4. CONNECTIVE TISSUE

Classification

The connective tissue provides the **supportive** and **connecting** framework (or **stroma**) for all the other tissues of the body. The connective tissue is formed by **cells** and the **extracellular matrix** (ECM). The ECM represents a combination of **collagens**, **noncollagenous glycoproteins**, and **proteoglycans** (**ground substance**) surrounding the cells of connective tissue. The cells of the connective tissue have important roles in the **storage of metabolites**, **immune and inflammatory responses**, and **tissue repair after injury**.

Unlike epithelial cells, which are almost free of intercellular material, **connective tissue cells are widely separated by components of the ECM**. In addition, epithelial cells lack direct blood and lymphatic supply, whereas connective tissue is directly supplied by blood and lymphatic vessels and nerves.

Connective tissue can be classified into three major groups (Figure 4-1): **embryonic connective tissue**, **adult connective tissue**, and **special connective tissue**.

Embryonic connective tissue is a loose tissue formed during early embryonic development. This type of connective tissue, found primarily in the **umbilical cord**, consists predominantly of a **hydrophilic ECM** and therefore has a jelly-like consistency. Because of this consistency, it is also called **mucoid connective tissue** or **Wharton's jelly**.

Adult connective tissue has considerable structural diversity because **the proportion of cells to fibers and of ground substance varies from tissue to tissue**. This variable cell-to-ECM ratio is the basis for the subclassification of adult connective tissue into two types of connective tissue proper:

1. **Loose** (or **areolar**) **connective tissue**
2. **Dense connective tissue**

Loose connective tissue contains **more cells than collagen fibers** and is generally found in the **mucosa** and **submucosa** of various organs and surrounding blood vessels, nerves, and muscles. This type of connective tissue facilitates dissection as performed by anatomists, pathologists, and surgeons.

Dense connective tissue contains **more collagen fibers than cells**. When the collagen fibers are preferentially oriented—as in tendons, ligaments, and the cornea—the tissue is called **dense regular connective tissue**. When the collagen fibers are **randomly oriented**—as in the dermis of the skin—the tissue is called **dense irregular connective tissue**.

In addition, **reticular** and **elastic fibers** predominate in irregular connective tissue.

Reticular connective tissue contains reticular fibers, which form the **stroma** of organs of the lymphoid-immune system (for example, lymph nodes and spleen), the hematopoietic bone marrow, and the liver. This type of connective tissue provides a delicate meshwork to allow passage of cells and fluid.

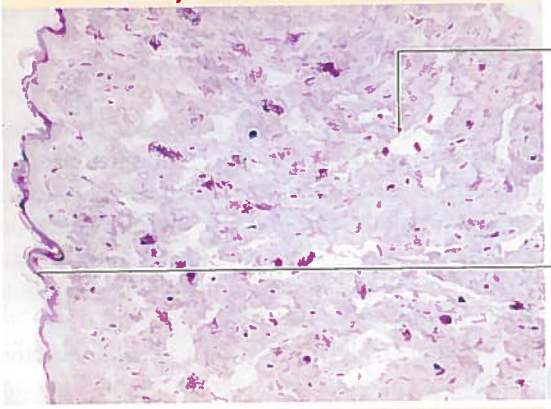
Elastic connective tissue contains irregularly arranged **elastic fibers** in ligaments of the vertebral column or concentrically arranged **sheets** or **laminae** in the wall of the aorta. This type of connective tissue provides **elasticity**.

The **special connective tissue** comprises types of connective tissue with special properties not observed in the embryonic or adult connective tissue proper. There are four types of special connective tissue (Figure 4-2):

1. **Adipose tissue**
2. **Cartilage**
3. **Bone**
4. **Hematopoietic tissue (bone marrow)**

Figure 4-1. Classification of connective tissue

Embryonic connective tissue



Nucleus of a mesenchymal cell embedded in an extracellular matrix rich in water-trapping proteoglycans

Amnion

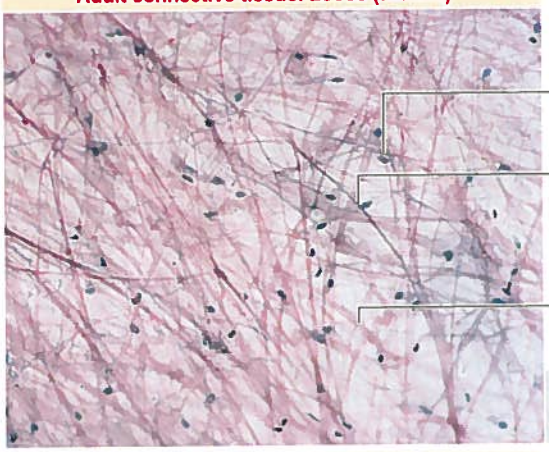
Umbilical cord

Embryonic connective tissue contains abundant extracellular matrix rich in **proteoglycans**.

Collagen and reticular fibers are also present but not abundant. Fusiform and stellate mesenchymal cells are widely spaced and surrounded by the extracellular matrix.

Embryonic connective tissue is present in the **umbilical cord (Wharton's jelly)** and in the **pulp of the developing tooth**.

Adult connective tissue: Loose (areolar)



Oval nucleus of a **fibroblast**

Elastic fibers are thin, straight, and branching

Collagen bundles are thick and wavy

Whole mount of mesentery

Adult connective tissue can be **loose** or **dense**.

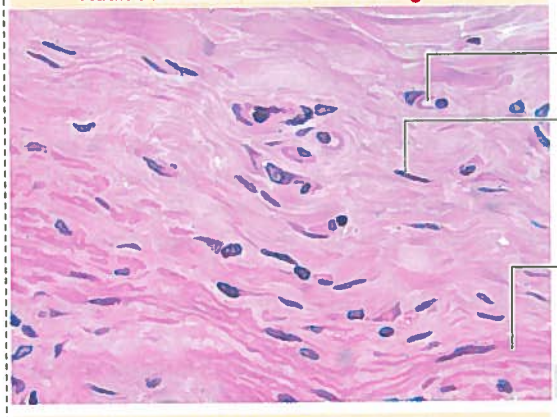
Dense connective tissue can be subclassified according to the orientation of the collagen fibers as **irregular** or **regular**.

