of induction have been meticulously dissected [22]. BMPs, however, have been demonstrated to function in multiple systems and stages of development [19]. The diversity and specificity, therefore, of cellular competence and response towards BMPs remain to be topics of intense investigation. The pleiotropic actions of BMPs can be concentration-dependent, and BMP signaling can be regulated by positive and negative cellular feedback events [20]. Concomitantly, BMP signals are modulated by BMP binding proteins and other growth and differentia-
Table 1

Selected genes causal to skeletal diseases and disorders

| OMIM #  | Gene name                        | Gene symbol | Diseases and disorders                                                                 |
|---------|----------------------------------|-------------|---------------------------------------------------------------------------------------|
| 114290  | Sry-related HMG-box gene 9       | SOX9        | Acampomelic campomelic dysplasia                                                       |
|         |                                  |             | Campomelic dysplasia                                                                   |
|         |                                  |             | Campomelic dysplasia with autosomal sex reversal                                       |
| 120110  | Collagen, type X alpha-1         | COL10A1     | Metaphyseal chondrodysplasia, Schmid type                                              |
|         |                                  |             | Spondylometaphyseal dysplasia, Japanese type                                           |
| 120140  | Collagen, type II alpha-1        | COL2A1      | Achondrogenesis, type II                                                               |
|         |                                  |             | Achondrogenesis-hypochondrogenesis, type II                                            |
|         |                                  |             | Hypochondrogenesis                                                                     |
|         |                                  |             | Kniest dysplasia                                                                       |
|         |                                  |             | Osteoarthritis with mild chondrodysplasia                                              |
|         |                                  |             | Spondyloepimetafysal dysplasia, Strudwick type                                         |
|         |                                  |             | Spondyloepiphyseal dysplasia, various types                                            |
|         |                                  |             | Spondylometaphyseal dysplasia, congenital type                                         |
|         |                                  |             | Spondyloephiperal dysplasia                                                            |
|         |                                  |             | Stickler syndrome, type I                                                              |
|         |                                  |             | Wagner syndrome                                                                        |
| 120150  | Collagen, type I alpha-1         | COL1A1      | Ehlers–Danlos syndrome, types I and VII                                               |
|         |                                  |             | Osteogenesis imperfecta, types I, II, III, and IV                                       |
| 120160  | Collagen, type I alpha-2         | COL1A2      | Ehlers–Danlos syndrome, type VII-B                                                     |
|         |                                  |             | Osteogenesis imperfecta, types II, III, and IV                                         |
|         |                                  |             | Osteogenesis imperfecta/Ehlers–Danlos crossover syndrome                               |
|         |                                  |             | Marfan syndrome, atypical                                                               |
| 120180  | Collagen, type III alpha-1       | COL3A1      | Arterial and aortic aneurysms                                                          |
|         |                                  |             | Ehlers–Danlos syndrome, types III and IV                                               |
| 120190  | Collagen, type V alpha-2         | COL5A2      | Ehlers–Danlos syndrome, types I and II                                                 |
| 120215  | Collagen, type V alpha-1         | COL5A1      | Ehlers–Danlos syndrome, types I, II, and mixed type                                    |
| 120260  | Collagen, type IX alpha-2        | COL9A2      | Epiphyseal dysplasia, multiple, type 2                                                 |
|         |                                  |             | Intervertebral disc disease                                                            |
| 120270  | Collagen, type IX alpha-3        | COL9A3      | Epiphyseal dysplasia, multiple, type 3                                                 |
|         |                                  |             | Epiphyseal dysplasia, multiple, with myopathy                                          |
| 120280  | Collagen, type XI alpha-1        | COL11A1     | Stickler syndrome, type II                                                             |
|         |                                  |             | Marshall syndrome                                                                      |
| 120290  | Collagen, type XI alpha-2        | COL11A2     | Sensorineural deafness, autosomal dominant nonsyndromic                               |
|         |                                  |             | Otospondylomegaephysal dysplasia                                                       |
|         |                                  |             | Stickler syndrome, type III                                                            |
|         |                                  |             | Weissenbacher–Zweymuller syndrome                                                      |
| 120380  | Matrix metalloproteinase 2       | MMP2        | Osteolysis, idiopathic, Saudi type                                                     |
| 121050  | Fibrillin 2                      | FBN2        | Contractural arachnodactyly, congenital                                                |
| 123101  | Muscle segment homeobox 2        | MSX2        | Craniosynostosis, Boston-type                                                         |
|         |                                  |             | Parietal foramina 1                                                                    |
| 125505  | Deoxyribonuclease I              | DNASE1      | Systemic lupus erythematosus susceptibility                                             |
| 133700  | Exostosin 1                      | EXT1        | Exostoses, multiple, type 1                                                            |
|         |                                  |             | Chondrosarcoma                                                                        |
| 133701  | Exostosin 2                      | EXT2        | Exostoses, multiple, type II                                                           |
| 134797  | Fibrillin 1                      | FBN1        | Marfan syndrome, various types                                                         |
|         |                                  |             | Ectopia lentis, familial                                                               |
|         |                                  |             | Marfanoid skeletal syndrome                                                            |
|         |                                  |             | Mass syndrome                                                                         |
|         |                                  |             | Shprintzen–Goldberg syndrome                                                           |

