

KELLEY & FIRESTEIN'S

Textbook of  
Rheumatology



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**Textbook of  
Rheumatology**

Tenth Edition

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TOR has borne the name of Dr. Kelley with pride for decades, recognizing his foresight and scholarship in creating the premier clinical and academic text in the discipline. With the passage of time, just as rheumatology has advanced, so too TOR has evolved. This has occurred driven particularly by the vision and energy of Dr. Firestein, our Editor-in-Chief. The co-editors consider it timely to acknowledge Dr. Firestein's remarkable contribution by renaming the textbook for this, its tenth edition.



Sincerest thanks to my wonderful wife, Linda, and our children, David and Cathy, for their patience and support. Also, the editorial help of our three Cavalier King Charles puppies, Winston, Humphrey, and Punkin, was invaluable.

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# Preface

## *Whither textbooks: Learning in the internet era*

The tenth edition of the *Textbook of Rheumatology* (TOR10) returns with the entire editorial team from the previous edition and a contributing group of truly outstanding experts in the field. Once again, we believe that this represents an authoritative—actually, *the* authoritative—resource for rheumatology trainees, clinicians, and researchers.

One key question that we are asked and actually ask ourselves is, “Why produce a textbook when you can log onto the internet and download a review article or original research in an instant?” Traditionalists may relish the shiny pages of a beautiful book, but this seems antiquated in the e-book era. However, we contend that a tome like TOR10 provides an entirely different experience for the reader. It is “moderated” by the editors; authors are carefully vetted, and their chapters are reviewed by multiple experts. The organization of TOR10 is also quite distinct. We do not divide a disease or topic into multiple short chapters written by several authors and covering narrow areas; instead, we purposely keep chapters broad enough to provide an integrated view of the literature. That does not mean that TOR10 chapters are broad or superficial. On the contrary, the level of scholarship is extraordinary, and the authors demonstrate their total mastery of the topic. We have

continued the principle of the founding editors to provide comprehensive references so that trainees appreciate the original, classic papers in our field. The publication cycle of textbooks is longer than the rapid fire of specific scientific discoveries or the theory du jour. This offers the distinct advantage of pause for thought. Paracelsus declared the physician’s greatest friend to be time. TOR10 delivers a considered appraisal over time of the evolving general concepts of rheumatology.

Even so, textbooks might seem anachronistic at this point. Not so for TOR10! We have migrated more and more to online or e-versions. While textbook paper copy distribution drifts downward, online access as measured by “clicks” has soared. These observations confirm our basic premise that our specialty still demands the content and care that goes into creating a unique resource like TOR10.

In the coming years, our book will continue to adapt to a changing learning environment. One thing that will not change, however, is the editors’ devotion to bringing readers an authoritative, well-written, and readable resource that can be trusted as the “gold standard” for our specialty to help them understand the science and practice of rheumatology. See you in a few years with TOR11!

**The Editors**

### CHAPTER 1

## Biology of the Normal Joint



Steven R. Goldring • Mary B. Goldring

#### KEY POINTS

Condensation of mesenchymal cells, which differentiate into chondrocytes, results in formation of the cartilage anlagen, which provides the template for the developing skeleton.

During development of the synovial joint, growth differentiation factor-5 regulates interzone formation, and interference with movement of the embryo during development impairs joint cavitation.

Members of the bone morphogenetic protein/transforming growth factor- $\beta$ , fibroblast growth factor, and Wnt families and the parathyroid hormone-related peptide/Indian hedgehog axis are essential for joint development and growth plate formation.

The synovial lining of diarthrodial joints is a thin layer of cells lacking a basement membrane and consisting of two principal cell types—macrophages and fibroblasts.

The articular cartilage receives its nutritional requirements via diffusion from the synovial fluid, and interaction of the cartilage with components of the synovial fluid contributes to the unique low-friction surface properties of the articular cartilage.

#### CLASSIFICATION OF JOINTS

Human joints, which provide the structures by which bones join with one another, may be classified according to the histologic features of the union and the range of joint motion. Three classes of joint design exist: (1) synovial or diarthrodial joints (Figure 1-1), which articulate with free movement, have a synovial membrane lining the joint cavity, and contain synovial fluid; (2) amphiarthroses, in which adjacent bones are separated by articular cartilage or a fibrocartilage disk and are bound by firm ligaments, permitting limited motion (e.g., the pubic symphysis, intervertebral disks of vertebral bodies, distal tibiofibular articulation, and sacroiliac joint articulation with pelvic bones); and (3) synarthroses, which are found only in the skull (suture lines) where thin, fibrous tissue separates adjoining cranial plates that interlock to prevent detectable motion before the end of normal growth, yet permit growth in childhood and adolescence.

Joints also can be classified according to the connective tissues that are present. Symphyses have a fibrocartilaginous

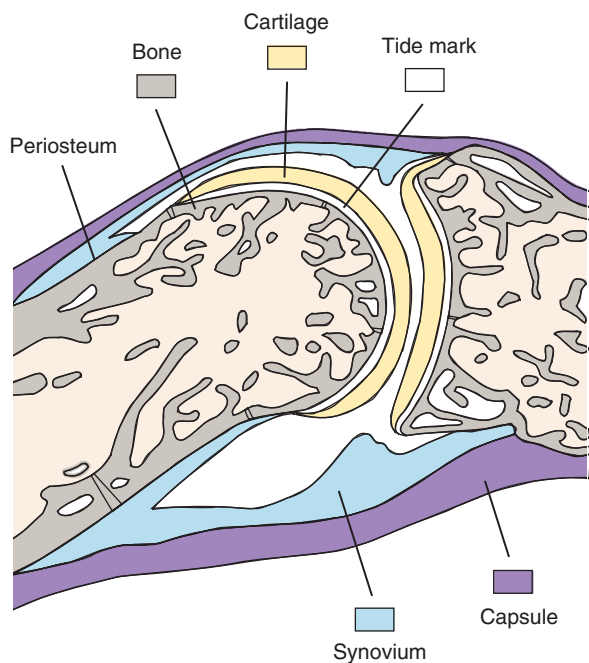
disk separating bone ends that are joined by firm ligaments (e.g., the symphysis pubis and intervertebral joints). In synchondroses, the bone ends are covered with articular cartilage, but no synovium or significant joint cavity are present (e.g., the sternomanubrial joint). In syndesmoses, the bones are joined directly by fibrous ligaments without a cartilaginous interface (the distal tibiofibular articulation is the only joint of this type outside the cranial vault). In synostoses, bone bridges are formed between bones, producing ankylosis.

The synovial joints are classified further according to their shapes, which include ball-and-socket (hip), hinge (interphalangeal), saddle (first carpometacarpal), and plane (patellofemoral) joints. These configurations reflect the varying functions, with the shapes and sizes of the opposing surfaces determining the direction and extent of motion. The various designs permit flexion, extension, abduction, adduction, or rotation. Certain joints can act in one (humeroulnar), two (wrist), or three (shoulder) axes of motion.

This chapter concentrates on the developmental biology and relationship between structure and function of a “prototypic,” “normal” human diarthrodial joint—the joint in which arthritis is most likely to develop. Most research that has been performed concerns the knee because of its accessibility, but other joints are described when appropriate.

#### DEVELOPMENTAL BIOLOGY OF THE DIARTHRODIAL JOINT

Skeletal development is initiated by the differentiation of mesenchymal cells that arise from three sources: (1) neural crest cells of the neural ectoderm that give rise to craniofacial bones; (2) the sclerotome of the paraxial mesoderm, or somite compartment, which forms the axial skeleton; and (3) the somatopleure of the lateral plate mesoderm, which yields the skeleton of the limbs.<sup>1</sup> The appendicular skeleton develops in the human embryo from limb buds, which are first visible at approximately 4 weeks of gestation. Structures resembling adult joints are generated at approximately 4 to 7 weeks of gestation.<sup>2</sup> Many other crucial phases of musculoskeletal development follow, including vascularization of epiphyseal cartilage (8 to 12 weeks), appearance of villous folds in synovium (10 to 12 weeks), evolution of bursae (3 to 4 months), and the appearance of periarticular fat pads (4 to 5 months).

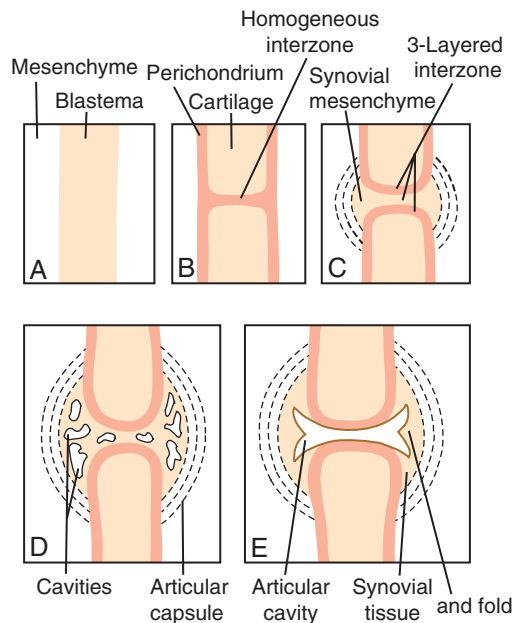


**Figure 1-1** A normal human interphalangeal joint, in sagittal section, as an example of a synovial, or diarthrodial, joint. The tidemark represents the calcified cartilage that bonds articular cartilage to the subchondral bone plate. (From Sokoloff L, Bland JH: *The musculoskeletal system*. Baltimore, Williams & Wilkins, 1975. Copyright 1975, the Williams & Wilkins Co, Baltimore.)

The upper limbs develop approximately 24 hours earlier than the analogous portions of the lower limbs. Proximal structures, such as the glenohumeral joint, develop before more distal ones, such as the wrist and hand. As a consequence, insults to embryonic development during limb formation affect a more distal portion of the upper limb than of the lower limb. Long bones form as a result of replacement of the cartilage template by endochondral ossification. The stages of limb development are well described by O’Rahilly and Gardner<sup>2,3</sup> and are shown in Figure 1-2. The developmental sequence of the events occurring during synovial joint formation and some of the regulatory factors and extra-cellular matrix components involved are summarized in Figure 1-3. The three main stages in joint development are interzone formation, cavitation, and morphogenesis, as described in detail in several reviews.<sup>4-9</sup>

## INTERZONE FORMATION AND JOINT CAVITATION

The structure of the developing synovial joint and the process of joint cavitation have been described in many classic studies performed on the limbs of mammalian and avian embryos.<sup>10</sup> In the human embryo, cartilage condensations, or chondrifications, can be detected at stage 17, when the embryo is small—approximately 11.7 mm long.<sup>2,3</sup> In the region of the future joint, after formation of the homogeneous chondrogenic interzone at 6 weeks (stages 18 and 19), a three-layered interzone is formed at approximately 7 weeks (stage 21), which consists of two chondrogenic, perichondrium-like layers that cover the opposing surfaces

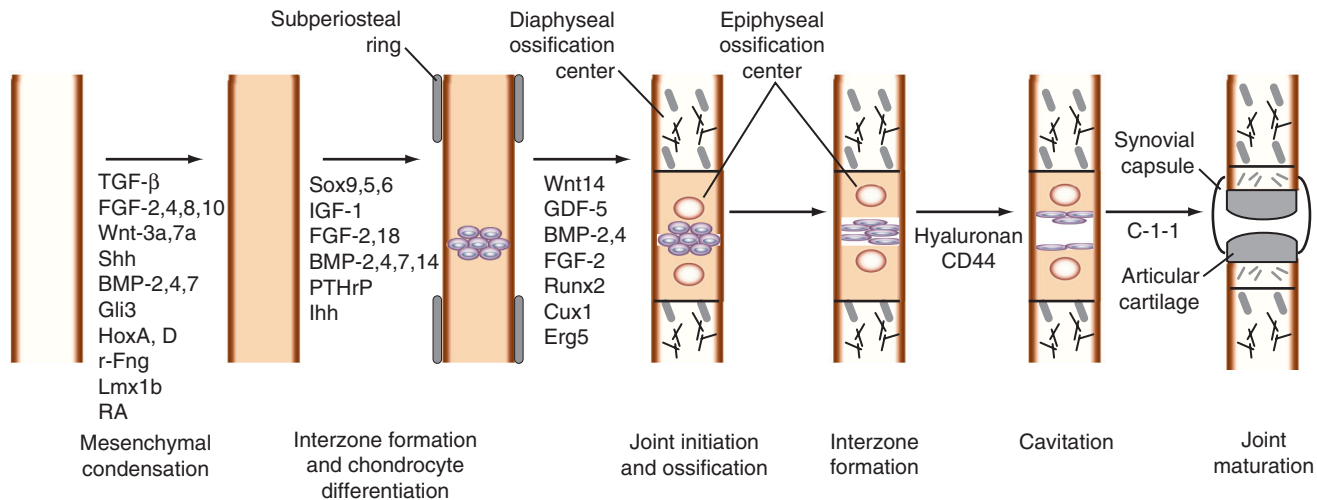


**Figure 1-2** The development of a synovial joint. **A**, Condensation. Joints develop from the blastema, not the surrounding mesenchyme. **B**, Chondrification and formation of the interzone. The interzone remains avascular and highly cellular. **C**, Formation of synovial mesenchyme. Synovial mesenchyme forms from the periphery of the interzone and is invaded by blood vessels. **D**, Cavitation. Cavities are formed in the central and peripheral interzone and merge to form the joint cavity. **E**, The mature joint. (From O’Rahilly R, Gardner E: *The embryology of movable joints*. In Sokoloff L, editor: *The joints and synovial fluid*, vol 1, New York, Academic Press, 1978.)

of the cartilage anlagen and are separated by a narrow band of densely packed cellular blastema that remains and forms the interzone. Cavitation begins in the central interzone at about 8 weeks (stage 23).

Although the cellular events associated with joint formation have been recognized for many years, only more recently have the genes regulating these processes been elucidated.<sup>6,7,9</sup> These genes include growth differentiation factor (GDF)-5 (also known as cartilage-derived morphogenetic protein-1) and Wnt14 (also known as Wnt9a), which are involved in early joint development. Two major roles have been proposed for Wnt14. First, it acts at the onset of joint formation as a negative regulator of chondrogenesis. Second, it facilitates interzone formation and cavitation by inducing the expression of GDF-5, autotaxin, lysophosphatidic acid, the bone morphogenetic protein (BMP) antagonist, chordin, and the hyaluronan receptor, CD44.<sup>4,11</sup> Paradoxically, application of GDF-5 to developing joints in mouse embryo limbs in organ culture causes joint fusion,<sup>12</sup> suggesting that temporospatial interactions among distinct cell populations are important for the correct response. The current view is that GDF-5 is required at the early stages of condensations, where it stimulates recruitment and differentiation of chondrogenic cells, and later, when its expression is restricted to the interzone.

The distribution of collagen types and proteoglycans in developing avian and rodent joints has been characterized histologically and by immunohistochemistry and in situ hybridization.<sup>9,13,14</sup> Types I and III collagen characterize the



**Figure 1-3** Development of long bones and diarthrodial joint formation from cartilage anlagen. BMP, Bone morphogenetic protein; C-C-1, Erg3 variant; CD44, cell determinant 44; Cux, cut-repeat homeobox protein; Erg, ETS-related gene 5; FGF, fibroblast growth factor; GDF, growth and differentiation factor; Gli, glioma-associated oncogene homolog; Hox, homeobox; IGF, insulin-like growth factor; Ihh, Indian hedgehog; Lmx1b, LIM homeodomain transcription factor 1b; PTHrP, parathyroid hormone-related protein; RA, retinoic acid; r-Fng, radical fringe; Runx, runt domain binding protein; Shh, Sonic hedgehog; Sox, SRY-related high mobility group-box protein; TGF- $\beta$ , transforming growth factor- $\beta$ ; Wnt, wingless type.

matrix produced by mesenchymal cells, which switch to the production of types II, IX, and XI collagens that typify the cartilaginous matrix at the time of condensation. The messenger RNAs (mRNAs) encoding the small proteoglycans, biglycan and decorin, may be expressed at this time, but the proteins do not appear until after cavitation in the regions destined to become articular cartilage. The interzone regions are marked by the expression of genes encoding type IIA collagen by chondrocyte progenitors in the perichondrial layers, type IIB and XI collagens by differentiated chondrocytes in the cartilage anlagen, and type I collagen in the interzone and in the developing capsule and perichondrium (Figure 1-4).<sup>15</sup>

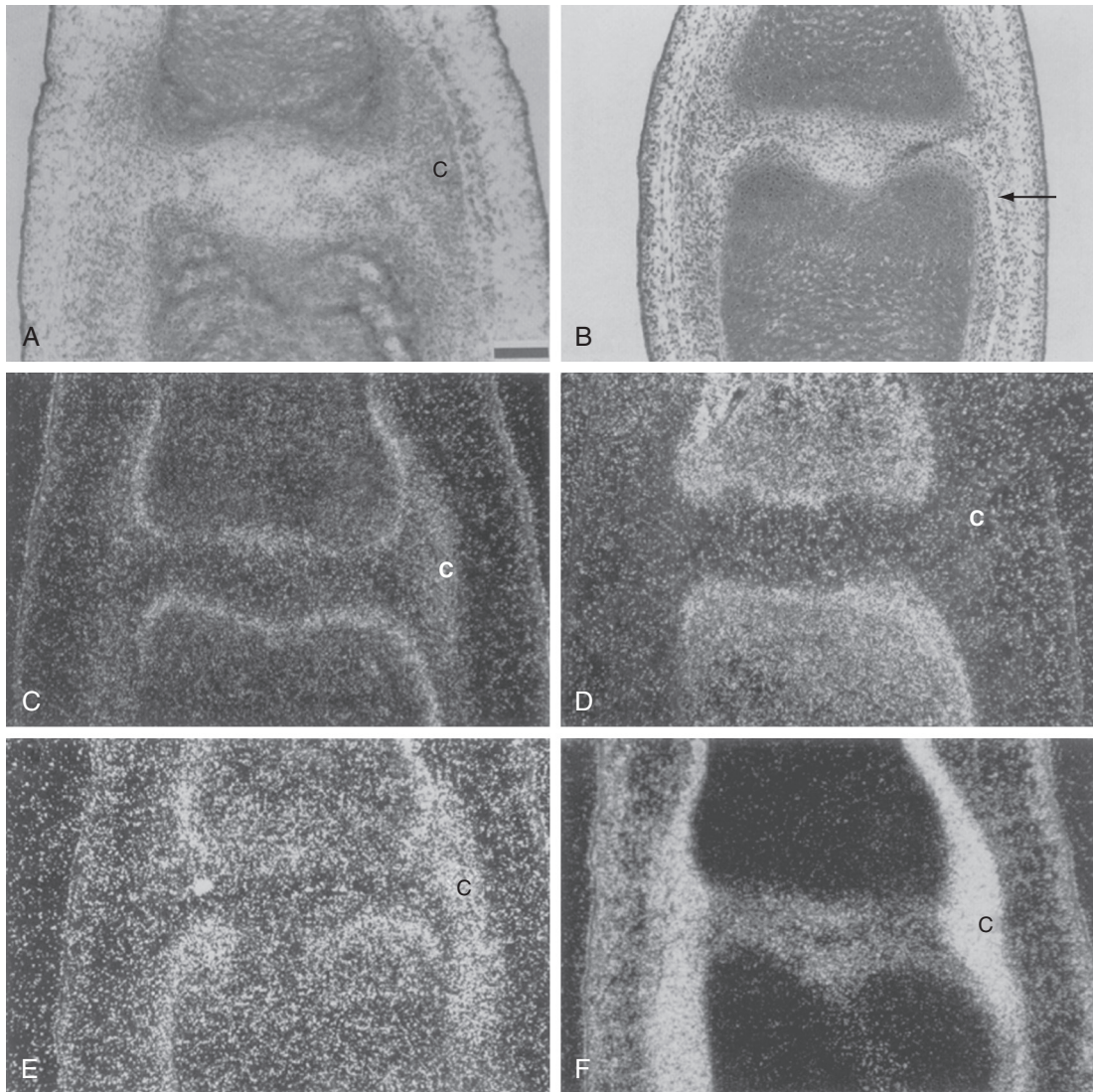
The interzone region contains cells in two outer layers, where they are destined to differentiate into chondrocytes and become incorporated into the epiphyses, and in a thin intermediate zone where they are programmed to undergo joint cavitation and may remain as articular chondrocytes.<sup>8</sup> These early chondrocytes arise from the same population, but unlike the other chondrocytes of the anlagen, they do not activate matrilin-1 expression and are destined to form the articular surface.<sup>16</sup> Fluid and macromolecules accumulate in this space and create a nascent synovial cavity. Blood vessels appear in the surrounding capsulosynovial blastemal mesenchyme before separation of the adjacent articulating surfaces. Although it was first assumed that these interzone cells should undergo necrosis or programmed cell death (apoptosis), many investigators have found no evidence of DNA fragmentation preceding cavitation. In addition, no evidence exists that metalloproteinases are involved in loss of tissue strength in the region undergoing cavitation. Instead, the actual joint cavity seems to be formed by mechanical changes induced by the synthesis of hyaluronan via uridine diphosphoglucose dehydrogenase (UDPGD) and hyaluronan synthase. Interaction of hyaluronan CD44 modulates cell migration, but the accumulation of hyaluronan and the associated mechanical influences play the

major role in forcing the cells apart and inducing rupture of the intervening extra-cellular matrix by tensile forces. This mechanism accounts partially for observations that joint cavitation is incomplete in the absence of movement.<sup>17-19</sup> Equivalent data from human embryonic joints are difficult to obtain,<sup>20</sup> but in all large joints in humans, complete joint cavities are apparent at the beginning of the fetal period.