Loose (areolar) connective tissue contains abundant elastic fibers and collagen bundles embedded in the ground substance.

Fibroblasts are recognized by their oval nuclear shape. **Mast cells**, **macrophages**, and **blood capillaries** can also be present (not shown in the micrograph).

Two types of fiber are present: **elastic fibers** and **collagen bundles**.

Adult connective tissue: Dense irregular



Blood capillary

Oval nucleus of a **fibroblast**

Collagen bundles are thick, wavy, and irregularly arranged

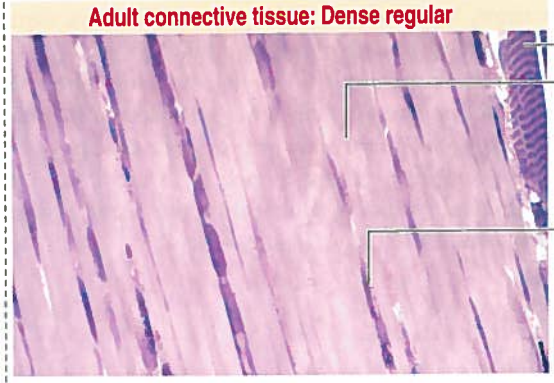
Dermis (skin)

Dense irregular connective tissue, found in the **dermis of the skin**, the **submucosa of the digestive tube**, and other sites, contains coarse, thick, and intertwined bundles of collagen fibers arranged in an irregular form.

Fibroblasts are sparse, separated by collagen bundles, and recognized by their oval nucleus.

Mast cells and **macrophages** can also be present (not shown in the micrograph).

Adult connective tissue: Dense regular



Skeletal muscle

Regularly arranged collagen bundles

Oval nucleus of a **fibrocyte** compressed by the regularly aligned collagen bundles

Tendon

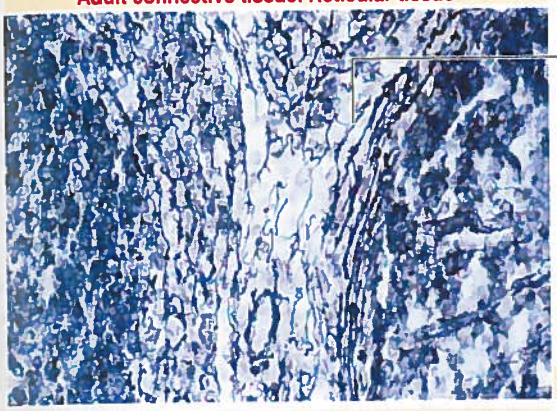
Dense regular connective tissue is found in **tendons** and **ligaments**.

This type of adult connective tissue consists of **regularly oriented parallel bundles of collagen fibers separated by linear rows of fibrocytes**.

The nuclei of fibrocytes appear as thin dark lines, and the cytoplasm is not visible at the light microscopic level.

Figure 4-2. Classification of connective tissue

Adult connective tissue: Reticular tissue



Reticular fibers (type III collagen) can be identified in the stroma of this lymphatic nodule after impregnation with **silver salts**. Reticular fibers are **argyrophilic**.

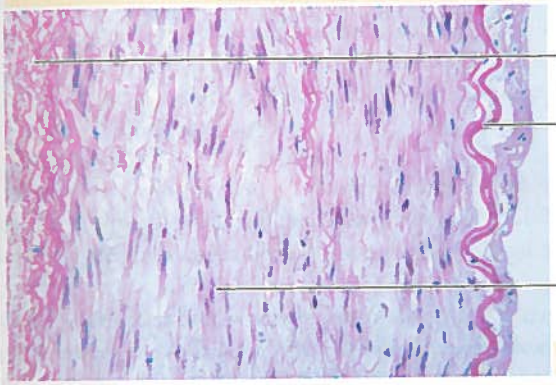
Lymphatic nodule

Reticular connective tissue is an adult-type connective tissue in which **reticular fibers** predominate. **Reticular connective tissue is characteristic of lymphatic tissues.**

Reticular fibers, synthesized by fibroblasts (also called **reticular cells**), are thin and branching structures.

Reticular fibers form a meshwork in which lymphoid cells are embedded.

Adult connective tissue: Elastic tissue



Elastic fibers are arranged in concentric and discontinuous sheets in the wall of this artery. In this section, elastic laminae appear as wavy pink bands.

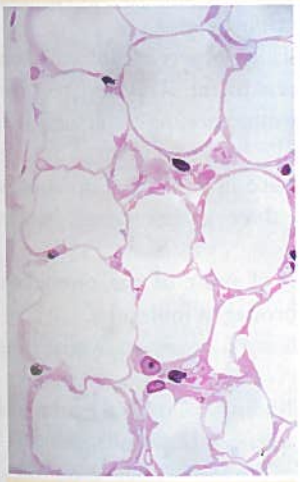
Smooth muscle cells

Artery

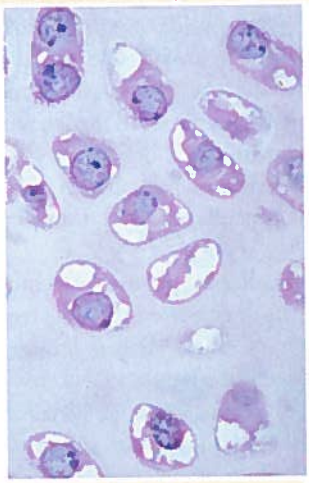
Elastic connective tissue is an adult-type connective tissue in which **elastic fibers** predominate. **Elastic connective tissue is characteristic of the walls of large blood vessels** and **ligaments.**

Elastic fibers in the wall of a blood vessel, synthesized by **smooth muscle cells**, form **discontinuous lamellae** or **membranes** in a concentric arrangement around the lumen.