Continued opposite
| OMIM #   | Gene name                                      | Gene symbol | Diseases and disorders                                                                 |
|---------|-----------------------------------------------|-------------|----------------------------------------------------------------------------------------|
| 134934  | Fibroblast growth factor receptor 3           | FGFR3       | Achondroplasia                                                                         |
|         |                                               |             | Crouzon syndrome with acanthosis nigricans                                            |
|         |                                               |             | Hypochondroplasia                                                                       |
|         |                                               |             | Muenke syndrome                                                                         |
|         |                                               |             | Multiple myeloma                                                                        |
|         |                                               |             | Saddan dysplasia                                                                        |
|         |                                               |             | Thanatophoric dysplasia, types I and II                                                |
| 136350  | Fibroblast growth factor receptor 1           | FGFR1       | Pfeiffer syndrome                                                                       |
| 139250  | Growth hormone 1                              | GH1         | Growth hormone deficiency                                                                |
|         |                                               |             | Isolated growth hormone deficiency, type I                                              |
|         |                                               |             | Kowarski syndrome                                                                       |
| 139320  | Guanine nucleotide-binding protein, alpha-stimulating activity polypeptide 1 | GNAS1       | McCune–Albright syndrome                                                                  |
|         |                                               |             | Albright hereditary osteodystrophy                                                       |
|         |                                               |             | Pituitary adenoma, ACTH-secreting                                                        |
| 142461  | Heparan sulfate proteoglycan of basement membrane, perlecan | HSPG2       | Schwartz–Jampel syndrome, type 1                                                          |
|         |                                               |             | Dyssegmental dysplasia, Silverman–Handmaker type                                           |
| 142958  | Homeobox A11                                  | HOXA11      | Radioulnar synostosis with amegakaryocytic thrombocytopenia                              |
| 147620  | Interleukin 6                                 | IL6         | Interleukin 6 polymorphism associated with systemic onset juvenile rheumatoid arthritis |
| 154870  | Matrix gamma-carboxyglutamic acid protein     | MGP         | Keutel syndrome                                                                         |
| 156845  | Microphthalmia-associated transcription factor | MITF        | Waardenburg syndrome, type IIA                                                            |
| 157660  | Mitochondrial RNA-processing endonuclease (RNA component of) | RMRP        | Tietz albinism–deafness syndrome                                                         |
| 168450  | Parathyroid hormone                           | PTH         | Hypoparathyroidism                                                                      |
| 168468  | Parathyroid hormone receptor 1                | PTHR1       | Metaphyseal chondrodysplasia, Murk Jansen type                                           |
|         |                                               |             | Chondrodysplasia, Blomstrand type                                                        |
| 176943  | Fibroblast growth factor receptor 2           | FGFR2       | Apert syndrome                                                                          |
|         |                                               |             | Beare–Stevenson cutis gyrata syndrome                                                    |
|         |                                               |             | Craniosynostosis, nonsyndromic unicoronal                                                |
|         |                                               |             | Crouzon syndrome                                                                        |
|         |                                               |             | Jackson–Weiss syndrome                                                                   |
|         |                                               |             | Pfeiffer syndrome                                                                        |
|         |                                               |             | Saethre–Chotzen syndrome                                                                 |
| 190180  | Transforming growth factor, beta-1            | TGFB1       | Camurati–Engelmann disease                                                               |
| 193500  | Paired box gene 3                             | PAX3        | Waardenburg syndrome, types I, II, and III                                               |
|         |                                               |             | Waardenburg syndrome with meningomyelocele                                               |
|         |                                               |             | Rhabdomyosarcoma, alveolar                                                               |
|         |                                               |             | Craniofacial–deafness–hand syndrome                                                      |