## CARTILAGE FORMATION AND ENDOCHONDRAL OSSIFICATION

The skeleton develops from the primitive, avascular, densely packed cellular mesenchyme, termed the *skeletal blastema*. Common precursor mesenchymal cells divide into chondrogenic, myogenic, and osteogenic lineages that determine the differentiation of cartilage centrally, muscle peripherally, and bone. The surrounding tissues, particularly epithelium, influence the differentiation of mesenchymal progenitor cells to chondrocytes in the cartilage anlagen. The cartilaginous nodules appear in the middle of the blastema, and simultaneously cells at the periphery become flattened and elongated to form the perichondrium. In the vertebral column, cartilage disks arise from portions of the somites surrounding the notochord, and nasal and auricular cartilage and the embryonic epiphysis form from the perichondrium. In the limb, the cartilage remains as a resting zone that later becomes the articular cartilage, or it undergoes terminal hypertrophic differentiation to become calcified (growth plate formation) and is replaced by bone (enchondral ossification). The latter process requires extra-cellular matrix remodeling and vascularization (angiogenesis). These events are controlled exquisitely by cellular interactions with the surrounding matrix, growth and differentiation factors, and other environmental factors that initiate or suppress cellular signaling pathways and transcription of specific genes in a temporospatial manner.





**Figure 1-4** In situ hybridization of a 13-day-old (stage 39) chicken embryo middle digit, proximal interphalangeal joint, midfrontal sections. **A**, Bright-field image showing developing joint and capsule (C). **B**, Equivalent paraffin section of opposite limb of same animal, showing onset of cavitation laterally (arrow). **C**, Expression of type IIA collagen messenger RNA (mRNA) in articular surface cells, perichondrium, and capsule. **D**, Type IIB collagen mRNA is expressed only in chondrocytes of the anlagen. **E**, Type XI collagen mRNA is expressed in the surface cells, perichondrium, and capsule, with lower levels in chondrocytes. **F**, Type I collagen mRNA is present in cells of the interzone and capsule. **C** through **F** images are dark field. Calibration bar = 1  $\mu\text{m}$ . (From Nalin AM, Greenlee TK Jr, Sandell LJ: Collagen gene expression during development of avian synovial joints: transient expression of types II and XI collagen genes in the joint capsule. *Develop Dyn* 203:352–362, 1995.)

### Condensation and Limb-Bud Formation

Formation of the cartilage anlage occurs in four stages: (1) cell migration, (2) aggregation regulated by mesenchymal-epithelial cell interactions, (3) condensation, and (4) chondrocyte differentiation. Interactions with the epithelium determine mesenchymal cell recruitment and migration, proliferation, and condensation.<sup>2,3,21</sup> The aggregation of chondroprogenitor mesenchymal cells into precartilage condensations was first described by Fell<sup>22</sup> and depends on signals initiated by cell-cell and cell-matrix interactions, the formation of gap junctions, and changes in the cytoskeletal architecture. Before condensation, the prechondrocytic mesenchymal cells produce extra-cellular matrix that is rich in hyaluronan and type I collagen and type IIA collagen, which contains the exon-2–encoded aminopropeptide

found in noncartilage collagens. The initiation of condensation is associated with increased hyaluronidase activity and the transient upregulation of versican, tenascin, syndecan, the cell adhesion molecules, neural cadherin (N-cadherin) and neural cell adhesion molecule (NCAM), which facilitate cell-cell interactions.<sup>21,23</sup>

Before chondrocyte differentiation, the cell-matrix interactions are facilitated by the binding of fibronectin to syndecan, thus downregulating NCAM and setting the condensation boundaries. Increased cell proliferation and extra-cellular matrix remodeling, with the disappearance of type I collagen, fibronectin, and N-cadherin and the appearance of tenascins, matrilins, and thrombospondins, including cartilage oligomeric matrix protein, initiate the transition from chondroprogenitor cells to a fully committed chondrocyte.<sup>1,23-25</sup> N-cadherin and NCAM disappear in

differentiating chondrocytes and are detectable later only in perichondrial cells. The differentiated chondrocytes then proliferate and become prehypertrophic when they stop proliferating and undergo the complex process of hypertrophic maturation. Whether the resting differentiated chondrocytes remain within cartilage elements in articular joints or other progenitors are the source of the articular chondrocyte has not been clarified completely.

Much of the current understanding of limb development is based on early studies in chickens and more recently in mice. The regulatory events are controlled by interacting patterning systems involving homeobox (Hox) transcription factors and fibroblast growth factor (FGF), hedgehog, transforming growth factor- $\beta$  (TGF- $\beta$ )/BMP, and Wnt pathways, each of which functions sequentially over time (see Figure 1-3).<sup>11,26-29</sup> The HoxA and HoxD gene clusters, which are crucial for the early events of limb patterning in the undifferentiated mesenchyme, are required for the expression of FGF-8 and Sonic hedgehog (Shh),<sup>30</sup> which modulate the proliferation of cells within the condensations.<sup>21</sup> BMP-2, BMP-4, and BMP-7 coordinately regulate the patterning of limb elements within the condensations depending on the temporal and spatial expression of BMP receptors and BMP antagonists, such as noggin and chordin, as well as the availability of BMP- and TGF- $\beta$ -induced SMADs (signaling mammalian homologues of *Drosophila* mothers against decapentaplegic).<sup>31</sup> BMP signaling is required for the formation of precartilaginous condensations and for the differentiation of precursors into chondrocytes,<sup>32,33</sup> acting in part by opposing effects on FGF actions.<sup>34</sup> Growth of the condensation ceases when noggin inhibits BMP signaling and permits differentiation to chondrocytes. The cartilage formed serves as a template for formation of cartilage elements in the vertebra, sternum, and rib, and for limb elongation or endochondral bone formation.

### Molecular Signals in Cartilage Morphogenesis and Growth Plate Development

The cartilage anlagen grow by cell division and deposition of the extra-cellular matrix and by apposition of proliferating cells from the inner chondrogenic layer of the perichondrium. The nuclear transcription factor, Sox9, is one of the earliest markers expressed in cells undergoing condensation and is required for the subsequent stage of chondrogenesis characterized by the deposition of matrix containing collagens II, IX, and XI and aggrecan in the cartilage anlagen.<sup>35</sup> The expression of SOX proteins depends on BMP signaling via BMPRI1A and BMPRI1B, which are functionally redundant and active in chondrocyte condensations, but not in the perichondrium.<sup>32</sup> Sox5 and Sox6 are required for the expression of Col9a1, aggrecan, link protein, and Col2a1 during chondrocyte differentiation.<sup>36</sup> The runt-domain transcription factor, Runx2 (also known as core binding factor, Cbfa1), is expressed in all condensations, including those that are destined to form bone.

Throughout chondrogenesis, the balance of signaling by BMPs and FGFs determines the rate of proliferation and the pace of the differentiation.<sup>28,34,37</sup> In the long bones, long after condensation, BMP-2, BMP-3, BMP-4, BMP-5, and BMP-7 are expressed primarily in the perichondrium, and only BMP-7 is expressed in the proliferating chondrocytes.<sup>37</sup>

BMP-6 is found later exclusively in hypertrophic chondrocytes along with BMP-2. More than 23 FGFs have been identified thus far.<sup>38</sup> The specific ligands that activate each FGF receptor (FGFR) during chondrogenesis in vivo have been difficult to identify because the signaling depends on the temporal and spatial location of not only the ligands but also the receptors.<sup>39</sup> FGFR2 is upregulated early in condensing mesenchyme and is present later in the periphery of the condensation along with FGFR1, which is expressed in surrounding loose mesenchyme. FGFR3 is associated with proliferation of chondrocytes in the central core of the mesenchymal condensation and overlaps with FGFR2. Proliferation of chondrocytes in the embryonic and postnatal growth plate is regulated by multiple mitogenic stimuli, including FGFs, which converge on cyclin D1.<sup>40</sup>

Early studies indicated that FGFR3 could serve as a master inhibitor of chondrocyte proliferation via Stat1 and the cell cycle inhibitor p21. More recent work has shown that FGFR3 activation downregulates AKT activity to decrease proliferation<sup>41</sup> and MEK activation leads to decreased chondrocyte differentiation.<sup>42</sup> The physiologic FGFR3 ligands are not known, but FGF-9 and FGF-18 are good candidates because they bind FGFR3 in vitro and are expressed in the adjacent perichondrium and periosteum, forming a functional gradient.<sup>28,43</sup> FGF-18-deficient mice have an expanded zone of proliferating chondrocytes similar to that in FGFR3-deficient mice, and FGF-18 can inhibit Indian hedgehog (Ihh) expression. As the growth plate develops, FGFR3 disappears and FGFR1 is upregulated in the prehypertrophic and hypertrophic zones, where FGF-18 and FGF-9 regulate vascular invasion by inducing vascular endothelial growth factor (VEGF) and VEGFR1 and terminal differentiation.<sup>38,44,45</sup>

The proliferation of chondrocytes in the lower proliferative and prehypertrophic zones is under the control of a local negative feedback loop involving signaling by parathyroid hormone-related protein (PTHrP) and Ihh.<sup>46</sup> Ihh expression is restricted to the prehypertrophic zone, and the PTHrP receptor is expressed in the distal zone of periarticular chondrocytes. The adjacent, surrounding perichondrial cells express the Hedgehog receptor patched (Ptch), which, upon Ihh binding, similar to Shh in the mesenchymal condensations, activates Smo and induces Gli transcription factors, which can feedback regulate Ihh target genes in a positive (*Gli1* and *Gli2*) or negative (*Gli3*) manner.<sup>47,48</sup> Ihh induces expression of PTHrP in the perichondrium, and PTHrP signaling stimulates cell proliferation via its receptor expressed in the periarticular chondrocytes.<sup>28,49</sup> More recent evidence indicates that Ihh also acts independently of PTHrP on periarticular chondrocytes to stimulate differentiation of columnar chondrocytes in the proliferative zone, whereas PTHrP acts by preventing premature differentiation into prehypertrophic and hypertrophic chondrocytes, suppressing premature expression of Ihh.<sup>50,51</sup> Ihh and PTHrP, by transiently inducing proliferation markers and repressing differentiation markers, function in a temporospatial manner to determine the number of cells that remain in the chondrogenic lineage versus the number that enter the endochondral ossification pathway.<sup>46</sup> Components of the extra-cellular matrix also contribute to regulation of the different stages of growth plate development, including chondrogenesis and terminal differentiation, by interacting



with signaling molecules and chondrocyte cell surface receptors.<sup>52</sup>

### Endochondral Ossification

The development of long bones from the cartilage anlagen occurs by a process termed *endochondral ossification*, which involves terminal differentiation of chondrocytes to the hypertrophic phenotype, cartilage matrix calcification, vascular invasion, and ossification (see Figure 1-3).<sup>28,49,53</sup> This process is initiated when the cells in the central region of the anlage begin to hypertrophy, increasing cellular fluid volume by almost 20 times. *Ihh* plays a pivotal role in regulating endochondral bone formation by synchronizing perichondrial maturation with chondrocyte hypertrophy, which is essential for initiating the process of vascular invasion. *Ihh* is expressed in prehypertrophic chondrocytes as they exit the proliferative phase and enter the hypertrophic phase, at which time they begin to express the hypertrophic chondrocyte marker, type X collagen and alkaline phosphatase. These cells are responsible for laying down the cartilage matrix that subsequently undergoes mineralization. Wnt/ $\beta$ -catenin signaling promotes chondrocyte maturation by a BMP-2-mediated mechanism and induces chondrocyte hypertrophy partly by enhancing matrix metalloproteinase (MMP) expression and potentially by enhancing *Ihh* signaling and vascularization.<sup>54</sup>

*Runx2*, which serves as a positive regulatory factor in chondrocyte maturation to hypertrophy, is expressed in the adjacent perichondrium and in prehypertrophic chondrocytes, but less in late hypertrophic chondrocytes, overlapping with *Ihh*, *COL10A1*, and *BMP-6*. *IHH* induces Gli transcription factors, which interact with *Runx2* and BMP-induced Smads, to regulate transcription and expression of *COL10A1*.<sup>55</sup> An essential role for *Runx2* in the process of chondrocyte hypertrophy is supported by the observation that the terminal differentiation is blocked in *Runx2*-deficient mice. A member of the myocyte enhancer factor (MEF) 2 family, *MEF2C*, stimulates hypertrophy partly by increasing *Runx2* expression.<sup>56</sup> The class II histone deacetylase, *HDAC4*, prevents premature hypertrophy by directly suppressing the activities of *Runx2* and *MEF2C*.<sup>57</sup> *HDAC4* is in turn regulated by *PTHrP* and salt-inducible kinase 3 (*SIK3*).<sup>58,59</sup> *Sox9*,<sup>60</sup> *FOXA2* and *FoxA3*,<sup>61</sup> *Runx3*,<sup>62</sup> *Zfp521*,<sup>63</sup> and peroxisome proliferator-activated receptor  $\gamma$  (*PPAR* $\gamma$ )<sup>64</sup> are also important transcriptional regulators of chondrocyte hypertrophy. *MMP-13*, a downstream target of *Runx2*, is expressed by terminal hypertrophic chondrocytes, and *MMP-13* deficiency results in significant interstitial collagen accumulation, leading to the delay of endochondral ossification in the growth plate with increased length of the hypertrophic zone.<sup>65,66</sup>

*Runx2* also is required for transcription activation of *COL10A1*, the gene encoding type X collagen, which is the major matrix component of the hypertrophic zone in the embryo and in the postnatal growth plate. Mutations in the *COL10A1* gene are associated with the dwarfism observed in human chondrodysplasias. These mutations affect regions of the growth plate that are under great mechanical stress, and it has been suggested that the defect in skeletal growth may be due partly to alteration of the mechanical integrity of the pericellular matrix in the hypertrophic zone, although

a role for defective vascularization also has been proposed. The extra-cellular matrix remodeling that accompanies chondrocyte terminal differentiation is thought to induce an alteration in the environmental stress experienced by hypertrophic chondrocytes, which eventually undergo apoptosis.<sup>67</sup> Whether chondrocyte hypertrophy with cell death is the ultimate fate of hypertrophic chondrocytes or whether hypertrophy is a transient process that precedes osteogenesis has been a subject of debate. However, recent genetic lineage tracing studies suggest that hypertrophic chondrocytes can survive at the chondro-osseous junction and become osteoblasts and osteocytes.<sup>68,69</sup>

Cartilage is an avascular tissue, and because the developing growth plate is relatively hypoxic, hypoxia inducible factor (HIF)-1 $\alpha$  is important for survival as chondrocytes transition to hypertrophy. Under normoxia, the cell content of HIF-1 $\alpha$ , -2 $\alpha$ , and -3 $\alpha$  is low because of oxygen-dependent hydroxylation by prolyl-hydroxylases, resulting in ubiquitination and degradation by the proteasome. In contrast, under hypoxia, prolyl-hydroxylase activity is reduced and the  $\alpha$  subunits heterodimerize with the constitutive  $\beta$ -subunit members known as aryl hydrocarbon receptor nuclear translocators (ARNTs). HIFs are transcription factors that bind to hypoxia-responsive elements (HREs) in responsive genes. HIF-2 $\alpha$  regulates endochondral ossification processes by directly targeting HREs within the promoters of the *COL10A1*, *MMP13*, and *VEGFA* genes.<sup>70</sup>

Vascular invasion of the hypertrophic zone is required for the replacement of calcified cartilage by bone. VEGF acts as an angiogenic factor to promote vascular invasion by specifically activating local receptors, including *Flk1*, which is expressed in endothelial cells in the perichondrium or surrounding soft tissues; *neuropilin 1* (*Npn1*), which is expressed in late hypertrophic chondrocytes; or *Npn2*, which is expressed exclusively in the perichondrium. VEGF is expressed as three different isoforms: VEGF188, a matrix-bound form, is essential for metaphyseal vascularization, whereas the soluble form, VEGF120 (VEGFA), regulates chondrocyte survival and epiphyseal cartilage angiogenesis, and VEGF164 can be either soluble or matrix bound and may act directly on chondrocytes via *Npn2*. VEGF is released from the extra-cellular matrix by MMPs, including *MMP-9*, membrane-type (MT)1-MMP (*MMP-14*), and *MMP-13*. *MMP-9* is expressed by endothelial cells that migrate into the central region of the hypertrophic cartilage.<sup>71</sup> *MMP-14*, which has a broader range of expression than *MMP-9*, is essential for chondrocyte proliferation and secondary ossification, whereas *MMP-13* is found exclusively in late hypertrophic chondrocytes. *Perlecan* (*Hspg2*), a heparan sulfate proteoglycan in cartilage matrix, is required for vascularization in the growth plate through its binding to the VEGFR of endothelial cells, permitting osteoblast migration into the growth plate.<sup>72</sup>

A number of ADAM (a disintegrin and metalloproteinase) proteinases are also emerging as important regulators in growth plate development. For example, *ADAM10* is a principle regulator of Notch signaling, which modulates endochondral ossification via *RBPjk* in chondrocytes<sup>73</sup> and promotes osteoclastogenesis at the chondro-osseous junction by regulating endothelial cell organization in the developing bone vasculature.<sup>74</sup> *ADAM17* is the critical proteinase mediating cellular shedding of TNF but also the epidermal



growth factor receptor (EGFR) ligands, including TGF- $\alpha$ . The EGFR signaling pathway induced by EGF and TGF- $\alpha$  plays a crucial role in the remodeling of the growth plate, where inactivation of EGFR results in the inability of hypertrophic chondrocytes to degrade the surrounding collagen matrix and to attract osteoclasts to invade and remodel the advancing growth plate under control of the osteoclast differentiation factor receptor activator of nuclear factor  $\kappa$ B (NF $\kappa$ B) ligand (RANKL).<sup>75,76</sup> Mice lacking ADAM17 in chondrocytes (*Adam17 $\Delta$ Ch*) show an expanded hypertrophic zone in the growth plate,<sup>77</sup> essentially phenocopying mice with defects in EGFR signaling in chondrocytes.<sup>75</sup> Tight regulation of EGFR signaling is important for cartilage and joint homeostasis, as shown in mice with cartilage-specific deletion of the mitogen-inducible gene 6 (MIG-6), a scaffold protein that binds EGFR and targets it for internalization and degradation.<sup>78</sup> These events of cartilage matrix remodeling and vascular invasion are required for the migration and differentiation of osteoclasts and osteoblasts, which remove the mineralized cartilage matrix and replace it with bone.