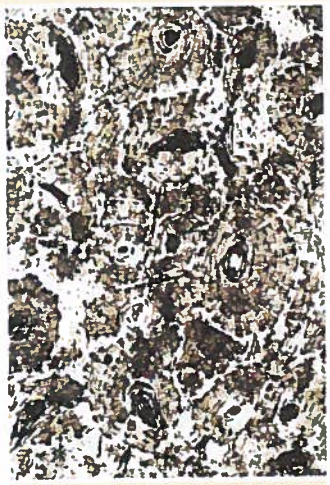
Special types of connective tissue



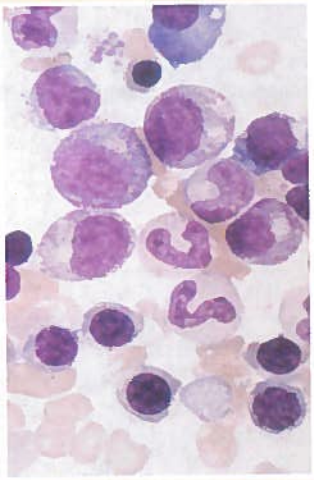
Adipose tissue



Cartilage



Bone



Hematopoietic tissue

Adipose tissue has more cells (called **adipose cells** or **adipocytes**) than collagen fibers and ground substance. This type of connective tissue is the most significant energy storage site of the body.

The **hematopoietic tissue** is found in the marrow of selected bones. This type of connective tissue is discussed in Chapter 6, Blood and Hematopoiesis.

Cartilage and bone are also regarded as **special connective tissue** but are traditionally placed in separate categories. Essentially, cartilage and bone are dense connective tissues with specialized cells and ground substance. An important difference is that cartilage has a **noncalcified ECM**, whereas the ECM of bone is

Box 4-A | Distribution of collagen

- **Type I collagen**

Present in **bone**, **tendon**, **dentin**, and **skin** as banded fibers with a transverse periodicity of 64 nm. This type of collagen provides tensile strength.

- **Type II collagen**

Observed in **hyaline** and **elastic cartilage** as fibrils thinner than type I collagen.

- **Type III collagen**

Present in the **reticular lamina of basement membranes**, as a component of reticular fibers. This is the first collagen type synthesized during wound healing and then is replaced by type I collagen.

Reticular fibers can be better recognized after impregnation with silver salts because reticular fibers are **argyrophilic** (silver-loving; Greek *argyros*, silver). Reticular fibers—and collagens in general—are glycoproteins and can be recognized with the **periodic acid–Schiff (PAS) reaction** because of their carbohydrate content.

Silver impregnation is a valuable tool in pathology for the recognition of distortions in the distribution of reticular fibers in alterations of lymphoid organs.

- **Type IV collagen**

Present in the **basal lamina**. This type of collagen does not form bundles. Single molecules of type IV collagen bind to one of the type IV collagen-binding sites of laminin.

- **Type V collagen**

Observed in **amnion** and **chorion** in the fetus and in muscle and tendon sheaths. **This type of collagen does not form banded fibrils.**

Box 4-B | Cell types making collagen

- The so-called **reticular cell** is a fibroblast that synthesizes reticular fibers containing type III collagen. Reticular fibers form the stroma of bone marrow and lymphoid organs.
- **The osteoblast (bone), chondroblast (cartilage), and odontoblast (teeth) also synthesize collagen.** These cell types are fibroblast equivalents in their respective tissues. Therefore, the synthesis of collagen is not limited to the fibroblast in connective tissue. In fact, **epithelial cells synthesize type IV collagen.**
- **A fibroblast may simultaneously synthesize more than one type of collagen.**
- **Smooth muscle cells**, found in the wall of arteries, intestine, the respiratory bronchial tree, and uterus, **can synthesize types I and III collagen.**

calcified. These two types of specialized connective tissue fulfill weight-bearing and mechanical functions that are discussed later (see Cartilage and Bone).

Cell components of connective tissue

The four major cell components of connective tissue are the **fibroblast**, the **macrophage**, the **mast cell**, and the **plasma cell**.

Under light microscopy, the **fibroblast** appears as a spindle-shaped cell with an elliptical nucleus. The cytoplasm is very thin and generally not resolved by the light microscope. Under **electron microscopy**, the fibroblast shows the typical features of a protein-secreting cell: a well-developed rough endoplasmic reticulum and a Golgi apparatus.

The fibroblast synthesizes and continuously secretes mature proteoglycans and glycoproteins and the precursor molecules of various types of collagens and elastin. Different types of collagen proteins and proteoglycans can be recognized as components of the **basement membrane**. As you may remember, **type IV collagen is found in the basal lamina** and **type III collagen** appears in the **reticular lamina** as a component of **reticular fibers** (see Boxes 4-A and 4-B). Heparan sulfate proteoglycans and the glycoprotein fibronectin are two additional products of the fibroblast that appear in the basement membrane. The protein collagen is a component of collagen and reticular fibers. However, elastic fibers do not contain collagen.

Collagen: Synthesis, secretion, and assembly

Collagens are generally divided into two categories: **fibrillar collagens** (forming fibrils with a characteristic banded pattern), and **nonfibrillar collagens** (see Box 4-C).

The synthesis of collagen starts in the rough endoplasmic reticulum (RER) following the typical pathway of synthesis for export from the cell (Figure 4-3).

Preprocollagen is synthesized with a **signal peptide** and released as **procollagen** within the cisterna of the RER. **Procollagen** consists of three polypeptide a chains, lacking the signal peptide, assembled in a **triple helix**.

Hydroxyproline and **hydroxylysine** are typically observed in collagen. Hydroxylation of proline and lysine residues occurs in the RER and requires ascorbic acid (vitamin C) as a cofactor. Inadequate wound healing is characteristic of **scurvy**, caused by a vitamin C deficiency.