| 203500  | Homogentisate 1,2-dioxygenase                 | HGD         | Alkaptonuria                                                                             |
| 217000  | Complement component 2                        | C2          | Complement component 2 deficiency                                                       |
| 222600  | Solute carrier family 26, member 2            | SLC26A2     | Achondrogenesis, type IB                                                                  |
|         |                                               |             | Atelosteogenesis, type II                                                                |
|         |                                               |             | Diastrophic dysplasia                                                                    |
| 249100  | Familial Mediterranean fever gene             | MEFV        | Familial Mediterranean fever                                                             |
| 277900  | ATPase, Cu(2+)-transporting, beta polypeptide | ATP7B       | Wilson Disease                                                                           |
| 300202  | Sedlin                                        | SEDL        | Spondyloepiphyseal dysplasia, late                                                        |
| 300300  | Bruton agammaglobulinemia tyrosine kinase      | BTK         | Agammaglobulinemia, X-linked associated with septic arthritis                           |
| OMIM # | Gene name                                      | Gene symbol | Diseases and disorders                                                                 |
|--------|-----------------------------------------------|-------------|----------------------------------------------------------------------------------------|
| 308000 | Hypoxanthine guanine phosphoribosyltransferase 1 | HPRT1       | Gout, HPRT-related                                                                     |
| 311850 | Phosphoribosylpyrophosphate synthetase 1       | PRPS1       | Gout, PRPS-related                                                                     |
| 312865 | Short stature homeobox                        | SHOX        | Short stature, idiopathic, Leri−Weill dyschondroosteosis, Langer mesomelic dysplasia    |
| 600211 | Runt-related transcription factor 2            | RUNX2       | Cleidocranial dysplasia                                                                |
| 600310 | Cartilage oligomeric matrix protein            | COMP        | Epiphyseal dysplasia, Pseudoachondroplasia                                             |
| 600725 | Sonic hedgehog                                | SHH         | Holoprosencephaly 3                                                                   |
| 600726 | Indian hedgehog                               | IHH         | Brachydactyly type A1                                                                  |
| 600856 | Cyclin-dependent kinase inhibitor 1C           | CDKN1C      | Beckwith−Wiedemann syndrome, Short stature, idiopathic                                  |
| 600946 | Growth hormone receptor                       | GHR         | Laron syndrome, Short stature, autosomal dominant and idiopathic                       |
| 601105 | Cathepsin K                                   | CTSK        | Pycnodysostosis                                                                       |
| 601146 | Growth/differentiation factor 5                | GDF5        | Acromesomelic dysplasia, Hunter−Thompson type, Brachydactyly, type C, Chondrodysplasia, Grebe type |
| 601199 | Calcium-sensing receptor                       | CASR        | Hypercalcuiic hypercalcemia, Hypercalcuiic hypocalcemia                                |
| 601309 | Patched                                       | PTCH        | Basal cell nevus syndrome, Basal cell carcinoma, sporadic                              |
| 601769 | Vitamin D receptor                            | VDR         | Vitamin D-resistant rickets, type II                                                   |
| 602337 | Receptor tyrosine kinase-like Orphan receptor 2 | ROR2        | Brachydactyly, type B1                                                                 |
| 602365 | Cathepsin C                                   | CTSC        | Robinow syndrome, autosomal recessive                                                   |
| 602727 | Chloride channel 7                            | CLCN7       | Osteopetrosis, Autosomal Recessive, Infantile Malignant                                 |
| 602991 | Noggin                                        | NOG         | Symphalangism, proximal                                                                |
| 603000 | Wnt1-inducible signaling pathway protein 3     | WISP3       | Arthropathy, progressive pseudorheumatoid of childhood                                 |
| 603499 | Tumor necrosis factor receptor superfamily, 11A | TNFRSF11A   | Expansile osteolysis, familial                                                         |
| 604142 | Tyro protein tyrosine kinase-binding protein   | TYROBP      | Paget disease of bone 2                                                                |
| 604283 | Proteoglycan 4                                | PRG4        | Camptodactyly−arthropathy−coxar vara−pericarditis syndrome                              |
| 604592 | T cell immune regulator 1                     | TCIRG1      | Osteopetrosis, autosomal recessive                                                     |
| 604831 | Ellis−Van Creveld syndrome gene                | EVC         | Ellis−Van Creveld syndrome, Weyers acroental dysostosis                                |
| 605145 | Ank                                           | ANKH        | Craniometaphyseal dysplasia, autosomal dominant                                         |
| 605380 | Fibroblast growth factor 23                   | FGF23       | Hypophosphatemic rickets, autosomal dominant                                          |
| 605420 | Aristaless-like 4                             | ALX4        | Parietal foramina 2                                                                   |
| 605740 | Sclerostin                                    | SOST        | Sclerosteosis                                                                         |