## DEVELOPMENT OF THE JOINT CAPSULE AND SYNOVIUM

The interzone and the contiguous perichondrial envelope, of which the interzone is a part, contain the mesenchymal cell precursors that give rise to other joint components, including the joint capsule, synovial lining, menisci, intracapsular ligaments, and tendons.<sup>7,9</sup> The external mesenchymal tissue condenses as a fibrous capsule. The peripheral mesenchyme becomes vascularized and is incorporated as the synovial mesenchyme, which differentiates into a pseudomembrane at about the same time as cavitation begins in the central interzone (stage 23, approximately 8 weeks). The menisci arise from the eccentric portions of the articular interzone. In common usage, the term *synovium* refers to the true synovial lining and the subjacent vascular and areolar tissue, up to—but excluding—the capsule. Synovial lining cells can be distinguished as soon as the multiple cavities within the interzone begin to coalesce. At first, these cells are exclusively fibroblast-like (type B) cells.

As the joint cavity increases in size, synovial-lining cell layers expand by proliferation of fibroblast-like cells and recruitment of macrophage-like (type A) cells from the circulation. The synovial lining cells express the hyaluronan receptor CD44 and UDPGD, the levels of which remain elevated after cavitation. This increased activity likely contributes to the high concentration of hyaluronan in joint fluids. Further synovial expansion results in the appearance of synovial villi at the end of the second month, early in the fetal period, which greatly increases the surface area available for exchange between the joint cavity and the vascular space. Cadherin 11 is an additional molecule expressed by synovial lining cells.<sup>79,80</sup> It is essential for establishment of synovial lining architecture during development, where its expression correlates with cell migration and tissue outgrowth of the synovial lining.

The role of innervation in the developing joint is not well understood. A dense capillary network develops in the subsynovial tissue, with numerous capillary loops that penetrate into the true synovial lining layer. The human syno-

vial microvasculature is already innervated by 8 weeks (stage 23) of gestation, around the time of joint cavitation. Evidence of neurotransmitter function is not found until much later, however, with the appearance of the sensory neuropeptide substance P at 11 weeks. The putative sympathetic neurotransmitter, neuropeptide Y, appears at 13 weeks of gestation, along with the catecholamine-synthesizing enzyme tyrosine hydroxylase. The finding that the *Slit2* gene, which functions for the guidance of neuronal axons and neurons, is expressed in the mesenchyme and in peripheral mesenchyme of the limb bud (stages 23 to 28) suggests that innervation is an integral part of synovial joint development.<sup>81</sup>

## DEVELOPMENT OF NONARTICULAR JOINTS

In contrast to articular joints, the temporomandibular joint develops slowly, with cavitation at a crown-rump length of 57 to 75 mm (i.e., well into the fetal stage). This slow development may occur because this joint develops in the absence of a continuous blastema and involves the insertion between bone ends of a fibrocartilaginous disk that arises from muscular and mesenchymal derivatives of the first pharyngeal arch. However, many of the same genes as those involved in articular joint development are involved in morphogenesis and growth of the temporomandibular joint.<sup>82</sup>

The development of other types of joints, such as synarthroses, is similar to that of diarthrodial joints except that cavitation does not occur, and synovial mesenchyme is not formed. In these respects, synarthroses and amphiarthroses resemble the “fused” peripheral joints induced by paralyzing chicken embryos, and they may develop as they do because relatively little motion is present during their formation.<sup>83</sup>

The intervertebral disk consists of a semiliquid nucleus pulposus (NP) in the center, surrounded by a multilayered fibrocartilaginous annulus fibrosus (AF), which is sandwiched between the cartilaginous end plates (EPs).<sup>84</sup> Between the EPs lies the vertebral body consisting of the growth plate, which later disappears, and the primary and secondary centers of ossification that fuse together. The cells in the NP arise from the embryonic notochord and the notochord orchestrates somatogenesis, from which arises the ventral mesenchymal sclerotome that forms the AF of the intervertebral disk, as well as the vertebral bodies and ribs.<sup>84</sup> The NP acts as the center for controlling cell differentiation in the AF and EP through Shh signaling, which is regulated by WNT signaling and, in turn, promotes growth and differentiation through downstream transcription factors, Brachyury and Sox9, and gene expression of extra-cellular matrix components.<sup>85,86</sup> The proteoglycans and collagens expressed during development of the intervertebral disk have been mapped and reflect the complex structure-function relationships that allow flexibility and resistance to compression in the spine.<sup>87</sup>

## DEVELOPMENT OF ARTICULAR CARTILAGE

In the vertebrate skeleton, cartilage is the product of cells from three distinct embryonic lineages. Craniofacial cartilage is formed from cranial neural crest cells; the cartilage of the axial skeleton (intervertebral disks, ribs, and sternum)

forms from paraxial mesoderm (somites); and the articular cartilage of the limbs is derived from the lateral plate mesoderm.<sup>1</sup> In the developing limb bud, mesenchymal condensations, followed by chondrocyte differentiation and maturation, occur in digital zones, whereas undifferentiated mesenchymal cells in the interdigital web zones undergo cell death. Embryonic cartilage is destined for one of several fates: It can remain as permanent cartilage, as on the articular surfaces of bones, or it can provide a template for the formation of bones by endochondral ossification. During development, chondrocyte maturation expands from the central site of the original condensation, which forms the cartilage anlage resembling the shape of the future bone, toward the ends of the forming bones. During joint cavitation, the peripheral interzone is absorbed into each adjacent cartilaginous zone, evolving into the articular surface. The articular surface is destined to become a specialized cartilaginous structure that does not normally undergo vascularization and ossification.<sup>4,8,9</sup>

More recent evidence indicates that postnatal maturation of the articular cartilage involves an appositional growth mechanism originating from progenitor cells at the articular surface, rather than an interstitial mechanism. During formation of the mature articular cartilage, the differentiated articular chondrocytes synthesize the cartilage-specific matrix molecules, such as type II collagen and aggrecan (see Chapter 3). Through the processes described previously, the articular joint spaces are developed and lined on all surfaces either by cartilage or by synovial lining cells. These two different tissues merge at the enthesis, the region at the periphery of the joint where the cartilage melds into bone, and where ligaments and the capsule are attached. In the postnatal growth plate, the differentiation of the perichondrium also is linked to the differentiation of the chondrocytes in the epiphysis to form the different zones of the growth plate, contributing to longitudinal bone growth. Once the growth plate closes in the human joint, the adult articular cartilage must be maintained by the resident chondrocytes with low-turnover production of matrix proteins.<sup>88,89</sup>

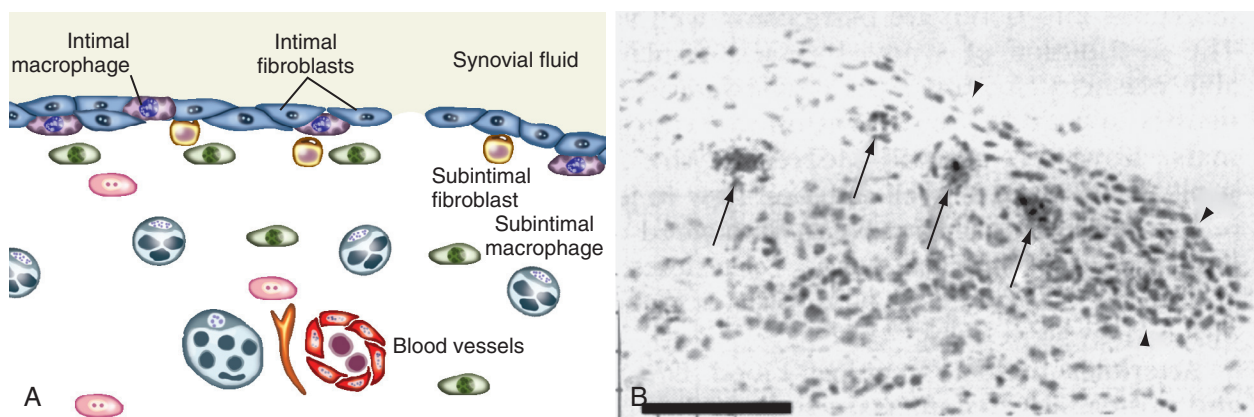
## ORGANIZATION AND PHYSIOLOGY OF THE MATURE JOINT

The unique structural properties and biochemical components of diarthrodial joints make them extraordinarily durable load-bearing devices. The mature diarthrodial joint is a complex structure, influenced by its environment and mechanical demands (see Chapter 6). Joints have structural differences that are determined by their different functions. The shoulder joint, which demands an enormous range of motion, is stabilized primarily by muscles, whereas the hip, which requires motion and antigravity stability, has an intrinsically stable ball-and-socket configuration. The components of the “typical” synovial joint are the synovium, muscles, tendons, ligaments, bursae, menisci, articular cartilage, and subchondral bone. The anatomy and physiology of muscles are described in detail in Chapter 5.

## SYNOVIUM

The synovium, which lines the joint cavity, is the site of production of synovial fluid that provides the nutrition for the articular cartilage and lubricates the cartilage surfaces. The synovium is a thin membrane between the fibrous joint capsule and the fluid-filled synovial cavity that attaches to skeletal tissues at the bone-cartilage interface and does not encroach on the surface of the articular cartilage. It is divided into functional compartments: the lining region (synovial intima), the subintimal stroma, and the neurovasculature (Figure 1-5). The synovial intima, also termed *synovial lining*, is the superficial layer of the normal synovium that is in contact with the intra-articular cavity. The synovial lining is loosely attached to the subintima, which contains blood vessels, lymphatics, and nerves. Capillaries and arterioles generally are located directly underneath the synovial intima, whereas venules are located closer to the joint capsule.

A transition from loose to dense connective tissue occurs from the joint cavity to the capsule. Most cells in the normal subintimal stroma are fibroblasts and macrophages, although



**Figure 1-5** **A**, Schematic representation of normal human synovium. The intima contains specialized fibroblasts expressing vascular cell adhesion molecule-1 (VCAM-1) and uridine diphosphoglucose (UDPG) and specialized macrophages expressing FcγRIIIa. The deeper subintima contains unspecialized counterparts. **B**, Microvascular endothelium in human synovium contains receptors for the vasodilator/growth factor substance P. Silver grains represent specific binding of [<sup>125</sup>I]Bolton Hunter–labeled substance P to synovial microvessels (arrows). Arrowheads indicate the synovial surface. Emulsion-dipped *in vitro* receptor autoradiography preparations with hematoxylin and eosin counterstain. Calibration bar = 1 μm. (**A**, from Edwards JCW: *Fibroblast biology: development and differentiation of synovial fibroblasts in arthritis*. *Arthritis Res* 2:344–347, 2000.)

adipocytes and occasional mast cells are present.<sup>90</sup> These compartments are not circumscribed by basement membranes but nonetheless have distinct functions; they are separated from each other by chemical barriers, such as membrane peptidases, which limit the diffusion of regulatory factors between compartments. Synovial compartments are unevenly distributed within a single joint. Vascularity is high at the enthesis where synovium, ligament, and cartilage coalesce. Far from being a homogeneous tissue in continuity with the synovial cavity, synovium is highly heterogeneous, and synovial fluid may be poorly representative of the tissue-fluid composition of any synovial tissue compartment. In rheumatoid arthritis, the synovial lining of diarthrodial joints is the site of the initial inflammatory process. This lesion is characterized by proliferation of the synovial lining cells, increased vascularization, and infiltration of the tissue by inflammatory cells, including B and T lymphocytes, plasma cells, and activated macrophages (see Chapter 69).<sup>91-94</sup> The roles of synovitis and synovial angiogenesis are also of current interest in relation to the progression and severity of joint damage in osteoarthritis (OA).<sup>95,96</sup>

## Synovial Lining

The synovial lining, a specialized condensation of mesenchymal cells and extra-cellular matrix, is located between the synovial cavity and stroma. In normal synovium, the lining layer is two to three cells deep, although intra-articular fat pads usually are covered by only a single layer of synovial cells, and ligaments and tendons are covered by synovial cells that are widely separated. At some sites, lining cells are absent, and the extra-cellular connective tissue constitutes the lining layer. Such “bare areas” become increasingly frequent with advancing age. Although the synovial lining is often referred to as the synovial membrane, the term *membrane* is more correctly reserved for endothelial and epithelial tissues that have basement membranes, tight intercellular junctions, and desmosomes. Instead, synovial lining cells lie loosely in a bed of hyaluronate interspersed with collagen fibrils; this is the macromolecular sieve that imparts the semipermeable nature of the synovium. The absence of any true basement membrane is a major determinant of joint physiology.

Early electron microscopic studies characterized lining cells as macrophage-derived type A and fibroblast-derived type B cells. High UDPGD activity and CD55 are used to distinguish type B synovial cells, whereas nonspecific esterase and CD68 typify type A cells. Normal synovium is lined predominantly by fibroblast-like synoviocytes, whereas macrophage-like synovial cells compose only 10% to 20% of lining cells (see Figure 1-5).<sup>92</sup>

Type A, macrophage-like synovial cells contain vacuoles, a prominent Golgi apparatus, and filopodia, but they have little rough endoplasmic reticulum. These cells express numerous cell surface markers of the monocyte-macrophage lineage, including CD16, CD45, CD11b/CD18, CD68, CD14, CD163, and the immunoglobulin (Ig)G Fc receptor, FcγRIIIa.<sup>90,92</sup> Synovial intimal macrophages are phagocytic and may provide a mechanism by which particulate matter can be cleared from the normal joint cavity. Similar to other tissue macrophages, these cells have little capacity to proliferate and are likely localized to the joint during develop-

ment. The op/op osteopetrotic mouse that is deficient in macrophages because of an absence of macrophage colony-stimulating factor also lacks synovial macrophages, suggesting that type A synovial cells are of a common lineage with other tissue macrophages. Although they represent only a small percentage of the cells in the normal synovium, the macrophages are recruited from the circulation during synovial inflammation, partly from subchondral bone marrow through vascular channels near the enthesis.

The type B, fibroblast-like synoviocytes contain fewer vacuoles and filopodia than type A cells and have abundant protein-synthetic organelles.<sup>92</sup> Similar to other fibroblasts, lining cells express genes encoding extra-cellular matrix components, including collagens, sulfated proteoglycans, fibronectin, fibrillin-1, and tenascin, and they express intra-cellular and cell surface molecules such as vimentin and CD90 (Thy-1). They have the potential to proliferate, although proliferation markers are rarely seen in normal synovium. In contrast to stromal fibroblasts, synovial intimal fibroblasts express UDPGD and synthesize hyaluronan, an important constituent of synovial fluid.<sup>90</sup> They also synthesize lubricin, which, together with hyaluronan, is necessary for the low-friction interaction of cartilage surfaces in the diarthrodial joint. Synovial lining cells bear abundant membrane peptidases on their surface that are capable of degrading a wide range of regulatory peptides, such as substance P and angiotensin II.

Normal synovial lining cells also express a rich array of adhesion molecules, including CD44, the principal receptor for hyaluronan; vascular cell adhesion molecule (VCAM)-1; intercellular adhesion molecule (ICAM)-1; and CD55 (decay-accelerating factor).<sup>92</sup> They are essential for cellular attachment to specific matrix components in the synovial lining region, preventing loss into the synovial cavity of cells subjected to deformation and shear stresses during joint movement. Adhesion molecules such as VCAM-1 and ICAM-1 potentially are involved in the recruitment of inflammatory cells during the evolution of arthritis. Cadherins mediate cell-cell adhesion between adjacent cells of the same type. The identification of cadherin-11 as a key adhesion molecule that regulates the formation of the synovial lining during development and the synoviocyte function postnatally has provided the opportunity to examine its role in inflammatory joint disease.<sup>79</sup> Cadherin-11 is highly expressed in fibroblast-like cells at the pannus-cartilage interface in rheumatoid synovium, where it plays a role in the invasive properties of the synovial fibroblasts,<sup>97</sup> and treatment with a cadherin-11 antibody or a cadherin-11 fusion protein reduces synovial inflammation and cartilage erosion in an animal model of arthritis.<sup>80</sup>

## Synovial Vasculature

The subintimal synovium contains blood vessels, providing the blood flow that is required for solute and gas exchange in the synovium itself and for the generation of synovial fluid.<sup>98</sup> The avascular articular cartilage also depends on nutrition in the synovial fluid, derived from the synovial vasculature. The vascularized synovium behaves similar to an endocrine organ, generating factors that regulate synoviocyte function and serving as a selective gateway that recruits cells from the circulation during stress and



inflammation. Finally, synovial blood flow plays an important role in regulating intra-articular temperature.

The synovial vasculature can be divided, on morphologic and functional grounds, into arterioles, capillaries, and venules. In addition, lymphatics accompany arterioles and larger venules.<sup>90</sup> Arterial and venous networks of the joint are complex and are characterized by arteriovenous anastomoses that communicate freely with blood vessels in periosteum and periarticular bone. As large synovial arteries enter the deep layers of the synovium near the capsule, they give off branches, which bifurcate again to form “microvascular units” in the subsynovial layers. The synovial lining region, the surfaces of intra-articular ligaments, and the entheses (in the angle of ligamentous insertions into bone) are particularly well vascularized.<sup>99</sup>

The distribution of synovial vessels, which were formed largely as a result of vasculogenesis during development of the joint, displays considerable plasticity. Vasculogenesis is a dynamic process that depends on the cellular interactions with regulatory factors and the extra-cellular matrix, which are also important in angiogenesis. In inflammatory arthritis, the density of blood vessels decreases relative to the growing synovial mass, creating a hypoxic and acidotic environment.<sup>100,101</sup> Angiogenic factors such as VEGF, acting via VEGF receptors 1 and 2 (Flt1 and Flk2), and basic FGF promote proliferation and migration of endothelial cells, a process that is facilitated by matrix-degrading enzymes and adhesion molecules such as integrin  $\alpha v \beta 3$  and E-selectin, expressed by activated endothelial cells. Vessel maturation is facilitated by angiopoietin-1 acting via the Tie-2 receptor. The angiogenic molecules are restricted to the capillary epithelium in normal synovium, but their levels are elevated in inflamed synovium in perivascular sites and areas remote from vessels.<sup>102,103</sup>

### Regulation of Synovial Blood Flow

Synovial blood flow is regulated by intrinsic (autocrine and paracrine) and extrinsic (neural and humoral) systems. Locally generated factors, such as the peptide vasoconstrictors angiotensin II and endothelin-1, act on adjacent arteriolar smooth muscle to regulate regional vascular tone.<sup>99</sup> Normal synovial arterioles are richly innervated by sympathetic nerves containing vasoconstrictors, such as norepinephrine and neuropeptide Y, and by “sensory” nerves that also play an efferent vasodilatory role by releasing neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP). Arterioles regulate regional blood flow. Capillaries and postcapillary venules are sites of fluid and cellular exchange. Correspondingly, regulatory systems are differentially distributed along the vascular axis. Angiotensin-converting enzyme, which generates angiotensin II, is localized predominantly in arteriolar and capillary endothelia and decreases during inflammation. Specific receptors for angiotensin II and for substance P are abundant on synovial capillaries, with lower densities on adjacent arterioles. Dipeptidyl peptidase IV, a peptide-degrading enzyme, is specifically localized to the cell membranes of venular endothelium. The synovial vasculature is not only functionally compartmentalized from the surrounding stroma but also highly specialized along its arteriovenous axis. Other unique characteristics of the normal synovial

vasculature include the presence of inducible nitric oxidase synthase-independent 3-nitrotyrosine, a reaction product of peroxynitrite, and the localization of the synoviocyte-derived CXCL12 chemokine on heparan sulfate receptors on endothelial cells, suggesting physiologic roles for these molecules in normal vascular function.