Packaging and secretion of **procollagen** take place in the Golgi apparatus. Upon secretion of procollagen, the following three events occur in the extracellular space:

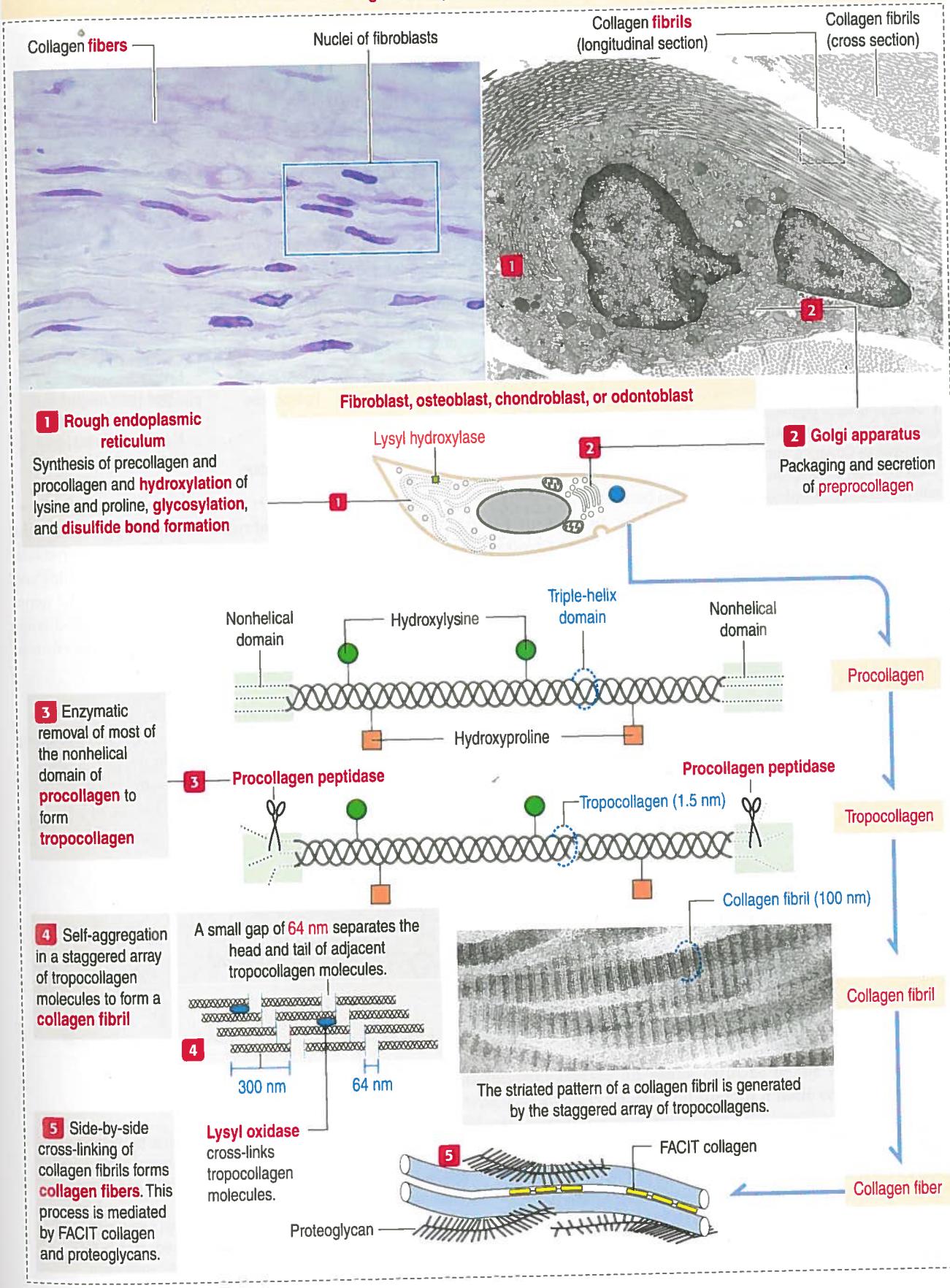
1. Enzymatic (**procollagen peptidase**) removal of most of the nonhelical endings of procollagen to give rise to soluble **tropocollagen** molecules.
2. Self-aggregation of tropocollagen molecules by a stepwise overlapping process to form **collagen fibrils**.
3. Cross-linking of tropocollagen molecules, leading to the formation of **collagen fibers**. **Lysyl oxidase** catalyzes cross-links between tropocollagens.

Groups of collagen fibers orient along the same axis to form **collagen bundles**. The formation of collagen bundles is guided by proteoglycans and other glycoproteins, including **FACIT** (for fibril-associated collagens with interrupted helices) collagens.

Clinical significance: Ehlers-Danlos syndrome

Ehlers-Danlos syndrome is clinically characterized by **hyperelasticity of the skin** (Figure 4-4) and **hypermobility of the joints**. The major defect resides in the synthesis, processing, and assembly of collagen. Several clinical subtypes are observed. They are classified by the degree of severity and the mutations in the collagen genes. For example, the vascular type form of Ehlers-Danlos syndrome—caused by a mutation in the *COL3A1* gene—is associated with severe vascular alterations leading to the development of varicose veins and spontaneous rupture