Mutations in a number of genes have been shown to cause congenital skeletal disorders, often with defects in cartilage formation as the primary basis. Others predispose the individual towards skeletal diseases such as arthritis. The completed sequence of the human genome opens the door for rapid identification of additional genetic mutations associated with human diseases and disorders. Functional genomics and the characterization of molecular mechanisms bridging genotypes to phenotypes are our challenges to realize solutions for the prevention, detection, diagnosis and therapy of these diseases and disorders. Data extracted from Online Mendelian Inheritance in Man (OMIM) [113].
tion factors, resulting in combinatorial signaling and divergent outcomes dependent on the modifiers, which can be either genetic or environmental [23].

**Sox9**

The Sry-type, high-mobility group (HMG)-box containing transcription factor SOX9 comes closest to serving the function of a master regulator of the chondrocyte lineage of any molecule yet characterized. Sox9 expression is directly induced by BMP signaling [24–26]. In humans, SOX9 haploinsufficiency (Online Mendelian Inheritance in Man [OMIM] number 114290) results in campomelic dysplasia (a lethal skeletal malformation syndrome) with XY sex reversal [27]. During embryogenesis, Sox9 is expressed in all chondroprogenitors, coincident with the expression of type II collagen. Sox9 regulates chondrogenesis through binding to essential DNA sequence motifs in chondrocyte-specific enhancer elements of the type II and type XI collagen genes and the cartilage-derived retinoic-acid-sensitive protein. Sox9 can even bind to, and activate, these DNA enhancer sequences in cartilage genes that have been transfected into nonchondrocytes [28–30]. Mouse embryonic stem cells with null mutations of Sox9 do not form cartilage in teratomas [31]. Animals that are heterozygous null for Sox9 exhibit defects in all cartilage primordia and present a phenotype similar to human campomelic dysplasia [32]. The phosphorylation of Sox9 by cAMP-dependent protein kinase A in response to parathyroid hormone-related peptide (PTHrP) signaling regulates the binding of Sox9 to responsive elements in the collagen promoters [33,34]. Furthermore, Sox9 is known to form complexes with L-Sox5 and Sox6, and may also interact with other chondrocyte-associated transcription factors [35]. The regulation of this key player in chondrogenesis, therefore, is at the level of expression, protein modification, complex formation, and transcriptional activation.

**Patterning and cell fate determination**

Chondrogenesis can be divided into two interdependent processes: patterning; and cell fate determination. Pattern formation is the process during which number, size, and shape of the cartilaginous template is delineated and established. Cell fate determination is the process by which the combinatorial interactions of genetic and environmental factors serve to direct the developmental progression of a cell lineage. Cell fate is progressively restricted, and tissue specificity is gradually committed. The actions of these determinants are dependent upon concentration, time, and position. Patterning and cell fate determination are governed by a series of tissue interactions, which include interactions between adjacent components of segmental structures, or between juxtaposed epithelium and mesenchyme. Chondrogenesis during craniofacial and limb development best illustrates the complexity and hierarchy of regulatory mechanisms underlying the developmental program. During vertebrate morphogenesis, CNCCs, as well as limb bud mesenchymal cells, respond to BMP4 [36]. Depending on the timing of exposure to BMP4, these mesenchymal cells may undergo apoptosis or chondrogenesis. The orchestration of the apoptotic and chondrogenic response results in the formation and delineation of cartilaginous structures in the developing face and limbs. Studies have shown that the regulation of BMP4-mediated divergent morphogenetic outcome is dependent on both positive and negative modulators, at the level of ligands, cytoplasmic signals, and transcription factors.