### JOINT INNERVATION

Dissection studies have shown that each joint has a dual nerve supply, consisting of specific articular nerves that penetrate the capsule as independent branches of adjacent peripheral nerves and articular branches that arise from related muscle nerves. The definition of joint position and the detection of joint motion are monitored separately and by a combination of multiple inputs from different receptors in varied systems. Nerve endings in muscle and skin and in the joint capsule mediate sensation of joint position and movement. Normal joints have afferent (sensory) and efferent (motor) innervations consisting of both unmyelinated and sensory thick myelinated A fibers in ligaments, fibrous capsule, menisci and adjacent periosteum, where they are thought to function primarily as sensors for pressure and movements. Sensory A and C fibers terminate as free nerve endings in the fibrous capsule, adipose tissue, ligaments, menisci, and the adjacent periosteum, where they are thought to act as nociceptors and contribute to the regulation of synovial microvascular function.

In normal synovium a dense network of fine unmyelinated nerve fibers follow the courses of blood vessels and extend into the synovial lining layers. These nerve fibers do not have specialized endings and are slow-conducting fibers; they may transmit diffuse, burning, or aching pain sensation. Sympathetic nerve fibers surround blood vessels, particularly in the deeper regions of normal synovium, and contain and release classic neurotransmitters, such as norepinephrine, and neuropeptides that are markers of sensory nerves including substance P, CGRP, neuropeptide Y, and vasoactive intestinal peptide.<sup>98,104</sup>

Afferent nerves containing substance P also have an efferent role in the synovium. Substance P is released from peripheral nerve terminals into the joint, and specific, G protein-coupled receptors for substance P are localized to microvascular endothelium in normal synovium. Abnormalities of articular innervation that are associated with inflammatory arthritis may contribute to the failure of synovial inflammation to resolve. Excessive local neuropeptide release may result in the loss of nerve fibers as a result of neuropeptide depletion. Synovial tissue proliferation without concomitant growth of new nerve fibers may lead to an apparent partial denervation of synovium. Studies in patients suggest that free nerve endings containing substance P may modulate inflammation and the pain pathway in OA. Afferent nerve fibers from the joint play an important role in the reflex inhibition of muscle contraction. Trophic factors generated by motor neurons, such as the neuropeptide CGRP, are important in maintaining muscle bulk and a functional neuromuscular junction. Decreases in motor neuron trophic support during articular inflammation probably contribute to muscle wasting.

Mechanisms of joint pain have been reviewed in detail.<sup>105-107</sup> In a noninflamed joint, most sensory nerve

fibers do not respond to movement within the normal range; these fibers are referred to as silent nociceptors. In an acutely inflamed joint, however, these nerve fibers become sensitized by mediators such as bradykinin, neurokinin 1, and prostaglandins (peripheral sensitization), and as a result, normal movements induce pain. Pain sensation is upregulated or downregulated further in the central nervous system, at the level of the spinal cord and in the brain, by central sensitization and “gating” of nociceptive input. Although the normal joint may respond predictably to painful stimuli, a poor correlation often exists between apparent joint disease and perceived pain in persons with chronic arthritis. Pain associated with joint movements within the normal range is a characteristic symptom described by patients with chronically inflamed joints caused by rheumatoid arthritis. Chronically inflamed joints may not be painful at rest, however, unless they are acutely inflamed.

The expression of substance P and CGRP are upregulated by nerve growth factor (NGF), which belongs to a family of neurotrophins that regulated neuronal growth during embryonic development.<sup>108</sup> Postnatally, NGF and the neurotrophins regulate neuronal regeneration and pain perception. In addition to promoting nerve growth and mediating pain perception, NGF can act together with VEGF to promote blood vessel formation. Angiogenesis and nerve growth thus are linked by common pathways involving NGF, VEGF, and neuropeptides such as CGRP, neuropeptide Y, and semaphorins.<sup>98,109,110</sup>

## TENDONS

Tendons are functional and anatomic bridges between muscle and bone.<sup>111,112</sup> Tendons focus the force of a large mass of muscle into a localized area on bone and, by splitting to form numerous insertions, may distribute the force of a single muscle to different bones. Tendons are formed of longitudinally arranged collagen fibrils embedded in an organized, hydrated proteoglycan matrix with blood vessels, lymphatics, and fibroblasts.<sup>113</sup> Cross-links between adjacent collagen chains or molecules contribute to the tensile strength of the tendon. Tendon collagen fibrillogenesis is initiated during early development by a highly ordered process of alignment involving the actin cytoskeleton and cadherin-11.<sup>114,115</sup> Many tendons, particularly those with a large range of motion, run through vascularized, discontinuous sheaths of collagen lined with mesenchymal cells resembling synovium. Gliding of tendons through their sheaths is enhanced by hyaluronic acid produced by the lining cells. Tendon movement is essential for the embryogenesis and maintenance of tendons and their sheaths.<sup>116,117</sup> Degenerative changes appear in tendons, and fibrous adhesions form between tendons and sheaths when inflammation or surgical incision is followed by long periods of immobilization. At the myotendinous junction, recesses between muscle cell processes are filled with collagen fibrils, which blend into the tendon. At its other end, collagen fibers of the tendon typically blend into fibrocartilage, mineralize, and merge into bone through a fibrocartilaginous transition zone termed the *enthesis*, or insertion site.<sup>118</sup>

Tendon fibroblasts synthesize and secrete collagens, proteoglycans, and other matrix components, such as fibronectin

and tenascin C, as well as MMPs and their inhibitors, which can contribute to the breakdown and repair of tendon components.<sup>113</sup> Collagen fibrils in tendon are composed primarily of type I collagen with some type III collagen, but there are regional differences in the distribution of other matrix components. The compressed region contains the small proteoglycans, biglycan, decorin, fibromodulin, and lumican, as well as the large proteoglycan versican. The major components in the tensile region of the tendon are decorin, microfibrillar type VI collagen, fibromodulin, and the proline and arginine-rich end leucine-rich repeat protein (PRELP). The presence of cartilage oligomeric matrix protein, aggrecan, and biglycan and collagen types II, IX, and XI is indicative of fibrocartilage. The collagen fiber orientation at the tendon-to-bone enthesis is important for maintaining microarchitecture by reducing the stress concentrations and shielding the outward splay of the insertion from the highest stresses.<sup>119</sup> Understanding the structure has implications for tendon repair because motion between a tendon graft and bone tunnel may impair early graft incorporation and lead to tunnel widening secondary to bone resorption.<sup>120,121</sup>

Failure of the muscle-tendon apparatus is rare; when it does occur, it is the result of enormous, quickly generated forces across a joint and usually occurs near the tendon insertion into bone.<sup>122</sup> Factors that may predispose to tendon failure are aging processes, including loss of extra-cellular water and the increase in intermolecular cross-links of collagen; tendon ischemia; iatrogenic factors, including injection of glucocorticoids; and deposition of calcium hydroxyapatite crystals within the collagen bundles. Alterations in collagen fibril composition and structure are associated with tendon degeneration during aging and may predispose to OA. Understanding the contributions in tendon development of Sox9, scleraxis, Mohawk, members of the TGF- $\beta$ /BMP superfamily, including GDF-5, and Wnt/ $\beta$ -catenin, and *lhh* signaling<sup>123,124</sup> may point to strategies for tendon repair.<sup>122,125</sup>

## LIGAMENTS

Ligaments, which provide a stabilizing bridge between bones, permit a limited range of movement.<sup>126</sup> Ligaments often are recognized only as hypertrophied components of the fibrous joint capsule and are structurally similar to tendons.<sup>127</sup> Although the fibers are oriented parallel to the longitudinal axis of both tissues, the collagen fibrils in ligaments are nonparallel and arranged in fibers that are oriented roughly along the long axis in a wavy, undulating pattern, or “crimp,” which can straighten in response to load. Some ligaments have a higher ratio of elastin to collagen (1:4) than do tendons (1:50), which permits a greater degree of stretch. Ligaments also have larger amounts of reducible cross-links, more type III collagen, slightly less total collagen, and more glycosaminoglycans compared with tendons. The cells in ligaments seem to be more metabolically active than the cells in tendons and have more plump cellular nuclei and higher DNA content.

During postnatal growth, the development of ligament attachment zones involves changes in the ratios and distribution of types I, III, and V collagen and the synthesis of type II collagen and proteoglycans by fibrochondrocytes

that develop from ligament cells at the attachment zone. Attachment zones are believed to permit gradual transmission of the tensile force between ligament and bone.

Ligaments play a major role in the passive stabilization of joints, aided by the capsule and, when present, menisci. In the knee, the collateral and cruciate ligaments provide stability when there is little or no load on the joint. As compressive load increases, there is an increasing contribution to stability from the joint surfaces themselves and the surrounding musculature. Injured ligaments generally heal, and structural integrity is restored by contracture of the healing ligament so it can act again as a stabilizer of the joint.

## BURSAE

The many bursae in the human body facilitate gliding of one tissue over another, much as a tendon sheath facilitates movement of its tendon. Bursae are closed sacs, lined sparsely with mesenchymal cells that are similar to synovial cells, but they are generally less well vascularized than synovium. Most bursae differentiate concurrently with synovial joints during embryogenesis. Throughout life, trauma or inflammation may lead to the development of new bursae, hypertrophy of previously existing ones, or communication between deep bursae and joints. In patients with rheumatoid arthritis, communications may exist between the subacromial bursae and the glenohumeral joint, between the gastrocnemius or semimembranosus bursae and the knee joint, and between the iliopsoas bursa and the hip joint. It is unusual, however, for subcutaneous bursae, such as the prepatellar bursa or olecranon bursa, to develop communication with the underlying joint.<sup>128</sup>

## MENISCI

The meniscus, a fibrocartilaginous, wedge-shaped structure, is best developed in the knee but also is found in the acromioclavicular and sternoclavicular joints, the ulnocarpal joint, and the temporomandibular joint. Until more recently, menisci were thought to have little function and a quiescent metabolism with no capability of repair, although early observations indicated that removal of menisci from the knee could lead to premature arthritic changes in the joint. Evidence from arthroscopic studies of patients with anterior cruciate ligament insufficiency indicates that disease of the medial meniscus correlates with that of the medial femoral cartilage. The meniscus is now considered to be an integral component of the knee joint that has important functions in joint stability, load distribution, shock absorption, and lubrication.<sup>129,130</sup>

The microanatomy of the meniscus is complex and age dependent.<sup>131</sup> The characteristic shapes of the lateral and the medial menisci are achieved early in prenatal development. At that time, the menisci are cellular and highly vascularized; with maturation, vascularity decreases progressively from the central margin to the peripheral margin. After skeletal maturity, the peripheral 10% to 30% of the meniscus remains highly vascularized by a circumferential capillary plexus and is well innervated. Tears in this vascularized peripheral zone may undergo repair and remodeling. The central portion of the mature meniscus is an avascular

fibrocartilage, however, without nerves or lymphatics, consisting of cells surrounded by an abundant extra-cellular matrix of collagens, chondroitin sulfate, dermatan sulfate, and hyaluronic acid. Tears in this central zone heal poorly, if at all.

Collagen constitutes 60% to 70% of the dry weight of the meniscus and is mostly type I collagen, with lesser amounts of types III, V, and VI. A small quantity of cartilage-specific type II collagen is localized to the inner, avascular portion of the meniscus. Collagen fibers in the periphery are mostly circumferentially oriented, with radial fibers extending toward the central portion. Elastin content is around 0.6%, and proteoglycan content is around 2% to 3% dry weight. Aggrecan and decorin are the major proteoglycans in the adult meniscus. Decorin is the predominant proteoglycan synthesized in the meniscus from young persons, whereas the relative proportion of aggrecan synthesis increases with age. Although the capacity of the meniscus to synthesize sulfated proteoglycans decreases after the teenage years, the age-related increases in expression of decorin and aggrecan mRNA suggest that the resident cells are able to respond quickly to alterations in the biomechanical environment.

The meniscus was defined originally as a fibrocartilage, based on the rounded or oval shape of most of the cells and the fibrous microscopic appearance of the extra-cellular matrix. Based on molecular and spatial criteria, three distinct populations of cells are recognized in the meniscus of the knee joint<sup>132</sup>:

1. The fibrochondrocyte is the most abundant cell in the middle and inner meniscus, synthesizing primarily type I collagen and relatively small amounts of type II and III collagens. It is round or oval in shape and has a pericellular filamentous matrix containing type VI collagen.
2. The fibroblast-like cells lack a pericellular matrix and are located in the outer portion of the meniscus. They are distinguished by long, thin, branching cytoplasmic projections that stain for vimentin. They make contact with other cells in different regions via connexin 43-containing gap junctions. The presence of two centrosomes, one associated with a primary cilium, suggests a sensory, rather than motile, function that could enable the cells to respond to circumferential tensile loads rather than compressive loads.
3. The superficial zone cells have a characteristic fusiform shape with no cytoplasmic projections. The occasional staining of these cells in the uninjured meniscus with  $\alpha$ -actin and their migration into surrounding wound sites suggest that they are specialized progenitor cells that may participate in a remodeling response in the meniscus and surrounding tissues.

Cell lineage tracing and gene profiling studies in mouse embryos have provided insight into the complexity of the meniscus and how it was formed.<sup>133,134</sup> Researchers have considerable interest in using this information to develop new strategies for meniscal generation.

## MATURE ARTICULAR CARTILAGE

Articular cartilage is a specialized connective tissue that covers the weight-bearing surfaces of diarthrodial joints.

The principal functions of cartilage layers covering bone ends are to permit low-friction, high-velocity movement between bones, to absorb the transmitted forces associated with locomotion, and to contribute to joint stability. Lubrication by synovial fluid provides frictionless movement of the articulating cartilage surfaces. Chondrocytes (see Chapter 3) are the single cellular component of adult hyaline articular cartilage and are responsible for synthesizing and maintaining the highly specialized cartilage matrix macromolecules. The cartilage extra-cellular matrix is composed of an extensive network of collagen fibrils, which confers tensile strength, and an interlocking mesh of proteoglycans, which provides compressive stiffness through the ability to absorb and extrude water. Numerous other noncollagenous proteins also contribute to the unique properties of cartilage (Table 1-1). Histologically, the tissue appears to be fairly homogeneous and clearly distinguished from the calcified cartilage and underlying subchondral bone (Figure 1-6). However, this appearance is misleading because significant topographical and regional differences exist in the molecular organization and composition of the articular cartilage, as described in Chapter 3.

## SUBCHONDRAL BONE

Subchondral bone is not a homogeneous tissue and consists of a layer of compact cortical bone and an underlying system of cancellous bone organized into a trabecular network.<sup>135,136</sup> The subchondral bone is separated from the overlying articular cartilage by a thin zone of calcified cartilage. The so-called tidemark defines the transition zone between the articular and calcified cartilage. This complex biocomposite of bone and calcified cartilage provides an optimal system for distributing loads that are transmitted from the weight-bearing surfaces that are lined by the hyaline articular cartilage. Although the tidemark was originally believed to form a barrier to fluid flow, evidence shows that biologically active molecules can transit this zone, providing a mechanism by which products produced by chondrocytes or bone cells can influence the activity of the other cell type.<sup>137,138</sup> In addition, further communication is provided via products released from vascular elements in channels that penetrate the calcified cartilage from the adjacent marrow space.<sup>139</sup> Under physiologic conditions the composition and structural organization of the subchondral bone and calcified cartilage are optimally adapted to transfer loads, but several conditions can lead to changes in the structural and functional properties of these tissues.

The subchondral bone undergoes continuous structural reorganization throughout postnatal life. These alterations are mediated by the coordinated activity of bone-resorbing osteoclasts and bone-forming osteoblasts that remodel and adapt the bone in response to local biomechanical and biological signals.<sup>140</sup> Several lines of evidence have established that osteocytes are the bone cell type that plays a key role in regulating the bone remodeling process.<sup>141,142</sup> Osteocytes are distributed throughout the mineralized bone matrix, forming an interconnected network that is ideally positioned to sense and respond to local and systemic stimuli. These effects are mediated via both cell-cell interactions with osteoclasts and osteoblasts but also via signaling through the release of soluble mediators. These products

**TABLE 1-1** Extra-cellular Matrix Components of Articular Cartilage\*

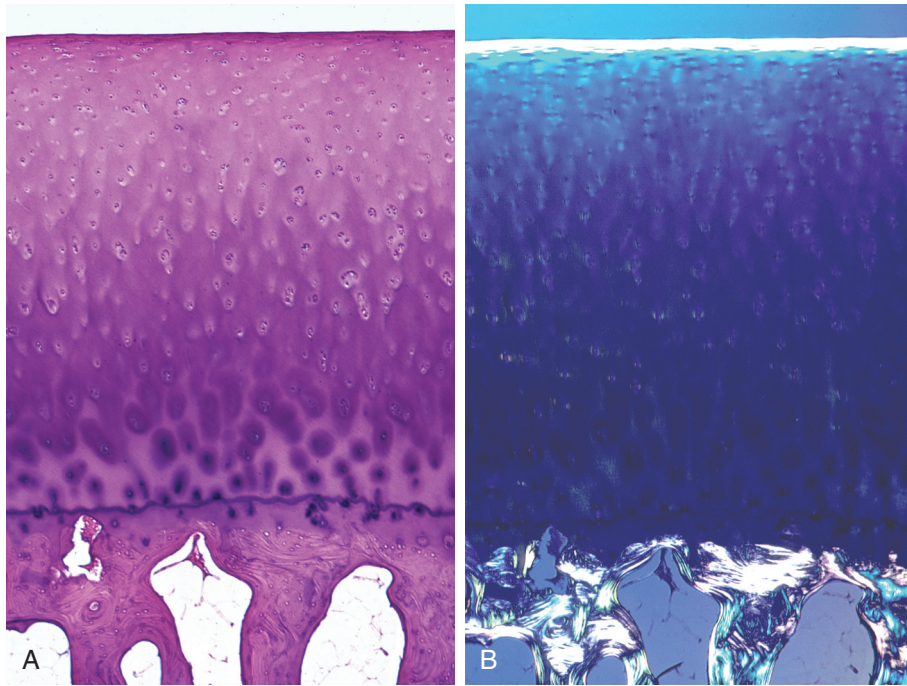
<b>Collagens</b>
Type II
Type IX
Type XI
Type VI
Types XII, XIV
Type X (hypertrophic chondrocyte)
<b>Proteoglycans</b>
Aggrecan
Versican
Link protein
Biglycan (DS-PGI)
Decorin (DS-PGII)
Epiphygan (DS-PGIII)
Fibromodulin
Lumican
Proline/arginine-rich and leucine-rich repeat protein (PRELP)
Chondroadherin
Perlecan
Lubricin (SZP)
<b>Other Noncollagenous Proteins (Structural)</b>
Cartilage oligomeric matrix protein (COMP) or thrombospondin-5
Thrombospondin-1 and thrombospondin-3
Cartilage matrix protein (matrilin-1) and matrilin-3
Fibronectin
Tenascin-C
Cartilage intermediate layer protein (CILP)
Fibrillin
Elastin
<b>Other Noncollagenous Proteins (Regulatory)</b>
Glycoprotein (gp)-39, YKL-40
Matrix Gla protein (MGP)
Chondromodulin-I (SCGP) and chondromodulin-II
Cartilage-derived retinoic acid-sensitive protein (CD-RAP)
Growth factors
<b>Cell Membrane-Associated Proteins</b>
Integrins ( $\alpha 1\beta 1$ , $\alpha 2\beta 1$ , $\alpha 3\beta 1$ , $\alpha 5\beta 1$ , $\alpha 6\beta 1$ , $\alpha 10\beta 1$ , $\alpha v\beta 3$ , $\alpha v\beta 5$ )
Anchoring CII (annexin V)
Cell determinant 44 (CD44)
Syndecan-1, 3, and 4
Discoidin domain receptor 2

\*The collagens, proteoglycans, and other noncollagenous proteins in the cartilage matrix are synthesized by chondrocytes at different stages during development and growth of cartilage. In mature articular cartilage, proteoglycans and other noncollagen proteins are turned over slowly, whereas the collagen network is stable unless exposed to proteolytic cleavage. Proteins that are associated with chondrocyte cell membranes also are listed because they permit specific interactions with extra-cellular matrix proteins. The specific structure-function relationships are discussed in Chapter 3 and described in Table 3-1.