Figure 4-3. Synthesis of collagen



Box 4-C | Characteristics of collagens

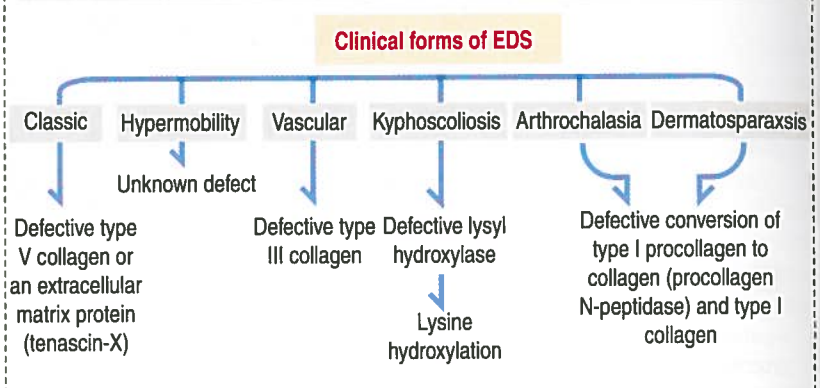
- Collagen is a three-chain fibrous protein in which the chains coil around each other (called a coiled-coil structure) like the strands of a rope. This triple-helix molecular organization generates a protein with considerable tensile strength.
- In **fibrillar collagen** (types I, II, III, and V), the completely processed molecule contains one triple helix, which accounts for almost the entire length of each molecule. Multiple triple helices of collagen fibers are aligned end-to-end and side-by-side in a regular arrangement. As a result, collagen fibers form dark and light periodic bands observed with the electron microscope.
- In **nonfibrillar collagens**, such as **type IV collagen**, several shorter triple-helical segments are separated by nontriple-helical domains and the N-terminal and C-terminal globular domains are not cleaved during protein processing.
- **Collagens form aggregates** (fibrils, fibers, or bundles) either alone or with extracellular matrix components. **Collagen fibrils and fibers** can be visualized with the electron microscope but not with the light microscope. **Collagen bundles** can be identified with the light microscope.

Figure 4-4. Ehlers-Danlos syndromes

A group of clinically and genetically diverse group of disorders resulting from defects in the synthesis and/or structure of collagen.

Abnormal collagen is devoid of tensile strength and skin is hyperextensible and vulnerable to trauma. The joints are hypermobile.

Collagen defects extend to blood vesels and the internal organs resulting in tissue rupture or detachment (retina).



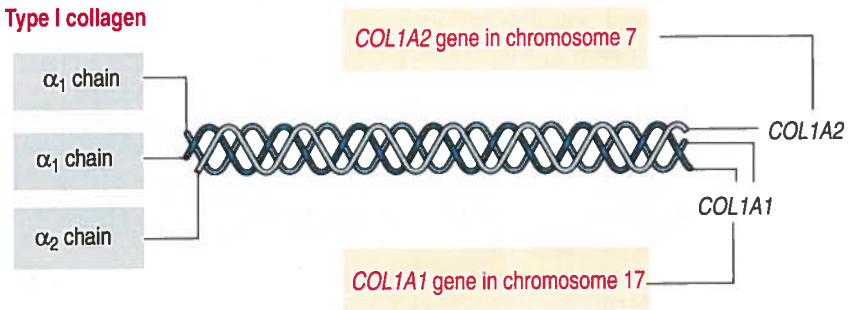
of major arteries. A deficiency in the synthesis of type III collagen, prevalent in the walls of blood vessels, is the major defect. Arthrochalasia and dermatosparaxsis types of Ehlers-Danlos syndrome display congenital dislocation of the hips and marked joint hypermobility. Mutations in the *COL1A1* and *COL1A2* genes (Figure 4-5), encoding type I collagen, and *procollagen N-peptidase* gene disrupt the cleavage site at the N-terminal of the molecule and affect the conversion of procollagen to collagen in some individuals.

Elastic fibers: Synthesis, secretion, and assembly

Like collagen, the synthesis of elastic fibers involves both the RER and the Golgi apparatus (Figure 4-6).