**Craniofacial development**

Cartilages of the craniofacial region are largely derived from CNCCs [37]. CNCCs are a specialized population of ectodermal cells in origin, and arise from the lateral margin of the developing hindbrain. At the early somite stage of the embryo, the hindbrain is segmented into compartments called rhombomeres. CNCCs that are generated from the hindbrain are thus segment-specific, and these cells undergo epithelial to mesenchymal transformation, leave the crest and migrate towards the forming face. The cells populate the branchial arches, expand, and eventually differentiate into cartilage and several other cell types. A series of experiments showed that CNCCs are responsive to BMP4 and, depending on the stage of development, there can be different outcomes. Instrumental to the differential responses is a homeodomain transcription factor, Msx2, which mediates craniofacial and limb morphogenesis [38,39]. A gain of function mutation in the human MSX2 gene (OMIM 123101) causes Boston-type craniosynostosis, while loss of function mutations in this gene cause parietal foramina type I [40]. Msx2 is present in all stages of CNCC development: formation; migration; and differentiation. In the developing rhombomeres, BMP4 and Msx2 are coexpressed in rhombomeres 3 and 5 and correlate with extensive CNCC apoptosis observed in these rhombomeres, which results in limited contribution of CNCC to the craniofacial skeleton [41]. Apoptosis is the mechanism of eliminating CNCCs, which are not necessary for differentiation, and the occurrence of apoptosis in alternate segments of the hindbrain serves to pattern the migrating CNCCs into three major streams. In these early premigratory crest cells, BMP4 induces Msx2 expression and causes apoptosis [41]. Overexpression of Msx2 along the cephalic neural tube results in increased apoptosis, suggesting that Msx2 is the mediator of BMP4 action [42]. In rhombomeres that produce a significant number of CNCCs, the action of BMP is restricted by a Wnt antagonist, cSFRP2 [43]. Taken together, the patterning of CNCC formation within the neural tube is regulated by both positive and negative signals modulated by BMP. At later stages, Msx2 functions as a repressor of chondrogenesis without inducing cell death [44]. During migration, Msx2 and Sox9 are coexpressed in migrating CNCCs. Msx2 serves to repress the function of Sox9.
such that these cells are allowed to migrate and arrive at their target site before overt differentiation occurs. A dominant negative form of Msx2 accelerates the rate and extent of chondrogenesis in CNCCs in cultures, demonstrating that, when the function of Msx2 is inhibited, cells are de-repressed, and allowed to differentiate [44]. Msx2 also functions as a repressor of chondrogenesis during the formation of Meckel’s cartilage in the mandibular division of the first branchial arch [26]. Overexpression of Msx2 in the developing mandible disrupts the formation of Meckel’s cartilage. Interestingly, Msx2 expression closely borders areas of cartilage differentiation and is tightly juxtaposed to the expression of Sox9. This suggests that Msx2 normally functions to delineate and define the boundaries for cartilage formation. Implanting BMP4-soaked beads in the developing mandible induces the expression of both Sox9 and Msx2 [26]. The relative level of expression of these antagonistic molecules, however, is dependent on positional cues within the mandible. These positional cues may be genes that are locally expressed in a specific region of the developing mandible. These genes may modify cellular competence to respond to BMP4, and consequently the expression pattern and profile of Sox9 and Msx2 induced by BMP4. It is this relative expression of Sox9 and Msx2 that determines whether ectopic cartilage will form around the bead. The regional molecular differences in the mandible that account for differential expression of Sox9 and Msx2 remain to be explored.

**Limb development**

During skeletogenesis in the developing limb bud, chondroprogenitor cells initiate their differentiation while neighboring cells undergo apoptosis, thus defining the boundaries of the developing skeletal elements. Mesenchymal condensations followed by chondrocyte differentiation and maturation occur in digital zones, whereas mesenchymal cells undergo apoptotic elimination in interdigital web zones to give rise to the delineation of the digits [45]. Failure of one of these processes results in limb malformations such as polydactyly or syndactyly of soft or hard tissues. BMPs regulate not only the chondrogenic and the apoptotic responses of the mesenchymal cells, but also specify digit identity, as well as participate in the generation, maintenance, and regression of the apical ectodermal ridge (a structure that governs the proximal–distal patterning of the limb bud) [46–48]. BMPs, however, do not pattern each region of the limb bud individually. Rather, evidence supports the hypothesis that BMPs participate in communicating cell fate decisions interactively between adjacent regions of the limb bud. Interdigital mesenchyme destined to undergo apoptosis in vivo produces cartilage when it is isolated away from the digits and developed in vitro [49,50]. Furthermore, digit identity is specified by the correspondingly more posterior interdigital tissue [48]. Interestingly, similar to the early patterning of the CNCC, Msx2 is also a mediator of BMP-regulated apoptosis in the interdigital mesenchyme [51]. Recent data suggest that the specificity of BMP for multiple actions during limb morphogenesis reflects different activities of the receptor subtypes transducing the BMP signal [52]. BMP receptor type IB appears to be the necessary mediator of BMP-induced chondrogenesis [53–55], although overexpression of the receptor, or constitutive activation of the receptor can also cause excessive apoptosis [56,57]. A significant challenge of future research is to distinguish between the downstream signaling pathways from the BMP receptor subtypes. These differences may provide a molecular basis for the specific and often antithetic responses elicited by BMPs within developing limb buds and other tissues.

**Chondrocyte maturation**

Embryonic cartilage is destined for one of several fates; it can remain as permanent cartilage, such as on the articular surfaces of bones, or it can provide a template for the formation of bones through the process of endochondral ossification (EO) [58,59]. Most of the bones of the axial and appendicular skeleton, and some of the bones of the craniofacial skeleton, develop through this process. The following two sections describe recent advances in understanding the molecular regulation of chondrocyte maturation during EO and joint formation.