DS-PG, Dermatan sulfate proteoglycan; SCGP, small cartilage-derived glycoprotein; SZP, superficial zone protein; YKL-40, 40KD chitinase 3-like glycoprotein.

include RANKL, the essential regulator of osteoclast differentiation and activity and its inhibitor osteoprotegerin (OPG),<sup>143,144</sup> as well as additional mediators, including prostanoids, nitric oxide, nucleotides, and a broad spectrum of growth factors and cytokines.<sup>145</sup> In addition to these factors, osteocytes also produce sclerostin and Dickkopf-related protein 1 (DKK-1), which are potent inhibitors of the Wnt/ $\beta$ -catenin pathway that contributes to the regulation of osteoblast-mediated bone formation.<sup>146</sup> The release of RANKL and OPG and the Wnt pathway regulators, DKK-1 and sclerostin, play a major role in controlling the





**Figure 1-6** Representative sections of normal human adult articular cartilage, showing nearly the same field in plain (A) and polarized (B) light. Note the clear demarcation of the articular cartilage from the calcified cartilage below the tidemark and the underlying subchondral bone. (Hematoxylin-eosin stain; original magnification  $\times 60$ .) (Courtesy Edward F. DiCarlo, MD, Pathology Department, Hospital for Special Surgery, New York, N.Y.)

adaptation of the subchondral bone to alterations in mechanical loading in both physiologic and pathologic conditions.

The maintenance of the structural and functional integrity of articular cartilage and subchondral bone under physiologic loading is evidence of the unique and intimate interaction of these tissues, but controversy remains with regard to the relationship between them in the pathogenesis of OA.<sup>147</sup> Radin and Rose<sup>148</sup> proposed that the initiation of early alterations in articular cartilage is caused by an increase in subchondral bone stiffness that adversely affects the function of articular chondrocytes, leading to deterioration in the properties of the articular cartilage and susceptibility to mechanical disruption. Alternatively, it has been proposed that changes in subchondral bone stiffness may be a result of cartilage deterioration.<sup>149-151</sup> The alterations in subchondral bone and cartilage that accompany the osteoarthritic process are not restricted to these tissues but also affect the zone of calcified cartilage, where there is evidence of vascular invasion, advancement of the calcified cartilage, and duplication of the tidemark that contributes to a decrease in articular cartilage thickness.<sup>152,153</sup> The penetration of the vascular channels from the subchondral bone and calcified cartilage into the deep zones of the articular cartilage permit exchange of fluids and soluble mediators between these tissues, providing an additional mechanism by which the subchondral bone and articular cartilage can affect the activity of cells within each of these tissues. These structural alterations in the articular cartilage and periarticular bone may also lead to modification of the contours of the adjacent articulating surfaces, further contributing to the adverse biomechanical environment.<sup>148,154-156</sup>

## SYNOVIAL FLUID AND NUTRITION OF JOINT STRUCTURES

The volume and composition of synovial fluid are determined by the properties of the synovium and its vasculature. Fluid in normal joints is present in small quantities (2.5 mL in the normal knee) sufficient to coat the synovial surface but not sufficient to separate one surface from the other. Tendon sheath fluid and synovial fluid are biochemically similar. Both are essential for the nutrition and lubrication of adjacent avascular structures, including tendons and articular cartilage, and for limiting adhesion formation and maintaining movement. Characterization and measurement of synovial fluid constituents have proved useful for the identification of locally generated regulatory factors, markers of cartilage turnover, and the metabolic status of the joint, as well as for the assessment of the effects of therapy on cartilage homeostasis. However, interpretation of such data requires an understanding of the generation and clearance of synovial fluid and its various components.

## GENERATION AND CLEARANCE OF SYNOVIAL FLUID

Synovial fluid concentrations of a protein represent the net contributions of synovial blood flow, plasma concentration, microvascular permeability, and lymphatic removal and its production and consumption within the joint space. Synovial fluid is a mixture of a protein-rich ultrafiltrate of plasma and hyaluronan synthesized by synoviocytes.<sup>91</sup> Generation of this ultrafiltrate depends on the differences between intracapillary and intra-articular hydrostatic pressures and



between colloid osmotic pressures of capillary plasma and synovial tissue fluid. Fenestrations (i.e., small pores covered by a thin membrane) in the synovial capillaries and the macromolecular sieve of hyaluronic acid facilitate rapid exchange of small molecules, such as glucose and lactate, assisted—in the case of glucose—by an active transport system. Proteins are present in synovial fluid at concentrations inversely proportional to molecular size, with synovial fluid albumin concentrations being about 45% of those in plasma. Concentrations of electrolytes and small molecules are equivalent to those in plasma.<sup>157</sup>

Synovial fluid is cleared through lymphatics in the synovium, assisted by joint movement. In contrast to ultrafiltration, lymphatic clearance of solutes is independent of molecular size. In addition, constituents of synovial fluid, such as regulatory peptides, may be degraded locally by enzymes, and low-molecular-weight metabolites may diffuse along concentration gradients into plasma. The kinetics of delivery and removal of a protein must be determined (e.g., using albumin as a reference solute) to assess the significance of its concentration in the joint.<sup>158</sup>

Hyaluronic acid is synthesized by fibroblast-like synovial lining cells, and it appears in high concentrations in synovial fluid at around 3 g/L, compared with a plasma concentration of 30  $\mu\text{g/L}$ .<sup>91</sup> Lubricin, a glycoprotein that assists articular lubrication, is another constituent of synovial fluid that is generated by the lining cells.<sup>159</sup> It is now believed that hyaluronan functions in fluid-film lubrication, whereas lubricin is the true boundary lubricant in synovial fluid (see later discussion). Because the volume of synovial fluid is determined by the amount of hyaluronan, water retention seems to be the major function of this large molecule.

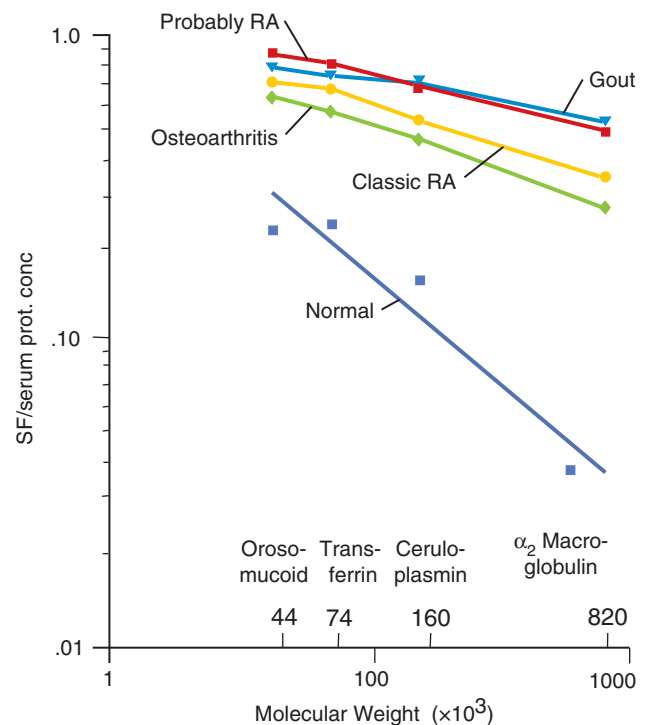
Despite the absence of a basement membrane, synovial fluid does not mix freely with extra-cellular synovial tissue fluid.<sup>96</sup> Hyaluronan may trap molecules within the synovial cavity by acting as a filtration screen on the surface of the synovial lining, resisting the movement of synovial fluid out from the joint space. Synovial fluid and its constituent proteins have a rapid turnover time (around 1 hour in normal knees), and equilibrium is not usually reached among all parts of the joint. Tissue fluid around fenestrated endothelium reflects plasma ultrafiltrate most closely, with a low content of hyaluronate compared with synovial fluid. Alternatively, locally generated or released peptides, such as endothelin and substance P, may attain much higher perivascular concentrations than those measured in synovial fluid. However, the turnover time for hyaluronan in the normal joint (13 hours) is an order of magnitude slower than that of small solutes and proteins. Association with hyaluronan may result in trapping of solutes within synovial fluid.

In normal joints, intra-articular pressures are slightly subatmospheric at rest (0 to  $-5$  mm Hg). During exercise, hydrostatic pressure in the normal joint may decrease further. Resting intra-articular pressures in rheumatoid joints are around 20 mm Hg, whereas during isometric exercise, they may increase to greater than 100 mm Hg, well above capillary perfusion pressure and, at times, above arterial pressure. Repeated mechanical stresses can interrupt synovial perfusion during joint movement, particularly in the presence of a synovial effusion.

## SYNOVIAL FLUID AS AN INDICATOR OF JOINT FUNCTION

In the absence of a basement membrane separating synovium or cartilage from synovial fluid, measurements of synovial fluid may reflect the activity of these tissues. A wide range of regulatory factors and products of synoviocyte metabolism and cartilage breakdown may be generated locally within the joint, resulting in marked differences between the composition of synovial fluid and plasma ultrafiltrate. Because little capacity exists for the selective concentration of solutes in synovial fluid, solutes that are present at higher concentrations than in plasma are probably synthesized locally. It is necessary, however, to know the local clearance rate to determine whether the solutes present in synovial fluid at lower concentrations than in plasma are generated locally.<sup>157,158</sup> Because clearance rates from synovial fluid may be slower than those from plasma, synovial fluid levels of drugs or urate may remain elevated after plasma levels have declined.

Plasma proteins are less effectively filtered in inflamed synovium, perhaps because of increased size of endothelial cell fenestrations or because interstitial hyaluronate-protein complexes are fragmented by enzymes associated with the inflammatory process. Concentrations of proteins, such as  $\alpha_2$  macroglobulin (the principal proteinase inhibitor of plasma), fibrinogen, and IgM, are elevated in inflammatory synovial fluids (see Figure 1-7), as are associated protein-bound cations. Membrane peptidases may limit the



**Figure 1-7** Ratio of the concentration of proteins in synovial fluid to that found in serum, plotted as a function of molecular weight. Larger proteins are selectively excluded from normal synovial fluid, but this macromolecular sieve is less effective in diseased synovium. Prot. conc., Protein concentration; RA, rheumatoid arthritis; SF, synovial fluid. (From Kushner I, Somerville JA: *Permeability of human synovial membrane to plasma proteins*. *Arthritis Rheum* 14:560, 1971. Reprinted with permission of the American College of Rheumatology.)

diffusion of regulatory peptides from their sites of release into synovial fluid. In inflammatory arthritis, fibrin deposits may retard flow between the tissue and the liquid phase.

Recently, Sohn and coworkers<sup>160</sup> analyzed synovial fluids and sera from a small series of patients with OA and rheumatoid arthritis using mass spectrometry and multiplex bead-based immunoassays. They identified more than a hundred proteins that were increased in the synovial fluid of patients with OA compared with healthy subjects. Of interest, they found that more than one-third of the proteins in the OA synovial fluid were plasma proteins. They speculated that the presence of these plasma proteins in the synovial fluid could be related to alterations in the endothelial barrier associated with local inflammation in the synovial tissue.

Gobezie and co-workers<sup>161</sup> utilized high-throughput mass spectroscopy-based proteomic analysis to define the protein expression profiles of high abundance synovial fluid proteins in healthy subjects and in patients with early and late OA. They identified 18 proteins that were significantly differentially expressed between the osteoarthritic and control groups. Although all of the differentially expressed proteins were present in the blood and could therefore enter the joint through alterations in vascular permeability associated with the disease state, these molecules were also products of synovial cells and chondrocytes, suggesting that they could be locally produced within the joint. More recently, Ritter et al.<sup>162</sup> utilized a more sensitive method based on gel electrophoresis and mass spectrometry to examine the synovial fluid proteome from patients with OA. They compared the proteomic results with mRNA expression profiles of joint tissues and demonstrated that many of the proteins were derived from synovium or cartilage, providing direct evidence that cells within the joint were a source of the synovial fluid products. Proteins associated with oxidative damage and activation of mitogen-activated protein kinases were among the high-abundance molecules in the OA synovial fluids. They also identified members of the pro-inflammatory complement cascade in the synovial fluid. Of interest, these molecules have been implicated in the pathophysiology of both OA and rheumatoid arthritis.<sup>163</sup>

## LUBRICATION AND NUTRITION OF THE ARTICULAR CARTILAGE

### Lubrication

Synovial fluid serves as a lubricant for articular cartilage and a source of nutrition for the chondrocytes. Lubrication is essential for protecting cartilage and other joint structures from friction and shear stresses associated with movement under loading. There are two basic categories of joint lubrication. In fluid-film lubrication, cartilage surfaces are separated by an incompressible fluid film, and hyaluronan functions as the lubricant. In boundary lubrication, specialized molecules attached to the cartilage surface permit surface-to-surface contact while decreasing the coefficient of friction. During loading, a noncompressible fluid film is trapped between opposing cartilage surfaces and prevents the surfaces from touching. Irregularities in the cartilage surface and its deformation during compression may augment this trapping of fluid. This stable film is approximately

0.1- $\mu\text{m}$  thick in the normal human hip joint, but it can be much thinner in the presence of inflammatory synovial fluids or with increased cartilage porosity.<sup>91</sup>

Lubricin is the major boundary lubricant in the human joint.<sup>159</sup> Lubricin is a glycoprotein, also called superficial zone protein or proteoglycan 4, that is synthesized by synovial cells, chondrocytes, meniscus, and tendon cells.<sup>164,165</sup> It has a molecular weight of 225,000, a length of 200 nm, and a diameter of 1 to 2 nm. Dipalmitoyl phosphatidylcholine, which constitutes 45% of the lipid in normal synovial fluid, acts together with lubricin as a boundary lubricant. In *boundary lubrication*, lubricin functions as a phospholipid carrier via a mechanism that is common to all tissues and protects the cartilage by reducing pathologic deposition of proteins at the cartilage surface.<sup>166</sup> The importance of lubricin in preserving cartilage homeostasis is illustrated by the study of persons with loss-of-function mutations in the lubricin gene, resulting in the camptodactyl-arthropathy-coxa varangericarditis syndrome, which is associated with the development of severe premature OA.<sup>167</sup> Of interest, long-term overexpression of lubricin in animal models of OA protects against both age-related and post-traumatic OA through inhibition of transcriptional programs that promote cartilage catabolism and chondrocyte hypertrophy.<sup>168</sup>

### Nutrition

As observed by Hunter in 1743,<sup>169</sup> normal adult articular cartilage contains no blood vessels. Vascularization of cartilage would be expected to alter its mechanical properties. Blood flow would be repeatedly occluded during weight bearing and exercise, with reactive oxygen species generated during reperfusion, resulting in repeated damage to cartilage matrix and chondrocytes. Chondrocytes synthesize specific inhibitors of angiogenesis that maintain articular cartilage as an avascular tissue.<sup>170-173</sup> As a result of the lack of adjacent blood vessels, the chondrocyte normally lives in a hypoxic and acidotic environment, with extra-cellular fluid pH values around 7.1 to 7.2,<sup>174</sup> and it uses anaerobic glycolysis for energy production.<sup>175,176</sup> High lactate levels in normal synovial fluid, compared with paired plasma measurements, partially reflect this anaerobic metabolism.<sup>176</sup> The two sources of nutrients for articular cartilage are the synovial fluid and subchondral blood vessels.

The synovial fluid and, indirectly, the synovial lining, through which synovial fluid is generated, are the major sources of nutrients for articular cartilage. Nutrients may enter cartilage from synovial fluid either by diffusion or by mass transport of fluid during compression-relaxation cycles.<sup>177</sup> Molecules as large as hemoglobin (65 kDa) can diffuse through normal articular cartilage,<sup>178</sup> and the solutes needed for cellular metabolism are much smaller. Diffusion of uncharged small solutes, such as glucose, is not impaired in matrices containing large amounts of glycosaminoglycans, and diffusivity of small molecules through hyaluronate is enhanced.<sup>179,180</sup>

Intermittent compression may serve as a pump mechanism for solute exchange in cartilage. The concept has arisen from observations that joint immobilization or dislocation leads to degenerative changes. In contrast, exercise increases solute penetration into cartilage in experimental systems.<sup>178</sup> During weight bearing, fluid escapes from the

load-bearing region by flow to other cartilage sites. When the load is removed, cartilage re-expands and draws back fluid, exchanging nutrients with waste materials.<sup>181</sup>

In a growing child, the deeper layers of cartilage are vascularized, such that blood vessels penetrate between columns of chondrocytes in the growth plate. It is likely that nutrients diffuse from these tiny end capillaries through the matrix to chondrocytes. Diffusion from subchondral blood vessels is not considered a major route for the nutrition of normal adult articular cartilage because of the barrier provided by its densely calcified lower layer. Nonetheless, partial defects may normally exist in this barrier,<sup>182</sup> and in arthritis, neovascularization of the deeper layers of articular cartilage may contribute to cartilage nutrition and to entry of inflammatory cells and cytokines.<sup>139,152,183</sup>

## CONCLUSION

Normal human synovial joints are complex structures that comprise interacting connective tissue elements that permit constrained and low-friction movement of adjacent bones. The development of synovial joints in the embryo is a highly ordered process involving complex cell-cell and cell-matrix interactions that lead to the formation of the cartilage anlage and interzone and joint cavitation. Understanding of the cellular interactions and molecular factors involved in cartilage morphogenesis and limb development has provided clues to understanding the functions of the synovium, articular cartilage, and associated structures in the mature joint.

The synovial joint is uniquely adapted to respond to environmental and mechanical demands. The synovial lining is composed of two to three cell layers, with no basement membrane separating the lining cells from the underlying connective tissue. The synovium produces synovial fluid, which provides nutrition and lubrication to the avascular articular cartilage. Normal articular cartilage contains a single cell type, the articular chondrocyte, which is responsible for maintaining the integrity of the extracellular cartilage matrix. This matrix consists of a complex network of collagens, proteoglycans, and other noncollagenous proteins, which provide tensile strength and compressive resistance. Proper distribution and relative composition of these proteins is required for the function of cartilage in protecting the subchondral bone from adverse environmental influences.

Maintenance of the unique composition and organization of each joint tissue is crucial for normal joint function, which is compromised in response to inflammation, biomechanical injury, and aging. Knowledge of the normal structure-function relationships within joint tissues is essential for understanding the pathogenesis and consequences of joint diseases.



Full references for this chapter can be found on [ExpertConsult.com](http://ExpertConsult.com).

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# Synovium

Douglas J. Veale • Gary S. Firestein

## KEY POINTS

The synovium provides nutrients to cartilage and produces lubricants for the joint.

The intimal lining of the synovium includes macrophage-like and fibroblast-like synoviocytes.

The sublining in normal synovium contains scattered immune cells, fibroblasts, blood vessels, and fat cells.

Fibroblast-like synoviocytes in the intimal lining produce specialized enzymes that synthesize lubricants such as hyaluronic acid.