Elastic fibers are synthesized by the **fibroblast** (in skin and tendons), the

Figure 4-5. Pathology of collagen: Molecular defects

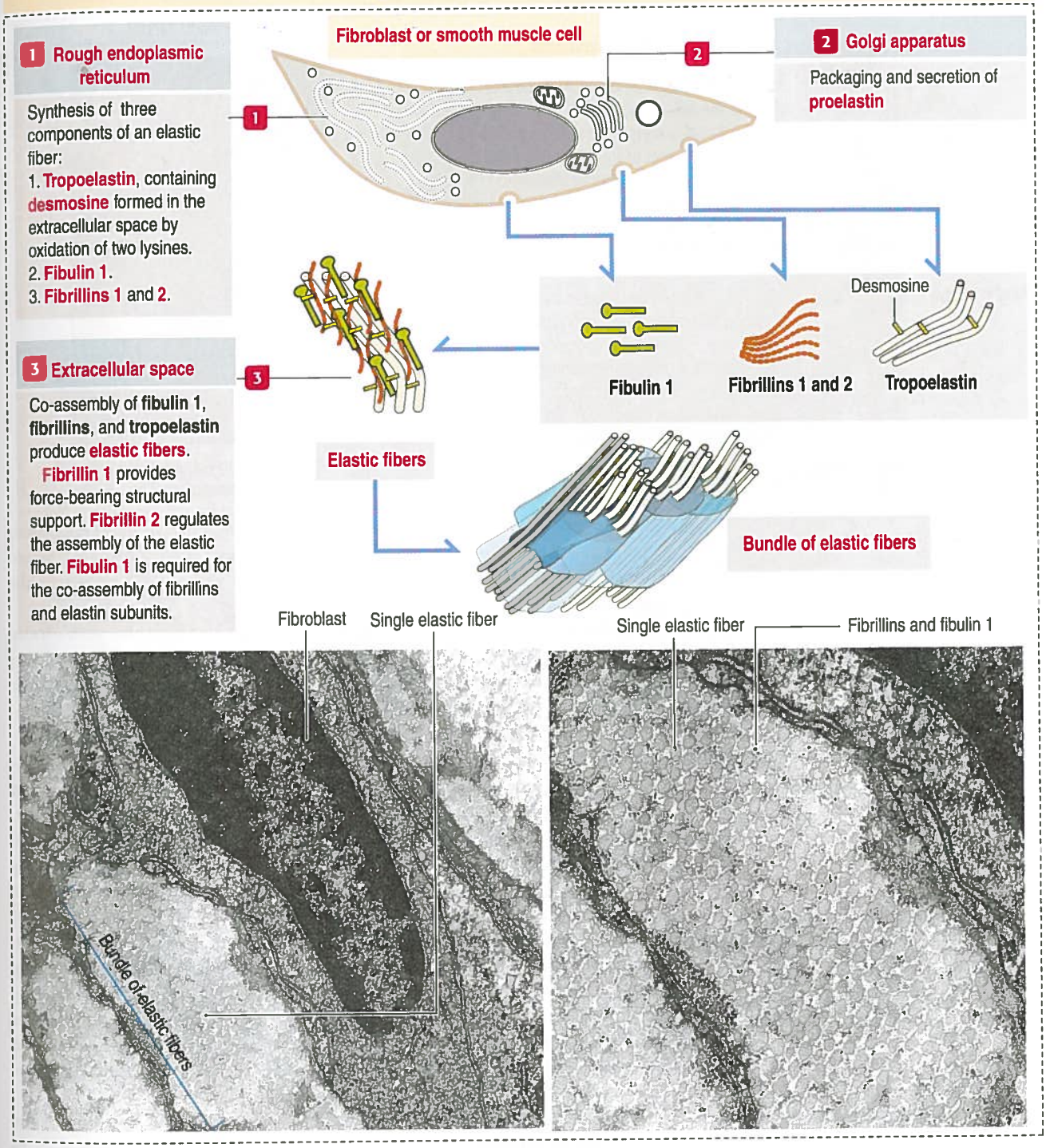


A mutation in ***COL1A1*** and ***COL1A2*** genes, encoding the α_1 and α_2 chains of type I collagen, respectively, involves cleavage sites for the N-terminal region of the molecule and interferes with the conversion of procollagen to collagen. This leads to defective cross-linking and a consequent reduction in the tensile strength of tendons (rich in type I collagen). This mutation is observed in some clinical forms of **Ehlers-Danlos syndrome**.

Strickler syndrome is characterized by myopia, hypoplasia of the lower jaw, and arthritis associated with dysplasia of the epiphyses. Type II collagen is abundant in cartilage and vitreous humor (eye). The ***COL2A1*** gene is mutated.

Osteogenesis imperfecta type I is associated with bone fragility. ***COL1A1*** point mutations determine a reduction in the production of type I collagen required for normal ossification.

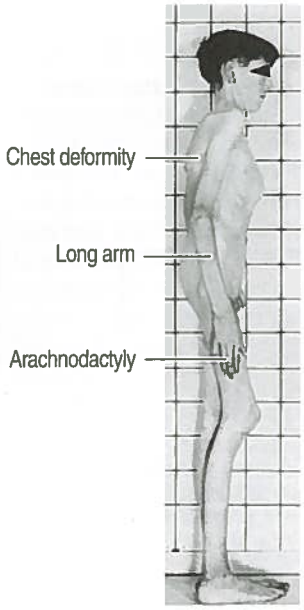
Figure 4-6. Synthesis of elastic fibers



chondroblast, the **chondrocyte** (in elastic cartilage of the auricle of the ear, epiglottis, larynx, and auditory tubes), and **smooth muscle cells** (in large blood vessels like the aorta and in the respiratory tree).

Proelastin, the precursor of elastin, is cleaved and secreted as **tropoelastin**. In the extracellular space, tropoelastin interacts with **fibrillins and fibulin 1** to organize **elastic fibers**, which aggregate to form **bundles of elastic fibers**. Tropoelastin contains a characteristic but uncommon amino acid: **desmosine**. Two lysine residues of tropoelastin are oxidized by lysyl oxidase to form a desmosine ring that cross-links two tropoelastin molecules. Cross-linking enables the stretching and recoil of tropoelastin, like rubber bands. **Elastic fibers do not**

Figure 4-7. Marfan syndrome

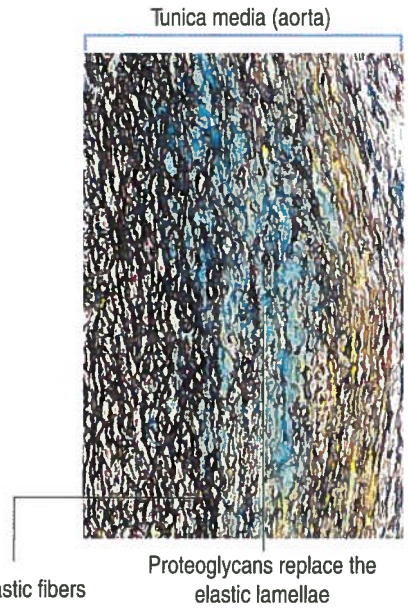


Patient with Marfan syndrome from McKusick VA: Heritable Disorders of Connective Tissue, 4th ed. St. Louis, Mosby, 1972.

Marfan syndrome

Marfan syndrome is an autosomal dominant disorder. Patients are tall, with long arms, legs, fingers, and toes (**arachnodactyly**). **Mitral valve prolapse**, **dilation of the root of the aorta** (detected by echocardiography), and **aortic dissection** (detected by MRI) are typical cardiovascular manifestations. Heart-related complications may shorten the life span of people with Marfan syndrome.

Etiology: an inherited defect in the gene encoding the protein **fibrillin-1** is responsible for the Marfan syndrome. Fibrillin-1 is a component of tropoelastin, a microfibril predominant in the aorta, skin, ligaments, and the ciliary zonular fibers of the lens. An increase in proteoglycans between the elastic lamellae weakens the wall of the aorta.



Microscopy from Weidner N, Cote RJ, Suster S, Weiss LM: Modern Surgical Pathology, St. Louis, Saunders, 2003.

contain collagen. Elastic fibers are produced during embryonic development and in adolescence but not so much in adults. Although elastic fibers are resilient during human life, many tissues decrease elasticity with age, in particular the skin, which develops wrinkles.

Under the light microscope, elastic fibers stain black or dark blue with **orcein**, a natural dye obtained from lichens.

Under the electron microscope, a cross section of an elastic fiber shows a dense core of elastin surrounded by microfibrils of **fibulin 1** and **fibrillins**.