**Endochondral ossification**

The anlagen of long bones develop as relatively homogeneous elongated masses of cartilage tissue surrounded by a perichondrium. Signaling between the perichondrium and the chondrocytes (discussed below) causes cells in the center of the anlagen to initiate progression in their maturation program to prepare a site of ossification. These chondrocytes undergo several rounds of more rapid proliferation, and then arrest in their cell cycle. The postmitotic cells change their morphology, alter their gene expression, and remodel their extracellular matrix to become hypertrophic chondrocytes (Fig. 1). Whereas proliferating and articular chondrocytes synthesize a cartilage matrix composed mostly of type II collagen, hypertrophic chondrocytes cease expressing type II and express type X collagen, which is recognized as a marker of hypertrophic cells in the chondrocyte lineage. The cartilage matrix also becomes mineralized, and the hypertrophic chondrocytes undergo apoptosis. Prior to their death, they deposit the angiogenic factor, vascular endothelial growth factor (VEGF), into their extracellular matrix, which promotes the invasion of blood vessels into the cartilage tissue [60]. The blood vessels bring chondroclasts, osteoblasts and osteoclasts into the new ossification center, which begin removing the mineralized cartilage matrix and forming bone tissue.

The process of chondrocyte maturation expands from this initial central site toward the ends of the forming bones, with the zones of chondrocyte proliferation, cell cycle
arrest, hypertrophy, and apoptosis arranged sequentially (Fig. 1). The perichondrium along the shaft differentiates into a collar of bone that expands toward the ends of the developing bone in pace with the advance of hypertrophic chondrocytes. During postnatal development, these cartilage structures, called growth plates, are 'sandwiched' between the bony metaphysis and epiphysis, and serve as factories for the rapid production of new bone. Regulation and coordination of the rates of chondrocyte proliferation, hypertrophic maturation, apoptosis, and bone collar formation are essential to normal bone morphogenesis. Human genetic disorders affecting EO, such as achondroplasia and chondrodysplasias, are relatively common. Positional cloning in affected pedigrees has contributed to the identification of genes that regulate bone development [59,61–63]. Further analysis of the regulatory mechanisms in animal models has provided an understanding of the interactions of these genes. Recent advances in understanding the regulation of EO have resulted from the studies of fibroblast growth factor receptors (FGFRs), Indian hedgehog (Ihh), PTHrP, BMPs and core-binding factor (Cbfa1). Although retinoids, nitric oxide, hypoxia, vitamin D, estrogens, and other small molecules, as well as extracellular matrix molecules and biomechanical signals contribute to the regulation of chondrocyte differentiation and maturation, this review will focus primarily on peptide and glycoprotein growth factor signaling pathways.

The PTHrP/Ihh pathway

Chondrocyte proliferation and maturation in the growth plate is regulated by a negative feedback loop of intercellular communication, mediated by the secreted signaling molecules PTHrP and Ihh [64,65]. PTHrP, a peptide hormone with homology to parathyroid hormone, is synthesized and secreted by periarticular perichondrial cells, and by chondrocytes later in development. It functions as a patterning molecule, inhibiting chondrocyte hypertrophy near the articular ends of the developing bone, thus maintaining a pool of proliferating cells [66]. Mutations in the PTH/PTHrP receptor that result in constitutive activation cause Jansen’s chondrodysplasia [67]. These patients have decreased skeletal growth, abnormal metaphases and other skeletal malformations (OMIM 156400). Ihh, a member of the hedgehog family of cell-surface-associated ligands is expressed in the postmitotic, prehypertrophic cells, and provides the signal to maintain PTHrP expression at the ends of the developing bone [65]. By inhibiting chondrocyte maturation, PTHrP downregulates Ihh in the cells near the ends of the bone. Ihh promotes chondrocyte proliferation and specifies growth in the long axis through PTHrP-dependent and PTHrP-independent mechanisms [68]. Loss of Ihh function by gene targeting in mice results in decreased chondrocyte proliferation, loss of PTHrP expression, and abnormal positioning of hypertrophic chondrocytes close to the articular surface [66]. Ihh is also necessary for the signaling between the postmitotic chondrocytes and the perichondrium to establish and advance the bone collar [66,69]. Point mutations in Ihh that may inhibit binding to its receptor causes shortening of the digits (brachydactyly type A-1; OMIM 112500), consistent with its role in chondrocyte proliferation and bone growth [70].

The BMP pathways

BMP6 may serve a direct role in regulating chondrocyte maturation, while other BMPs may contribute to signaling between the chondrocytes and the perichondrium. BMP6 is expressed in prehypertrophic and hypertrophic chondrocytes, and several other BMPs are expressed in the perichondrium [71–74]. Treatment of chondrogenic cultures with BMP6 promotes the expression of type X collagen and alkaline phosphatase [75]. Misexpression of constitutively active BMP receptor type IA in developing limbs, however, delays chondrocyte maturation, and like Ihh overexpression, upregulates PTHrP [56,65]. This suggests that BMP stimulation of its receptor in the diaphy-

![Figure 1](http://arthritis-research.com/content/4/2/094)
seal perichondrium mediates the signaling between Ihh from the prehypertrophic chondrocytes and PTHrP expression in the periarticular perichondrium.