## STRUCTURE

The synovium is a membranous structure that extends from the margins of articular cartilage and lines the capsule of diarthrodial joints, including the temporomandibular joint<sup>1</sup> and the facet joints of vertebral bodies (Figure 2-1).<sup>2</sup> The healthy synovium covers intra-articular tendons and ligaments, as well as fat pads, but not articular cartilage or meniscal tissue. Synovium also ensheaths tendons where they pass beneath ligamentous bands and bursas that cover areas of stress such as the patella and the olecranon. The synovial membrane is divided into two general regions: the intima, or synovial lining, and the subintima, otherwise referred to as the *sublining*. The intima represents the interface between the cavity containing synovial fluid and the subintimal layer. No well-formed basement membrane separates the intima from the subintima. In contrast to the pleura or pericardium, it is not a true lining because it lacks tight junctions, epithelial cells, and a well-formed basement membrane. The subintima is composed of fibrovascular connective tissue and merges with the densely collagenous fibrous joint capsule.

## Synovial Lining Cells

The synovial intimal layer is composed of synovial lining cells (SLCs), which are arrayed on the luminal aspect of the joint cavity. SLCs, termed *synoviocytes*, are one to three cells deep, depending on the anatomic location, and extend 20 to 40  $\mu\text{m}$  beneath the lining layer surface. The major and minor axes of SLCs measure 8 to 12  $\mu\text{m}$  and 6 to 8  $\mu\text{m}$ , respectively. The SLCs are not homogeneous and are conventionally divided into two major populations, namely, type A (macrophage-like) synoviocytes and type B (fibroblast-like) synoviocytes.<sup>3</sup>

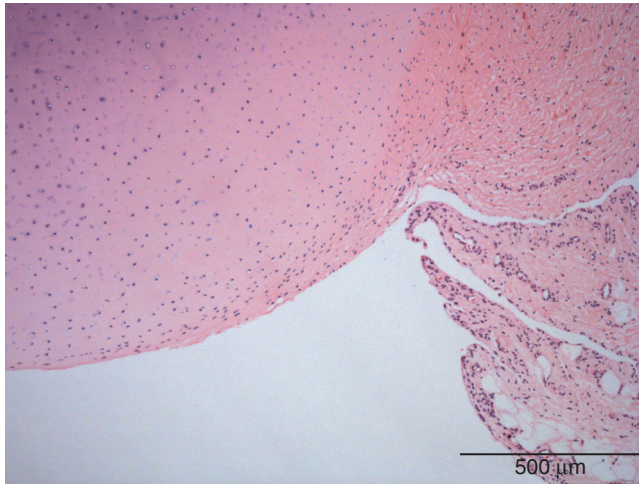
## Ultrastructure of Synovial Lining Cells

Transmission electron microscopic analysis shows that the intimal cells form a discontinuous layer, and thus the subintimal matrix can directly contact the synovial fluid (Figure 2-2). The existence of two distinct cell types—type A and type B SLCs—was originally described by Barland and associates,<sup>4</sup> and several lines of evidence, including animal models, detailed ultrastructural studies, and immunohistochemical analyses, indicate that these cells represent macrophages (type A SLCs) and fibroblasts (type B SLCs). Studies of SLC populations in a variety of species, including humans, have found that macrophages make up anywhere from 20% and fibroblast-like cells approximately 80% of the lining cell.<sup>5,6</sup> The existence of the two cell types has been substantiated by similar findings in a wide variety of species, including hamsters, cats, dogs, guinea pigs, rabbits, mice, rats, and horses.<sup>6-14</sup>

Distinguishing different cell populations that form the synovial lining requires immunohistochemistry or transmission light microscopy. At an ultrastructural level, type A cells are characterized by a conspicuous Golgi apparatus, large vacuoles, and small vesicles, and they contain little rough endoplasmic reticulum, giving them a macrophage-like phenotype (Figure 2-3A and B). The plasma membrane of type A cells possesses numerous fine extensions, termed *filopodia*, that are characteristic of macrophages. Type A cells occasionally cluster at the tips of the synovial villi; this uneven distribution explains, at least in part, early reports that suggested that type A cells were the predominant intimal cell type.<sup>4,8</sup> However, the distribution is highly variable and can differ depending on the joint evaluated or even within an individual joint.

Type B SLCs have prominent cytoplasmic extensions that extend onto the surface of the synovial lining (Figure 2-3C and D).<sup>15</sup> Frequent invaginations are seen along the plasma membrane, and a large indented nucleus relative to the area of the surrounding cytoplasm is also a feature. Type B cells have abundant rough endoplasmic reticulum widely distributed in the cytoplasm, and the Golgi apparatus, vacuoles, and vesicles are generally inconspicuous, although some cells have small numbers of prominent vacuoles at their apical aspect. Type B SLCs are known to contain longitudinal bundles of different-sized filaments, supporting their classification as fibroblasts. Desmosomes and gap-like junctions have been described in rat, mouse, and rabbit synovium, but the existence of these structures in human SLCs has never been documented. Although occasional reports describe an intermediate synoviocyte phenotype, it





**Figure 2-1** The cartilage-synovium junction. Hyaline articular cartilage occupies the *left half* of this image, and fibrous capsule and synovial membrane occupy the *right half*. A sparse intimal lining layer with a fibrous subintima can be observed extending from the margin of the cartilage across the capsular surface to assume a more cellular intimal structure with areolar subintima.



**Figure 2-2** Transmission electron photomicrograph of synovial intimal lining cells. The cell *on the left* exhibits the dendritic appearance of a synovial intimal fibroblast (type B cell). Other overlying fibroblast dendrites can be observed. Intercellular gaps allow the synovial fluid to be in direct contact with the synovial matrix.

is likely that these cells are functionally conventional type A or B cells.<sup>16,17</sup>

### Immunohistochemical Profile of Synovial Cells

**Synovial Macrophages.** Synovial macrophages and fibroblasts express lineage-specific molecules that can be detected by immunohistochemistry. Synovial macrophages express common hematopoietic antigen CD45 (Figure 2-4A); monocyte/macrophage receptors CD163 and CD97; and lysosomal enzymes CD68 (Figure 2-4B), neuron-specific esterase, and cathepsins B, L, and D. Cells expressing CD14, a molecule that acts as a co-receptor for the detection of bacterial lipopolysaccharide and is expressed by circulating monocytes and monocytes newly recruited to tissue, are rarely seen in the healthy intimal layer, but small numbers are found close to venules in the subintima.<sup>18-24</sup>

The Fc $\gamma$  receptor, Fc $\gamma$ RIII (CD16), which is expressed by Kupffer cells of the liver and type II alveolar macrophages of the lung, is expressed on a subpopulation of synovial macrophages.<sup>25-27</sup> The synovial macrophage population also expresses the class II major histocompatibility complex

(MHC) molecule, which plays an important role in the immune response. More recently, the macrophages, which are responsible for the removal of debris, blood, and particulate material from the joint cavity and possess antigen-processing properties, have been found to express Z39Ig, a complement-related protein that is a cell surface receptor and immunoglobulin superfamily member involved in the induction of human leukocyte antigen, DR subregion (HLA-DR) and implicated in phagocytosis and antigen-mediated immune responses.<sup>28-30</sup>

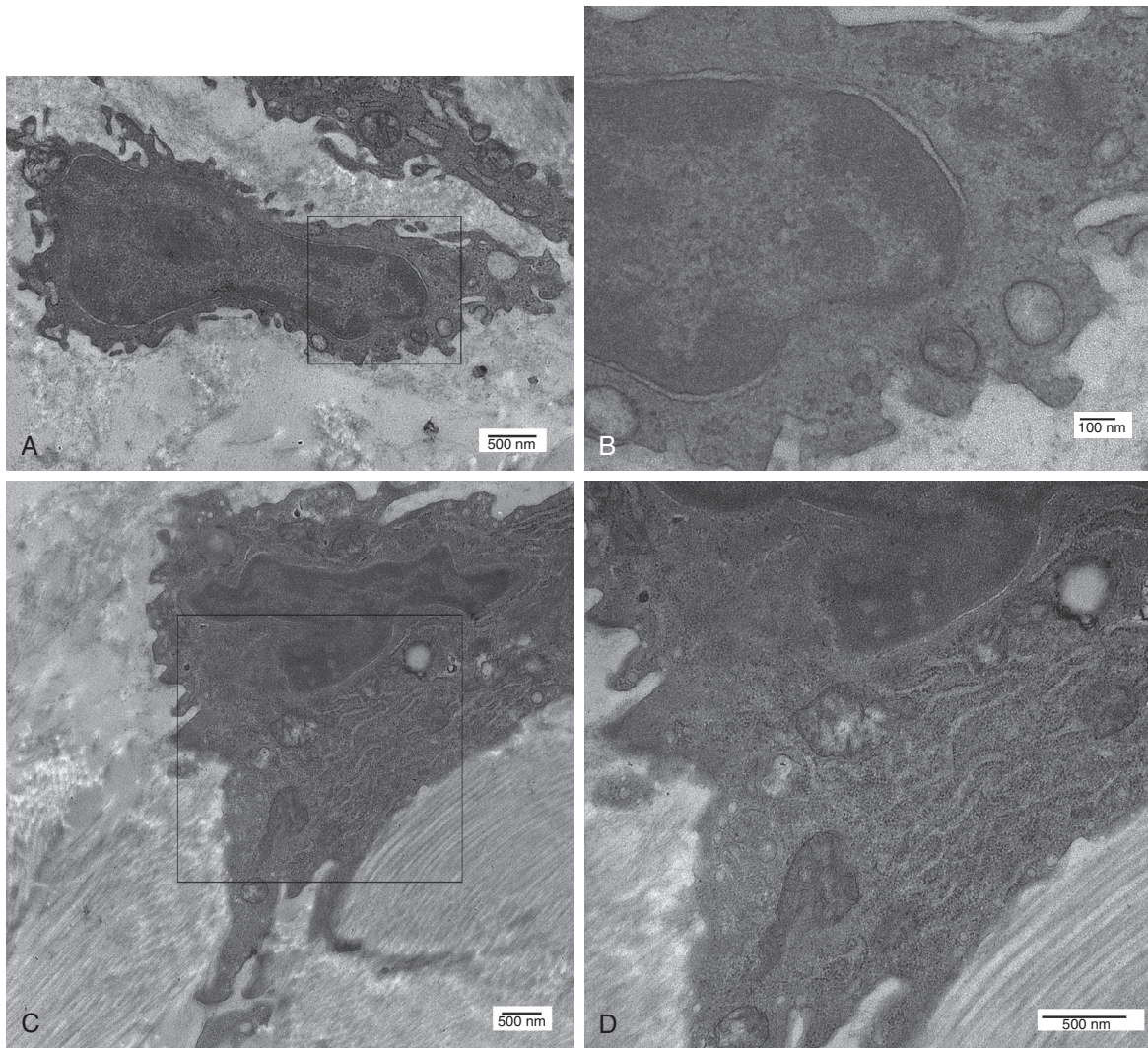
Expression of the  $\beta$ 2 integrin chains CD18, CD11a, CD11b, and CD11c varies; CD11a and CD11c may be absent or weakly expressed on a few lining cells.<sup>31,32</sup> Osteoclasts, which are tartrate resistant and acid phosphatase positive and express the  $\alpha$ V $\beta$ 3 vitronectin and calcitonin receptors, do not appear in the normal synovium.

**Synovial Intimal Fibroblasts.** Synovial intimal and subintimal fibroblasts are indistinguishable by light microscopy. They generally are considered to be closely related in terms of cell lineage, but because of their different microenvironments, they do not always share the same phenotype. They possess prominent synthetic capacity and produce the essential joint lubricants hyaluronic acid (HA) and lubricin.<sup>33</sup> Intimal fibroblasts express uridine diphosphoglucose dehydrogenase (UDPGD), an enzyme involved in HA synthesis that is a relatively specific marker for this cell type. UDPGD converts UDP-glucose to UDP-glucuronate, one of the two substrates required by HA synthase for assembly of the HA polymer.<sup>34</sup> CD44, the nonintegrin receptor for HA, is expressed by all SLCs.<sup>32,35,36</sup>

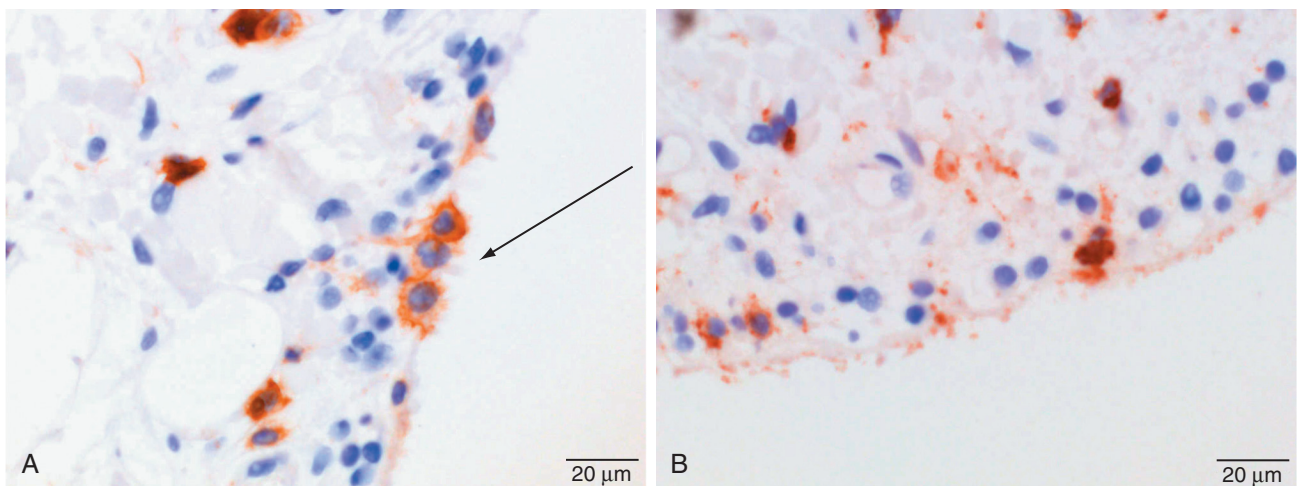
Synovial fibroblasts also synthesize normal matrix components, including fibronectin, laminin, collagens, proteoglycans, lubricin, and other identified and unidentified proteins. They have the capacity to produce large quantities of metalloproteinases, metalloproteinase inhibitors, prostaglandins, and cytokines. This capacity must provide essential biologic advantages, but the complex physiologic mechanisms relevant to normal function are incompletely delineated. Expression of selected adhesion molecules on synovial fibroblasts probably facilitates the trafficking of some cell populations, such as neutrophils, into the synovial fluid and the retention of others, such as mononuclear leukocytes, in the synovial tissue. Expression of metalloproteinases, cytokines, adhesion molecules, and other cell surface molecules is strikingly increased in inflammatory states.

Specialized intimal fibroblasts express many other molecules that also might be expressed by the intimal macrophage population or by most subintimal fibroblasts, including decay-accelerating factor (CD55), vascular cell adhesion molecule-1,<sup>33,37-40</sup> and cadherin-11.<sup>41,42</sup> PGP.95, a neuronal marker, might be specific for type B synoviocytes in some species.<sup>43</sup> Decay-accelerating factor, which is also expressed on many other cells (most notably erythrocytes), as well as bone marrow cells, interacts with CD97, a glycoprotein that is present on the surface of activated leukocytes, including intimal macrophages, and is thought to be involved in signaling processes early after leukocyte activation.<sup>44,45</sup> In contrast, Fc $\gamma$ RIII is expressed by macrophages only when they are in close contact with decay-accelerating factor-positive fibroblasts or decay-accelerating factor-coated fibrillin-1 microfibrils in the extra-cellular matrix.<sup>26</sup>





**Figure 2-3** Transmission electron photomicrographs of synovial intimal macrophages (type A cells) and fibroblasts (type B cells). **A**, Low-powered magnification shows the surface fine filopodia, characteristic of macrophages, and a smooth-surfaced nucleus. **B**, The boxed area in **A** is shown at a higher magnification, revealing numerous vesicles, characteristic of macrophages. Absence of rough endoplasmic reticulum also is noted. **C**, The convoluted nucleus along with the prominent rough endoplasmic reticulum (boxed area) is characteristic of a synovial intimal fibroblast (type B cell). **D**, The rough endoplasmic reticulum is shown at greater magnification.



**Figure 2-4** Photomicrographs depicting synovial intimal macrophages by immunohistochemistry. Macrophages are decorated with CD45 (arrow in **A**) and CD68 (**B**), which are markers that identify hematopoietic cells (CD45) and macrophages (CD68).

Toll-like receptors (TLRs) are also expressed on intimal fibroblasts, including TLR2, which is activated by serum amyloid A (among other ligands), leading to angiogenesis and cell invasion that is mediated, at least in part, via the Tie2 signaling pathway.<sup>46,47</sup> Cadherins are a class of tissue-restricted transmembrane proteins that play important roles in homophilic intercellular adhesion and are involved in maintaining the integrity of tissue architecture. Cadherin-11, which was cloned from rheumatoid arthritis (RA) synovial tissue, is expressed in normal synovial intimal fibroblasts but not in intimal macrophages. Fibroblasts transfected with cadherin-11 form a lining-like structure in vitro, which implicates this molecule in the architectural organization of the synovial lining.<sup>41,42,48</sup> This suggestion is supported by the observation that cadherin-deficient mice have a hypoplastic synovial intimal lining and are resistant to inflammatory arthritis.<sup>49</sup> When fibroblasts expressing cadherin-11 are embedded in laminin microparticles, they migrate to the surface and form an intimal lining-like structure.<sup>50</sup> If macrophage lineage cells are included in the culture, they can co-localize with fibroblasts on the surface. Therefore, the organization of the synovial lining, including the distribution of type A and B cells, is orchestrated by fibroblast-like synoviocytes.

$\beta$ 1 and  $\beta$ 3 integrins are present on all SLCs, forming receptors for laminin (CD49f and CD49b), types I and IV collagen (CD49b), vitronectin (CD51), CD54 (a member of the immunoglobulin superfamily), and fibronectin (CD49d and CD49e). CD31 (platelet-endothelial cell adhesion molecule), a member of the immunoglobulin superfamily expressed on endothelial cells, platelets, and monocytes, is weakly expressed on SLCs.<sup>32</sup>

### Turnover of Synovial Lining Cells

Proliferation of SLCs in humans is low; when normal human synovial explants have a labeling index of approximately 0.05% to 0.3%<sup>51</sup> when exposed to 3H thymidine.

This labeling index bears a striking contrast to labeling indices of approximately 50% for bowel crypt epithelium. Similar evidence of low proliferation has been found in the synovium of rats and rabbits. The proportion of SLCs expressing the proliferation marker Ki67 is between 1 in 2800 and 1 in 30,000, confirming the relatively slow rate of in situ proliferation.<sup>52</sup> Proliferating cells are generally synovial fibroblasts,<sup>22,53</sup> a finding consistent with the concept that type A synovial cells are terminally differentiated macrophages. Mitotic activity of SLCs is low in inflammatory conditions, such as RA—a condition associated with SLC hyperplasia. Some investigators<sup>54</sup> have reported only rare mitotic figures in RA synovium samples.