Clinical significance: Marfan syndrome

Marfan syndrome is an autosomal dominant disorder in which the elastic tissue is weakened. Defects are predominantly observed in three systems: the **ocular**, **skeletal**, and **cardiovascular systems**. The **ocular defects** include **myopia** and **detached lens** (ectopia lentis). The skeletal defects (Figure 4-7) include long and thin arms and legs (**dolichostenomelia**), hollow chest (**pectus excavatum**), scoliosis, and elongated fingers (**arachnodactyly**).

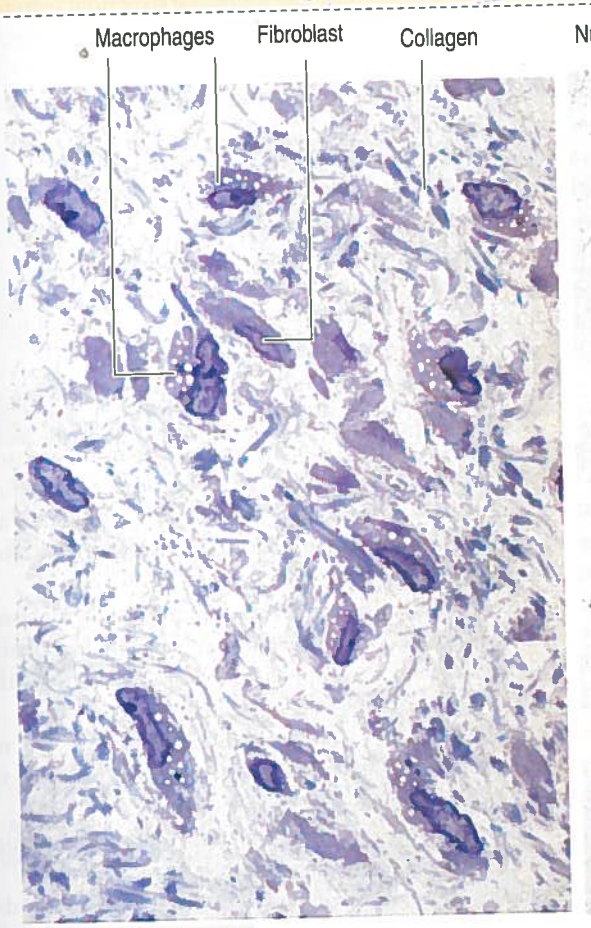
Cardiovascular abnormalities are life-threatening. Patients with Marfan syndrome display **prolapse of the mitral valve** and **dilation of the ascending aorta**. Dilation of the aorta and peripheral arteries may progress to dissecting aneurysm (Greek *aneurysma*, widening) and rupture. Medical treatment, such as administration of β-adrenergic blockers to reduce the force of systolic contraction in order to diminish stress on the aorta, and limited heavy exercise increase the survival rate of patients with Marfan syndrome.

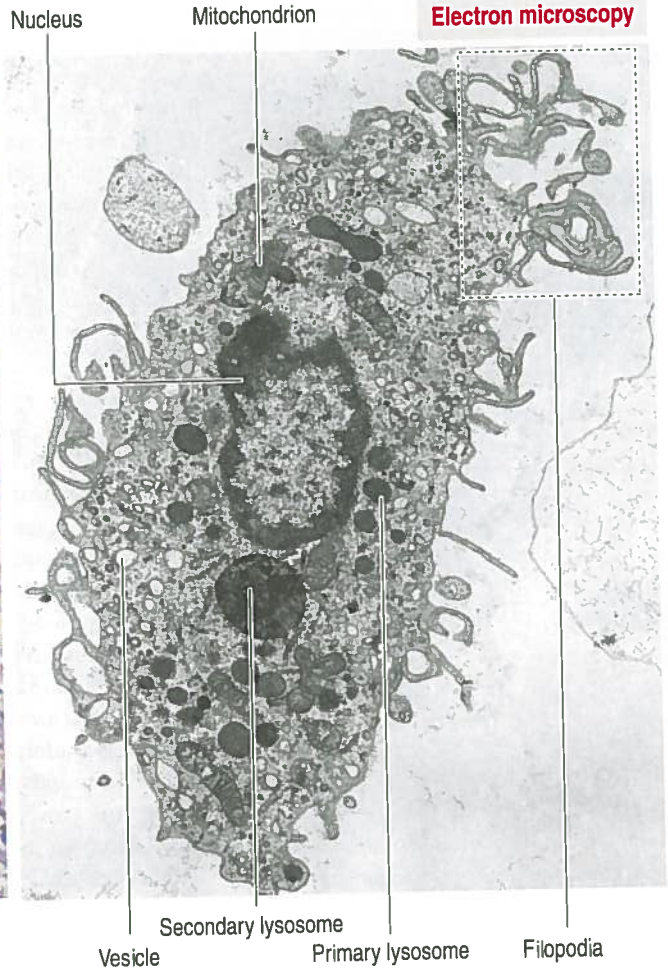
Defects observed in Marfan syndrome are caused by poor recoiling of the elastic lamellae dissociated by an increase in proteoglycans (see Figure 4-7). In the skeletal system, the periosteum, a relatively rigid layer covering the bone, is abnormally elastic and does not provide an oppositional force during bone development, resulting in skeletal defects.

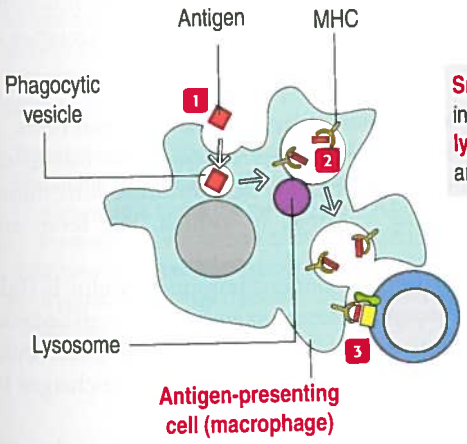
A mutation of the fibrillin 1 gene on chromosome 15 is responsible for Marfan syndrome. Fibrillin is present in the aorta, suspensory ligaments of the lens (see Chapter 9, Sensory Organs: Vision and Hearing), and the periosteum (see Bone). A homologous fibrillin 2 gene is present on chromosome 5. Mutations in the fibrillin 2 gene cause a disease called congenital contractural arachnodactyly. This disease affects the skeletal system, but ocular and cardiovascular defects are not observed.