The fibroblast growth factor pathways
Of the high affinity receptors for fibroblast growth factors (FGFs), three of the five family members FGFR1, FGFR2 and FGFR3 regulate skeletal development. The importance of FGFR3 in regulating chondrocyte proliferation and maturation has been revealed by analysis of patients with activating mutations in this gene, which causes achondroplasia (OMIM 100800), hypochondroplasia (OMIM 146000), thanatophoric dysplasias (OMIM 187600), and other skeletal and soft tissue disorders, depending on the mutation (OMIM 134934)[63]. Su et al. [76] demonstrated that chondrocytes from a fetus with thanatophoric dysplasia type II exhibited increased activation of the transcription factor STAT1, and increased expression of the cyclin-dependent kinase inhibitor p21(Waf1/Cip1), a STAT-regulated gene. This suggests that mutations in FGFR cause defects in EO by inhibiting chondrocyte proliferation. Subsequent studies in mice and tissue culture cells have supported the hypothesis that increased FGFR activity disrupts the normal pattern of cartilage growth and maturation, at least in part by signaling through Stat molecules and increasing cyclin-dependent kinase inhibitor expression [77–82]. Furthermore, increased FGFR signaling causes premature apoptosis of growth plate chondrocytes in a Stat1-dependent manner [82,83]. Conversely, the FGFR3 null mutant mice exhibit increased chondrocyte proliferation and increased bone growth [84]. Interestingly, Ihh and PTHrP expression is decreased in mice expressing activated mutant FGFR3 or in wild type metatarsals grown in culture in the presence of FGF2 [85,86]. This suggests interactions between the FGF signaling pathway and the PTHrP/Ihh pathways in regulating chondrocyte proliferation and maturation, although the precise mechanisms of these interactions is not clear.

The Cbfa1 pathway
Cbfa1 (also called Runx2, PEBP2A or Osf2; OMIM 600211) is a critical gene in the regulation of skeletal development as it is necessary for endochondral and intramembranous bone formation, and it is sufficient to induce premature and ectopic chondrocyte hypertrophy [87–90]. Cbfa1 encodes a transcription factor containing a conserved runt domain, that is expressed in mesenchymal condensations, chondrocytes, and cells of the osteoblast lineage [90–93]. Heterozygous loss of function mutations in Cbfa1 cause cleidocranial dysplasia (OMIM 119600), a syndrome that includes clavicle hypoplasia or aplasia, failure in closure of the anterior fontanel, and other skeletal and dental malformations [94]. In addition to the essential role that Cbfa1 plays in osteoblast differentiation, it also regulates chondrocyte maturation. Loss of Cbfa1 by gene targeting in mice results in a complete lack of bone formation and a lack of chondrocyte hypertrophy in most of the skeleton [87,88,92,93]. Ectopic expression of Cbfa1 in nonhypertrophic chondrocytes of transgenic mice promotes their hypertrophic differentiation and disrupts joint formation [90,95]. Cbfa1 is a direct regulator of osteocalcin and other genes in osteoblasts, and may also directly regulate hypertrophic-chondrocyte-specific genes [89]. VEGF, which is normally expressed in hypertrophic chondrocytes, is not expressed in the chondrocytes of Cbfa1 null mutant mice. Furthermore, VEGF expression is upregulated by Cbfa1 in fibroblasts in tissue culture [96]. These data suggest that Cbfa1 is an important regulator of EO, controlling chondrocyte maturation, osteoblast differentiation, and angiogenesis in the developing bone.

The regulation of Cbfa1 expression and activity serves as a point of convergence of multiple signaling pathways. Cbfa1 expression is upregulated by BMP2, BMP4 or BMP7 treatment of multipotential, skeletal, or myogenic cell lines [91,97–99]. The regulation of Cbfa1 by BMP may be mediated by Msx2, since Cbfa1 expression is decreased in Msx2 null mutant mice [39]. Negative regulators of Cbfa1 expression in osteogenic cells include glucocorticoid, 1,25(OH)2D3, and TGF-β [91,100,101]. The function of Cbfa1 is repressed by its association with Smad3 in TGF-β stimulated cells [101]. Cbfa1 expression is upregulated in transgenic mice carrying an activated mutant FGFR1, and upregulated in a mesenchymal cell line treated with FGF2 or FGF8 [102]. Ihh may be both a regulator of Cbfa1 expression and a target of Cbfa1 transcriptional activity. Cbfa1 expression and bone collar formation is dependent on Ihh expression in prehypertrophic chondrocytes [66,69]. Expression of Cbfa1 in nonhypertrophic chondrocytes induces Ihh expression in these cells and eventual hypertrophy [90,95]. Further study of the regulation of Cbfa1, its protein interactions, and the targets of its transcriptional activity will contribute to the detailed characterization of the molecular mechanisms regulating EO.