Apart from the knowledge that synovial fibroblasts proliferate slowly, little is known about their natural life span, recruitment, or mode of death. Apoptosis is likely involved with maintaining synovial homeostasis, but cultured fibroblast-like synoviocytes tend to be resistant to apoptosis, and very few intimal lining cells display evidence of completed apoptosis by ultrastructural analysis or by labeling for fragmented DNA. The paucity of normal synovium samples for evaluation and the rapid clearance of apoptotic cells could confound the analysis.<sup>55</sup>

### Origin of Synovial Lining Cells

There is little doubt that the type A SLC population is bone marrow-derived and represents cells of the mononuclear phagocyte system.<sup>4</sup> Studies in the Beige (bg) mouse, which harbors a homozygous mutation that confers the presence of giant lysosomes in macrophages, have confirmed the bone marrow origin of these cells.<sup>56,57</sup> Normal mice with bone marrow depleted through irradiation were rescued with bone marrow cells obtained from the bg mouse. Electron microscopic analysis of the synovium from recipient animals revealed that type A SLCs contained the giant lysosomes of the donor bg mouse and that these structures were never identified in type B cells. These findings provide powerful evidence that (1) type A SLCs represent macrophages, (2) they are recruited from the bone marrow, and (3) they are a distinct lineage from type B SLCs.

In addition to immunohistochemistry, several lines of evidence support the concept that type A SLCs are recruited from the bone marrow:

- The osteopetrotic (op/op) mouse, a spontaneously occurring mutant that fails to produce macrophage colony-stimulating factor because of a missense mutation in the *CSF1* gene,<sup>58-60</sup> has low numbers of circulating and resident macrophage colony-stimulating factor-dependent macrophages, including those in the synovium.
- Type A cells in rat synovium do not populate the joint until after the development of synovial blood vessels.<sup>22</sup>
- Type A SLCs are conspicuous around vessels in the synovium in neonatal mice.<sup>6</sup>
- When synovial explants are placed in culture, the reduction in type A SLCs is explained in part by their migration into the culture medium—an observation that reflects the process of migration of macrophages into the synovial fluid in vivo.<sup>1,61</sup>
- Macrophages constitute up to 80% of the cells found around venules in inflammatory conditions such as RA and are cleared rapidly (<48 hours) after successful treatment but will re-accumulate from the circulation if relapse occurs.<sup>62</sup>

Type B intimal cells represent a resident fibroblast population in the synovial lining, but little is known about the cells from which they derive and about how their recruitment is regulated. The existence of mesenchymal stem cells in the synovium suggests that these cells might differentiate into the synovial lining fibroblast. To date, a specific transcription factor directing mesenchymal stem cell differentiation into the synovial fibroblast, similar to factors required for commitment by this multipotential population into bone (*cbfa-1*), cartilage (*Sox-9*), and fat (peroxisome proliferator-activated receptor  $\gamma$  [PPAR $\gamma$ ]), has not been identified.

Several important signaling pathways are activated in the inflamed synovium, including nuclear factor- $\kappa$ B (NF- $\kappa$ B), Janus kinase/signal transducer and activator of transcription (JAK/STAT), Notch, and hypoxia-inducible factor 1,  $\alpha$  subunit (HIF-1 $\alpha$ ). NF- $\kappa$ B is a key transcriptional regulator in the inflamed synovium.<sup>63</sup> NF- $\kappa$ B signaling is complex and may be activated by cytokines, cell surface



adhesion molecules, and hypoxia.<sup>63,64</sup> NF- $\kappa$ B activation could facilitate synovial hyperplasia by promoting proliferation and inhibiting apoptosis of RA fibroblast-like synoviocytes. One of the key roles of NF- $\kappa$ B is to protect RA fibroblast-like synoviocytes against apoptosis, possibly by countering the cytotoxicity of tumor necrosis factor (TNF) and Fas ligand.<sup>65</sup>

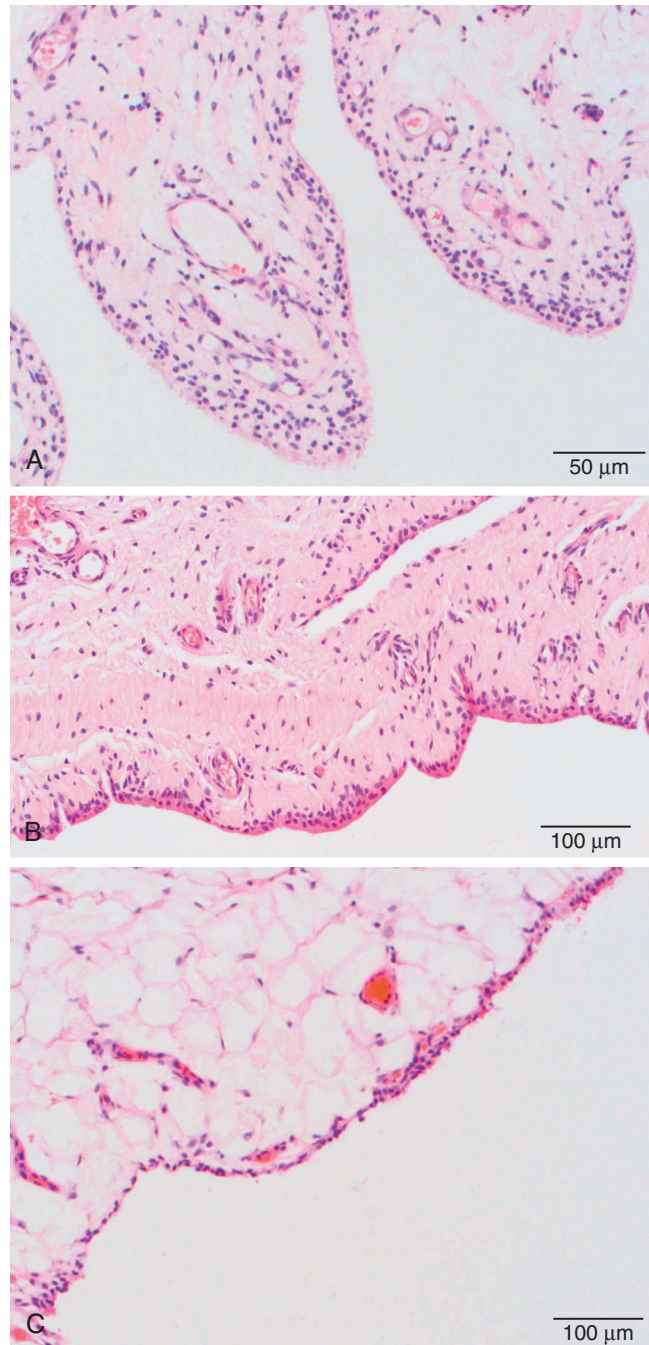
JAK/STAT, Notch, and HIF-1 $\alpha$  signaling pathways are also evident in inflamed synovium. STAT3 expression in the synovium correlates with synovitis and is activated by interleukin (IL)-6<sup>66</sup> but also indirectly by TNF. Notch signaling pathway components are predominantly localized to perivascular/vascular regions<sup>67</sup> and are regulated by vascular endothelial growth factor (VEGF) and ang2, which is consistent with the role of mediation of angiogenesis by Notch in inflammation and cancer.<sup>67,68</sup> Interestingly, hypoxia induces activation of phospho (p)-STAT3/p-STAT1, NF- $\kappa$ B, and Notch in synovial cells.<sup>69</sup> Furthermore, Notch/HIF-1 $\alpha$  interactions in RA synoviocytes are in part mediated through STAT3 activation,<sup>70</sup> possibly through competition of STAT3 with von Hippel–Lindau tumor suppressor for binding to HIF-1 $\alpha$ . Although no direct link between NF- $\kappa$ B and HIF-1 $\alpha$  has been demonstrated in the inflamed joint, preferential activation of the canonical NF- $\kappa$ B pathway occurs in RA synovial tissue obtained from patients with more hypoxic joints.<sup>69</sup>

### Subintimal Layer

SLCs are not separated from the underlying subintima by a well-formed basement membrane composed of the typical trilaminar structure seen beneath epithelial mucosa. Nevertheless, most components of basement membrane are present in the extra-cellular matrix surrounding SLCs. These components include tenascin X, perlecan (a heparan sulfate proteoglycan), type IV collagen laminin, and fibrillin-1.<sup>71,72</sup> Of note is the absence of laminin-5 and integrin  $\alpha 3\beta 3\gamma 2$ , which are components of epithelial hemidesmosomes.<sup>73</sup>

The subintima is composed of loose connective tissue of variable thickness and variable proportions of fibrous/collagenous and adipose tissue, depending on the anatomic site. Under normal healthy conditions, inflammatory cells are virtually absent from the subintima, apart from a sprinkling of macrophages and scattered mast cells.<sup>74</sup> Human synovial tissue is a rich source of mesenchymal stem cells, and although it is unknown which compartment contains this cell population, some cells have the ability to self-renew and differentiate into bone, cartilage, and fat *in vitro*—a phenomenon that reflects the ability of the cell to regenerate *in vivo*.<sup>75-77</sup>

Three categories of subintima are well defined: areolar, fibrous, and fatty/adipose types. Under the light microscope, areolar-type subintima, the most commonly studied, generally is found in larger joints in which there is free movement (Figure 2-5A). It is composed of fronds with a cellular intimal lining and loose connective tissue in the subintima, with little in the way of dense collagen fibers, and a rich vasculature. The fibrous subintima is composed of scant, dense, fibrous, poorly vascularized connective tissue, and it has an attenuated layer of SLCs (Figure 2-5B). The adipose type, which contains abundant mature fat cells and has a



**Figure 2-5** Photomicrographs of different morphologic types of synovial tissue. All photomicrographs show an intimal layer of one to two cells in depth. **A**, The areolar synovium is composed of villous fronds. Beneath the intimal lining layer is cellular loose fibrovascular fatty subintima. **B**, The fibrous synovium comprises dense collagenous material in the subintimal layer. **C**, The subintimal layer of the fatty synovial tissue is composed of less cellular mature adipose tissue with little collagen deposition.

single layer of SLCs, is seen more commonly with aging and in intra-articular fat pads (Figure 2-5C).

The subintima contains types I, III, V, and VI collagen, glycosaminoglycans, proteoglycans, and extra-cellular matrices, including tenascin and laminins. Integrin receptors for collagens, laminin, and vitronectin are absent or at best weakly expressed by subintimal cells. In contrast,



receptors for fibronectin (CD49d and CD49e) are detected, and CD44, the HA receptor, is strongly expressed in most subintimal cells.  $\beta 2$  integrins are largely limited to perivascular areas, particularly in the subintimal zone, as is CD54.<sup>78</sup>

### Subintimal Vasculature

The vascular supply to the synovium is provided by many small vessels and is shared in part by the joint capsule, epiphyseal bone, and other perisynovial structures. Arteriovenous anastomoses communicate freely with the vascular supply to the periosteum and to periarticular bone. As large synovial arteries enter the deep layers of the synovium near the capsule, they branch to form microvascular units in the more superficial subsynovial layers. Precapillary arterioles probably play a major role in controlling circulation to the lining layer. The surface area of the synovial capillary bed is large, and because it runs only a few cell layers deep to the surface, it has a role in trans-synovial exchange of molecules. The intimal lining, however, is devoid of blood vessels. While few in number, studies have shown that vessels in the normal synovium have an intact pericyte layer, suggesting vessel stability, in contrast to the inflamed joint, where a mix of mature and immature vessels were observed. Neural cell adhesion molecule (NCAM) deficiency and oxidative DNA damage suggest that vessels may remain in a plastic state even after pericyte recruitment.<sup>79,80</sup> After TNF blockade, synovial blood vessels become more stable and resemble normal synovium.

Numerous physical factors influence synovial blood flow. Heat promotes blood flow through synovial capillaries. Exercise enhances synovial blood flow to normal joints but may reduce the clearance rate of small molecules from the joint space. Experiments have shown a substantial vascular reserve capacity in normal articulations. Immobilization reduces synovial blood flow, and pressure on the synovial membrane can act to tamponade the synovial blood supply.

Vascular endothelial lining cells express CD34 and CD31 (Figure 2-6A). They also express receptors for the major components of basement membrane, including laminin and collagen IV, and the integrin receptors CD49a (laminin and collagen receptors), CD49d (fibronectin receptor), CD41, CD51 (vitronectin receptor), and CD61 (the  $\beta 3$  integrin subunit). Endothelial cells express CD44, the HA receptor, and CD62P (P-selectin), which acts as a receptor that supports binding of leukocytes to activated platelets and endothelium. They are only weakly positive in uninflamed synovium, however, for expression of CD54 (intercellular adhesion molecule-1), a receptor for  $\beta 2$  integrins expressed by many leukocytes. The endothelial cells of capillaries in the superficial zone of the subintima are strongly positive for HLA-DR expression by immunohistochemistry, whereas cells in the larger vessels in the deep aspect of the membrane are negative.<sup>32,34</sup>

Hypoxia might be a key driver of endothelial cell activation and blood vessel formation in the inflamed joint. This theory was originally proposed in 1970,<sup>81</sup> when a synovial fluid electrode was used to demonstrate that a partial pressure of  $O_2$  in a knee joint affected by RA was 26.5 mm Hg, which was significantly lower than that in

joints affected by osteoarthritis (42.9 mm Hg) or traumatic effusions (63 mm Hg). This observation was supported by studies showing increased glycolytic metabolism in the joint suggestive of increased metabolic activity. Low  $pO_2$  in the inflamed synovial membrane was confirmed with  $pO_2$  probes, with mean levels approximately 3% compared with normal joints at 7%.<sup>82</sup> The degree of hypoxia in synovium affected by RA and normal synovium was inversely related to the number of blood vessels observed and their level of maturity. In patients responding to TNF blockade, the  $pO_2$  increased, thus improving oxygenation to a level similar to that of normal joints.

### Subintimal Lymphatics

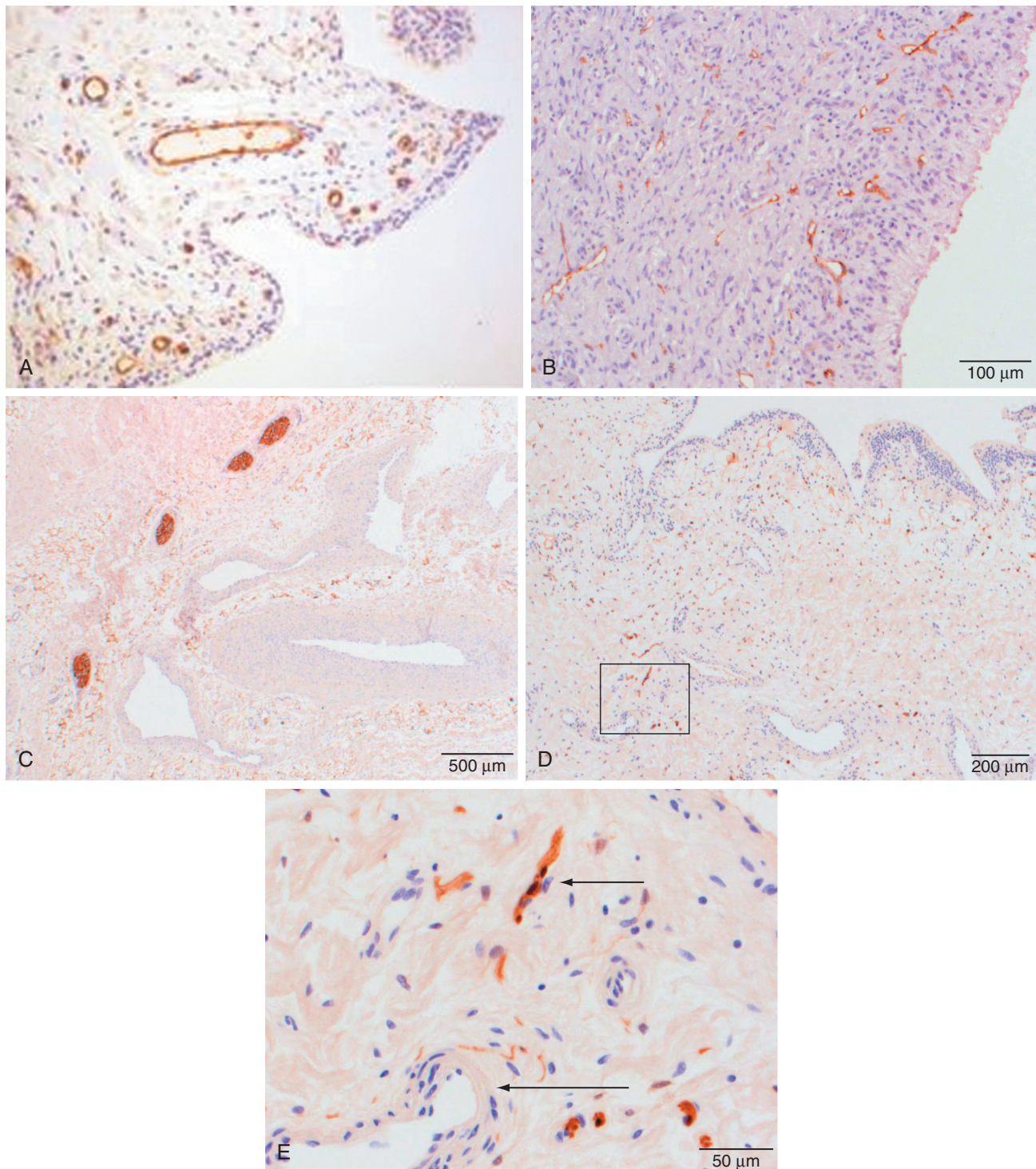
Detailed analysis of the number and distribution of lymphatic vessels has been made possible by the use of the antibody to the lymphatic vessel endothelial HA receptor (LYVE-1) (Figure 2-6B).<sup>83</sup> This antibody is highly specific for lymphatic endothelial cells in lymphatic vessels and lymph node sinuses and does not react with endothelial cells of capillaries and other blood vessels that express CD34 and factor VIII-related antigen. Expression of LYVE-1 in lymphatic endothelial cells has been used as a marker to show that lymphatic vessels are less common in the fibrous synovium compared with areolar and adipose variants of human subsynovial tissue. Detection of this molecule reveals that lymphatics are present in the superficial, intermediate, and deeper layers of synovial membrane in synovium from healthy persons or patients with osteoarthritis and joints affected by RA, although the number in the superficial subintimal layer is low in normal synovium. Little difference in the distribution and number is noted between normal and osteoarthritis synovium, which is characterized by lack of villous hypertrophy. Lymphatic channels are plentiful, however, in the subintimal layer in the presence of villous edema hypertrophy and chronic inflammation.

### Subintimal Nerve Supply

The synovium has a rich network of sympathetic and sensory nerves. The former, which are myelinated and detected with the antibody against S-100 protein, terminate close to blood vessels, where they regulate vascular tone (Figure 2-6C through E). Sensory nerves respond to proprioception and pain via large myelinated nerve fibers and via small ( $<5 \mu m$ ) unmyelinated or myelinated fibers with unmyelinated free nerve ends (nociceptors). The latter are immunoreactive in the synovium for neuropeptides, including substance P, calcitonin gene-related peptide, and vasoactive intestinal peptides.<sup>84,85</sup>

## FUNCTION

Synthetic and protective functions of individual synovial cell populations are multiple and complex. The composite synovial structure, which includes cell populations and their products, vasculature, nerves, and the intercellular matrix, possesses several specialized functions that are essential for normal joint movement, synovial fluid formation, chondrocyte nutrition, and cartilage protection at multiple



**Figure 2-6** Photomicrographs of synovium show lymphovascular and nervous structures by immunohistochemistry. **A** and **B**, Areolar synovium featuring thin-walled vessels are highlighted with antibody to CD31 (**A**), and lymphatic vessels in an inflamed synovium are highlighted with antibody to lymphatic vessel endothelial HA receptor (LYVE-1) (**B**). **C**, Deep in the synovial subintima, close to the joint capsule, medium-sized neurovascular bundles are present with nerves highlighted by antibody to S-100. **D**, Within the more superficial synovium, small nerves decorated with S-100 are identified. **E**, The boxed area in **D** is shown at a higher magnification. The upper arrow is directed at a nerve; the lower arrow is directed at a small vessel.

anatomic locations. These functions must be preserved over a lifetime to maintain maximal mobility and independence. Absence of essential constituents of synovial fluid or inadequate cartilage protection results in early articular malfunction, which may progress to local or generalized joint failure.