Joint formation
Another fate for embryonic skeletal cartilage is the formation of joints. The cartilage template for the developing limb skeleton forms as a continuous, branched cartilage element from the humerus/femur to the digit rays, with only a few skeletal elements formed from independent condensations [103,104]. These cartilage structures are then segmented through the differentiation and apoptosis of chondrocytes to form joint cavities, through a process called cavitation. Concurrently, adjacent chondrocytes and perichondrial cells differentiate to form articular cartilage and other joint-associated tissues [105,106].

Cartilage-derived morphogenetic protein 1 (CDMP1) and its mouse homologue, Gdf5, are members of the TGF-β
superfamily, related to BMPs. Null mutation of Gdf5 causes shortening of the digits and defects in joint formation in the limbs as seen in the classical mouse mutant line brachypodium (bp) [105,107]. Mutations in CDMP1 cause the human skeletal disorder Grebe type chondrodysplasia (OMIM 200700), Hunter-Thompson type acromesomelic dysplasia (OMIM 201250) and brachydactyly type C (OMIM 113100), which all include shortened or missing phalanges. Gdf5 is normally expressed in developing joints. It is one of the earliest markers of joint formation and is strongly expressed throughout cartilage [108–110]. Gli3, a transcription factor that functions in the hedgehog signaling pathway, is also expressed in developing joints, and its expression pattern is expanded in bp mice [110]. Focal application of exogenous Gdf5 protein to the cartilage digit rays of bp mouse limb buds in culture inhibits the expanded expression of Gli3 [110]. This suggests that CDMP1/Gdf5 provides an important signal for the sites of joint formation, and this signal regulates the expression of genes that control chondrocyte differentiation.

Recent studies by Hartmann and Tabin [111] demonstrated the importance of Wnt-14 in initiating joint formation and in the spacing of joints within the cartilage condensation. Wnt-14 is a member of the large Wnt family of secreted glycoproteins that bind to receptors of the frizzled family. Wnt-14 is expressed in the early joint-forming regions of the developing chicken limb in a pattern similar to Gdf5. In fully developed joints, however, Wnt-14 is expressed in the joint capsule and synovial membrane, while Gdf5 is restricted to the joint fibrocartilaginous cartilage. Ectopic expression of Wnt-14 in developing digits induces morphological and molecular changes indicative of ectopic joint formation, including inhibition of cartilage differentiation, and upregulation of Gli3, Gdf5, autotaxin, chordin, Wnt-4 and CD44rel expression in relative patterns similar to those seen in normal joint development [111]. Furthermore, ectopic Wnt-14 expression represses adjacent endogenous joint development in the same cartilage condensation. This suggests that joint initiation by Wnt-14 activates a signal that regulates the positioning of the next joint in the patterning of the digits.

Study of the Wnt/frizzled pathway will contribute to an understanding of skeletal patterning and joint formation and may also provide molecular characterization of cellular changes in rheumatoid arthritis. Wnt-5a and frizzled 5 are both overexpressed in the synovial tissues of rheumatoid arthritis patients when compared to normal joint tissue or tissue from osteoarthritic joints [112]. This may suggest a change in the state of differentiation of synoviocytes that contributes to progression of the disease.

Future directions
Skeletal morphogenesis depends greatly on the patterning and formation of cartilage, and the subsequent remodeling of cartilage into bones or joints. Molecules such as BMPs regulate important steps at different times in the life cycle of cartilage. This may suggest that BMPs instruct chondrocytes when to change their patterns of gene expression and behavior rather than providing specific instructions of which genes to express. The set of genes ready for expression may be determined by the history of signals to which the cell has been exposed, and the collection of receptors, signaling molecules and transcription factors accumulated in response to those signals. During chondrogenesis and endochondral ossification, chondrocyte proliferation is regulated by FGFs, BMPs, PTHrP, Ihh, cell–cell and cell–matrix adhesion, and biomechanical signals. These multiple concurrent signals converge on the regulation of cell cycle progression and cell differentiation. Further study of the interaction of cartilage-associated transcription factors such as Msx2 and Cbfa1 with cell-cycle regulators will contribute to an understanding of the important connection between proliferation and differentiation. Tissue engineering for the treatment of skeletal diseases and disorders will depend on effective tools for expanding populations of chondrocytes while maintaining or restoring their state of differentiation.

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