### Joint Movement

Four characteristics of the synovium are essential for joint movement: deformability, porosity, nonadherence, and lubrication. In a healthy person, the synovium is a highly deformable structure that facilitates movement between



other adjacent, nondeformable structures within the joint. This unique facility of the synovium to enable movement between tissues rather than within tissues has been emphasized<sup>86</sup> and can be attributed to the presence of a free surface that allows synovial tissue to remain separated from adjacent tissues. The ensuing space is maintained by the presence of synovial fluid.

### Deformability

The deformability of normal synovium is considerable because it must accommodate the extreme positional range available to the joint and its adjacent tendons, ligaments, and capsule. When a finger is flexed, the palmar synovium of each interphalangeal joint contracts while the dorsal synovium expands, and as the finger extends, the reverse mechanism occurs. This normal contraction and expansion of synovium seems to involve a folding and unfolding component and an elastic stretching and relaxation of the tissue. During repeated rapid movement, the synovial lining cannot be pinched between cartilage surfaces and for it to successfully retain its integrity and the integrity of synovial blood vessels and lymphatics. Deformability also limits the extent of synovial ischemia-reperfusion injury during joint motion by maintaining a relatively low intra-articular pressure.

### Porosity

The synovial microvasculature and the intimal lining must be porous to permit robust diffusion of nutrients to cartilage. The structure of the intimal lining is ideal for this requirement because of the relatively disorganized basement membrane and lack of tight junctions. Plasma components freely diffuse into the intra-articular space, and most plasma components, including proteins, are present in synovial fluid at about one-third to one-half the plasma concentration.

### Nonadherence

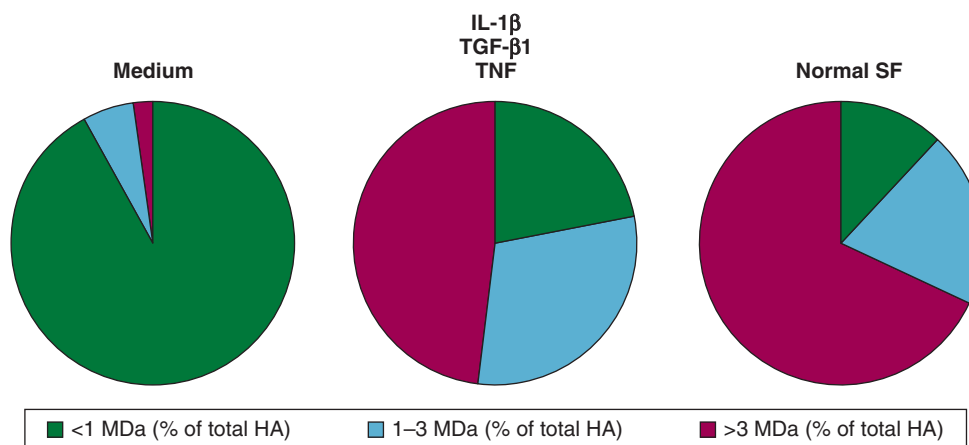
The third important characteristic of the synovium that facilitates joint movement is its nonadherence to opposing

surfaces. Intimal cells on the synovial surface adhere to underlying cells and matrix but do not adhere to opposing synovial and cartilage surfaces. The mechanism that preserves this phenomenon of nonadherence is unknown and might involve the arrangement of cell surface and tissue matrix molecules, such as collagen, fibronectin, and HA. Alternatively, nonadherence may result in part from regular movement of the normal synovial lining.

### Lubrication

The fourth characteristic of synovium that is essential for joint motion is an efficient lubrication mechanism to facilitate movement of cartilage on cartilage. The mechanisms of joint lubrication are complex and are an integral component of synovial physiology. In an articulating joint, cartilage is subjected to numerous compressive and frictional forces every day. Friction and wear can never be eliminated from a functioning joint. Adult chondrocytes do not normally divide *in vivo*, and damaged cartilage has limited capacity for self-repair. For a joint to maintain its function throughout a lifetime of use, protective biologic mechanisms, such as lubrication, help minimize wear and damage that result from normal daily activities. Synovial membrane may also contribute to concentration of lubricants in synovial fluid, because it is a semi-permeable membrane. These functions have recently been replicated by a polytetrafluoroethylene membrane that can be used in a bioreactor system to modulate lubricant retention in bioengineered synovial fluid. Synoviocytes adherent to such membranes may serve as a source of lubricant and a barrier for lubricant transport.<sup>87</sup> Furthermore, cytokines can stimulate normal lubricant production 40- to 80-fold in such bioreactor systems (Figure 2-7).<sup>88</sup>

*Boundary lubrication* refers to the protective effect of particular lubricating molecules adsorbing to a surface and repelling its opposing interface.<sup>89</sup> Bearing surfaces must generate a mutual repulsion to be lubricated in the boundary mode. Boundary lubricants exert their effects by changing the physicochemical characteristics of a surface, and they reduce articular friction and wear by providing a smooth and slippery coating. Friction is reduced by an interposed



**Figure 2-7** The molecular size of synovial fluid hyaluronic acid (HA) (Normal SF), supernatants of cultured synoviocytes (Medium), and synoviocytes stimulated with IL-1 $\beta$ , TGF- $\beta$ 1 TNF. Note that cells stimulated with the cytokine cocktail closely approximate HA of normal SF compared with control cells, with high molecular weight species that promote a low friction environment. (Data from Blewis ME, Lao BJ, Schumacher BL, et al: *Interactive cytokine regulation of synoviocyte lubricant secretion*. Tissue Eng Part A 16:1329–1337, 2010.)

film of protective fluid that allows one surface to ride freely over another. The cartilage matrix is integral to this phenomenon because it is fluid filled and compressible. Loaded cartilage extrudes lubricant fluid from its surface, and expressed fluid contributes to the separation of the two articulating surfaces. Scanning electron microscopy has shown a continuous film of fluid, only 100 nm thick, that separates one surface from the other, preventing direct abrasive contact.<sup>90</sup> This ultrathin coating of lubricant resists distraction of the two articulating surfaces, enhancing joint stability. In healthy joints, another essential advantage of an intra-articular lubrication system is the effective prevention of pinching of adjacent, well-vascularized synovial membrane, a feature that is lost in the inflamed joint in which synovial membrane adheres to the cartilage surface.

**Hyaluronic Acid.** HA, a high-molecular-weight polysaccharide, is a major component of synovial fluid and cartilage.<sup>91</sup> It is produced in large amounts by mechanosensitive, fibroblast-like synoviocytes.<sup>92,93</sup> HA, which has three mammalian forms designated HAS1, HAS2, and HAS3,<sup>94</sup> is synthesized by HA synthase at the plasma membrane and is extruded directly into the extra-cellular compartment. HA synthase activity and HA secretion are stimulated by pro-inflammatory cytokines, including IL-1 $\beta$  and TGF- $\beta$ .<sup>92,95,96</sup> Interestingly, although the levels of cytokines are increased in arthritic joints, the synovial fluid concentration of HA decreases.<sup>97</sup> HA is also synthesized by many other skeletal cells and is an important component of extracellular matrices. It is simultaneously a solid phase matrix element of cartilage and other tissues and a fluid phase element in the synovial space under normal and abnormal conditions.

HA has many biologic functions, which include effects on cell growth, migration, and adhesion. The regulatory role of HA is mediated through HA-binding proteins and receptors, including CD44, which are present on the cell surfaces of chondrocytes, lymphocytes, and other mononuclear cell populations. HA plays a crucial role in morphogenesis and in wound healing. HA also is a vital structural component of the synovial lining, and it has an essential role in the induction of joint cavitation during embryogenesis. HA, which is produced by synovium, was originally thought to be primarily a joint lubricant, and it is generally accepted that it plays a major physiologic role in maintaining synovial fluid viscosity. HA is important in normal joint function, not least through its capacity to provide effective shock absorption. It has been suggested that HA is a particularly important viscohydrodynamic lubricant at low-load interfaces, such as synovium-on-synovium and synovium-on-cartilage.<sup>98</sup> Synovial fluid HA, acting in combination with albumin, has a role in the attenuation of fluid loss from the joint cavity, particularly during periods of increased pressure, which can occur during sustained joint flexion.<sup>99-101</sup>

**Lubricin.** Compelling evidence suggests that lubricin, which was first described in the 1970s,<sup>102</sup> is the factor primarily responsible for boundary lubrication of diarthrodial joints.<sup>103</sup> Lubricin, a large secreted, mucin-like proteoglycan with an apparent molecular weight of 280 kDa, is a product of the gene proteoglycan 4 (*PRG4*). It is a major component of synovial fluid and is present at the cartilage surface.

The gene is highly expressed by human synovial fibroblasts and by superficial zone chondrocytes.<sup>104</sup> Lubricin is closely related to superficial zone protein, megakaryocyte-stimulating factor, and hemangiopoietin, which are encoded by the same gene but can differ in terms of post-translational modification. Superficial zone protein is expressed by SLCs and by superficial zone chondrocytes at the cartilage surface but not by intermediate or deep zone chondrocytes.<sup>105</sup> It has been suggested that lubricin may bind to the much longer hyaluronate polymers, distributing shear stress and stabilizing essential lubricant molecules.<sup>106</sup>

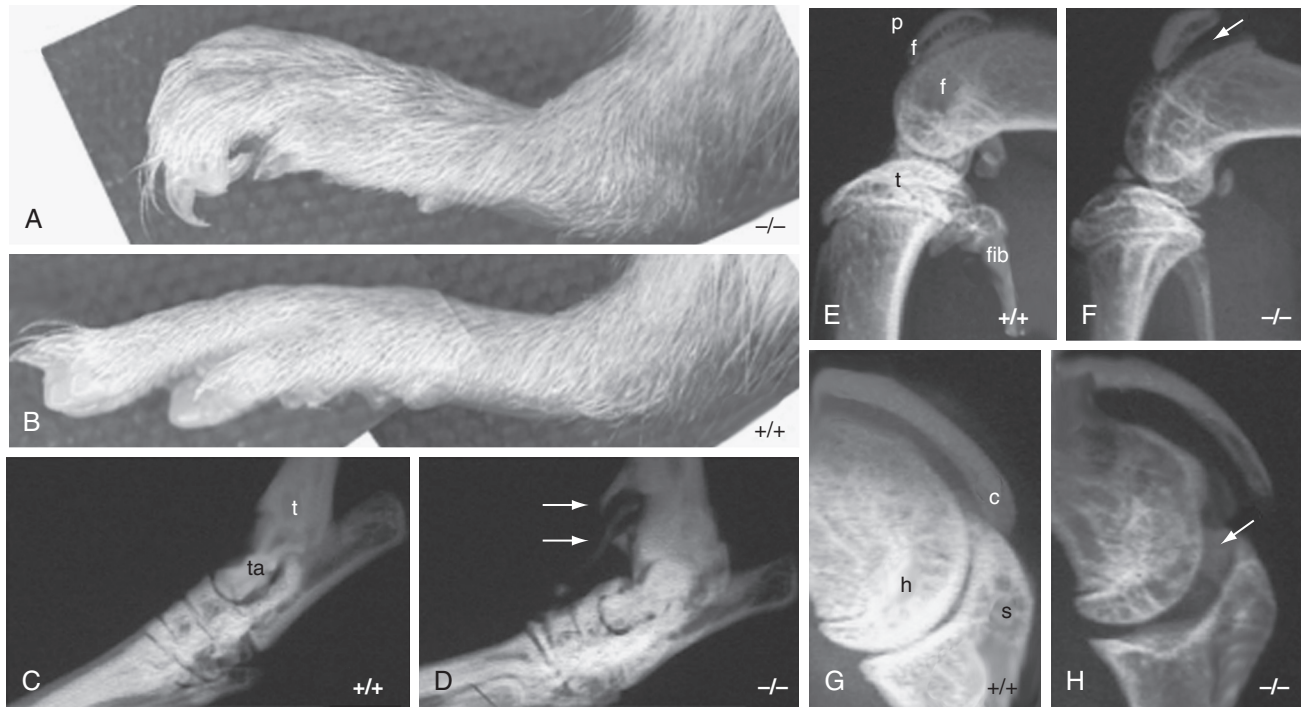
In an experimental model, lubricin seemed to have multiple functions in articulating joints and tendons, including protection of cartilage surfaces from protein deposition and cell adhesion and inhibition of synovial cell overgrowth.<sup>107</sup> *Prp4*<sup>-/-</sup> mice, which were consistently normal at birth, showed progressive loss of superficial zone chondrocytes and increasing synovial cell hyperplasia (Figure 2-8). The essential role of lubricin in maintaining joint integrity was shown by the identification of disease-causing mutations in patients with the autosomal-recessive disorder camptodactyly-arthropathy-coxa vara-pericarditis (CACP) syndrome.<sup>108</sup> CACP is a large joint arthropathy associated with the absence of lubricin from synovial fluid and ineffective boundary lubrication provided by the synovial fluid (Figure 2-9).<sup>106,109</sup> In other studies of lubricin biology and joint integrity, experimental injury resulted in reduced synovial fluid lubricin concentrations, decreased boundary lubricating ability, and increased cartilage matrix degradation, each of which could be attributed to trauma-induced inflammatory processes.<sup>104</sup>

Other investigators have argued against the primacy of lubricin in joint lubrication by proposing that surface-active phospholipid, which is also secreted by intimal fibroblasts, is the essential boundary lubricant that reduces cartilage friction to remarkably low levels.<sup>110</sup> It was hypothesized that lubricin acts as the carrier of surface-active phospholipid to articular cartilage but is not the lubricant per se, a function that is similar to that of the well-characterized alveolar surfactant binding proteins in the lung.

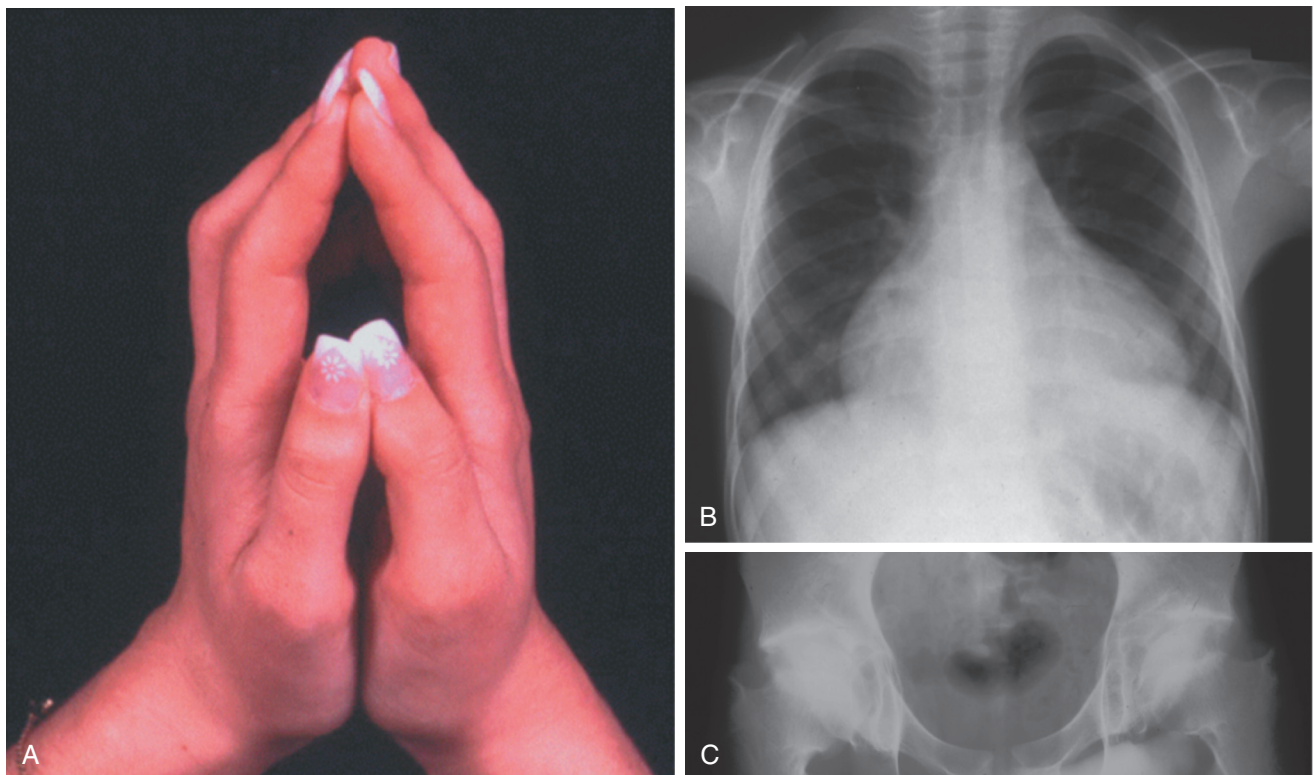
## Synovial Fluid Formation

In healthy people a constant volume of synovial fluid is important during joint movement as a cushion for synovial tissue and as a reservoir of lubricant for cartilage. Many of the soluble components and proteins in synovial fluid exit the synovial microcirculation through pores or fenestrations in the vascular endothelium, then diffuse through the interstitium before entering the joint space. Synovial fluid is in part a filtrate of plasma to which additional components, including HA and lubricin, are added and removed by the SLCs (Figure 2-10). As noted earlier, concentrations of electrolytes and small molecules in synovial fluid are similar to those in plasma. Synovial permeability to most small molecules is determined by a process of free diffusion through the double barrier of endothelium and interstitium, limited mainly by the intercellular space between SLCs. For most small molecules, synovial permeability is inversely related to the dimensions of the molecule.

Experimental evidence suggests that the exchange of small solutes is determined predominantly by the synovial



**Figure 2-8** Clinical appearance and radiographic changes in *Prg4*<sup>-/-</sup> mice. **A** and **B**, Photographs of the hind paws of 6-month-old *Prg4*<sup>-/-</sup> (**A**) and wild-type (**B**) mice. Note the curved digits in the mutant mouse and swelling at the ankle joint. **C** and **D**, Radiographs of the ankle joint of 9-month-old wild-type (**C**) and *Prg4*<sup>-/-</sup> mice (**D**). Structures corresponding to the tibia (t) and talus (ta) are indicated. Note the calcification of structures adjacent to the ankle (arrows in **D**). **E**, Lateral knee radiograph of a 4-month-old wild-type mouse. Structures corresponding to the patella (p), femoral condyle (f), tibial plateau (t), and fibula (fib) are indicated. **F**, Lateral knee radiograph of a 4-month-old *Prg4*<sup>-/-</sup> mouse. Note the increased joint space between the patella and femur (arrow) and osteopenia of the patella, femoral condyles, and tibial plateau. **G**, Shoulder radiograph of a 4-month-old wild-type mouse. Structures corresponding to the humeral head (h), glenoid fossa of the scapula (s), and lateral portion of the clavicle (c) are indicated. **H**, Shoulder radiograph of a 4-month-old *Prg4*<sup>-/-</sup> mouse. Note the increased joint space between the humerus and scapula (arrow) and osteopenia of the humeral head. (From Rhee DK, Marcelino J, Baker M, et al: *The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth*. *J Clin Invest* 115:622–631, 2005.)



**Figure 2-9** Clinical features of camptodactyly–arthropathy–coxa vara–pericarditis (CACP) syndrome. **A**, The characteristic deformity of the hands is shown. **B**, A chest radiograph shows an enlarged cardiac outline caused by pericarditis. **C**, radiograph of the pelvis highlights coxa vara in a boy with CACP. (**B** and **C**, Courtesy Ronald Laxer, MD, Hospital for Sick Children, Toronto, Ontario, Canada.)