Pour en savoir plus!

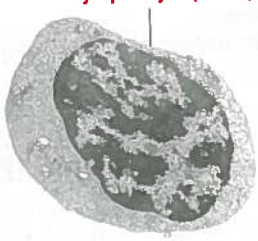
Figure 4-8. Macrophages







Small lymphocytes are present in the connective tissue. **Large lymphocytes** or **immunoblasts** are found in lymphoid tissues.



Macrophages as antigen-presenting cells

1 A macrophage takes up an antigen that is stored within a phagocytic vesicle.

2 A lysosome fuses with the phagocytic vesicle and the antigen is broken down into small peptide fragments, which bind to a receptor molecule—called the **major histocompatibility complex (MHC)**.

3 The phagocytic vesicle fuses with the plasma membrane, and the antigen is presented to a **lymphocyte** (T cell derived from the thymus).

Pour en savoir plus!

Macrophages

Macrophages have **phagocytic** properties and derive from monocytes, cells formed in the bone marrow (Figure 4-8).

Monocytes circulate in blood and migrate into the connective tissue, where they differentiate into macrophages. Macrophages have specific names in certain organs; for example, they are called **Kupffer cells** in the liver, **osteoclasts** in bone, and **microglial cells** in the central nervous system. Macrophages migrate to the site of inflammation, attracted by certain mediators, particularly C5a (a member

Box 4-D | Metachromasia

- The granules of the mast cell have a staining property known as **metachromasia** (Greek *meta*, beyond; *chroma*, color).
- After staining with a metachromatic dye, such as **toluidine blue**, the mast cell granules stain with a color that is different from the color of the dye (purple-red instead of blue).
- This phenomenon is determined by a change in the electronic structure of the dye molecule after binding to the granular material. In addition, mast cell granules are PAS positive because of their glycoprotein nature.

Pour en savoir plus!

of the complement cascade; see Chapter 10, Immune-Lymphatic System).

Macrophages in the connective tissue have the following structural features:

1. They contain abundant **lysosomes** required for the breakdown of phagocytic materials.

2. Active macrophages have numerous **phagocytic vesicles** (or **phagosomes**) for the transient storage of ingested materials.

3. The nucleus has an irregular outline.

Macrophages of the connective tissue have three major functions:

1. **To turn over senescent fibers and ECM material.**

2. The **presentation of antigens to lymphocytes as part of inflammatory and immunologic responses** (see Chapter 10, Immune-Lymphatic System).

3. **Production of cytokines** (for example, **interleukin-1**, an activator of helper T cells, and **tumor necrosis factor–α**, an inflammatory mediator).

Mast cells

Like macrophages, **mast cells originate in the bone marrow** from precursor cells lacking cytoplasmic granules. When mast cell precursors migrate into the connective tissue or the lamina propria of mucosae, they proliferate and accumulate cytoplasmic granules. **Mast cells and basophils circulating in blood derive from the same progenitor in the bone marrow.**

The **mast cell** is the source of **vasoactive mediators** contained in **cytoplasmic granules** (Figure 4-9). These granules contain **histamine**, **heparin**, and **chemotactic mediators** to attract monocytes, neutrophils, and eosinophils circulating in blood to the site of mast cell activation.

Leukotrienes are vasoactive products of mast cells. **Leukotrienes are not present in granules; instead, they are released from the cell membrane of the mast cells as metabolites of arachidonic acid.**

There are two populations of mast cells: **mucosal mast cells** (found predominantly in the intestine and lungs), and **connective tissue mast cells.**

Connective tissue mast cells differ from mucosal mast cells in the number and size of **metachromatic** (see Box 4-D) cytoplasmic granules, which tend to be more abundant in connective tissue mast cells. Although these two cell populations have the same cell precursor, the definitive structural and functional characteristics of mast cells depend on the site of differentiation (mucosa or connective tissue).

Clinical significance: Mast cells and allergic hypersensitivity reactions

The secretion of specific vasoactive mediators plays an important role in the regulation of vascular permeability and bronchial smooth muscle tone during **allergic hypersensitivity reactions** (for example, in **asthma**, **hay fever**, and **eczema**).

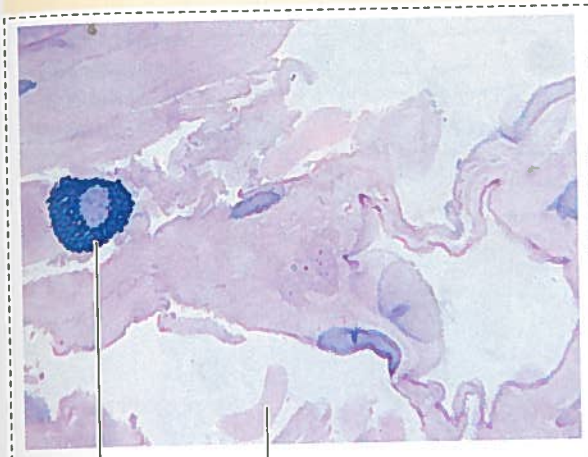
The surface of **mast cells** and **basophils** contains immunoglobulin E (IgE) receptors. Antigens bind to two adjacent IgE receptors and the mast cell becomes IgE-sensitized. An IgE-sensitized mast cell releases Ca^{2+} from intracellular storage sites and the content of the cytoplasmic granules is rapidly discharged by a process known as **degranulation**.

The release of histamine during asthma (Greek *asthma*, panting) causes dyspnea (Greek *dyspnoia*, difficulty with breathing) triggered by the histamine-induced spasmodic contraction of the smooth muscle surrounding the bronchioles and the hypersecretion of goblet cells and mucosal glands of bronchi.

During **hay fever**, histamine increases vascular permeability leading to edema (excessive accumulation of fluid in intercellular spaces).

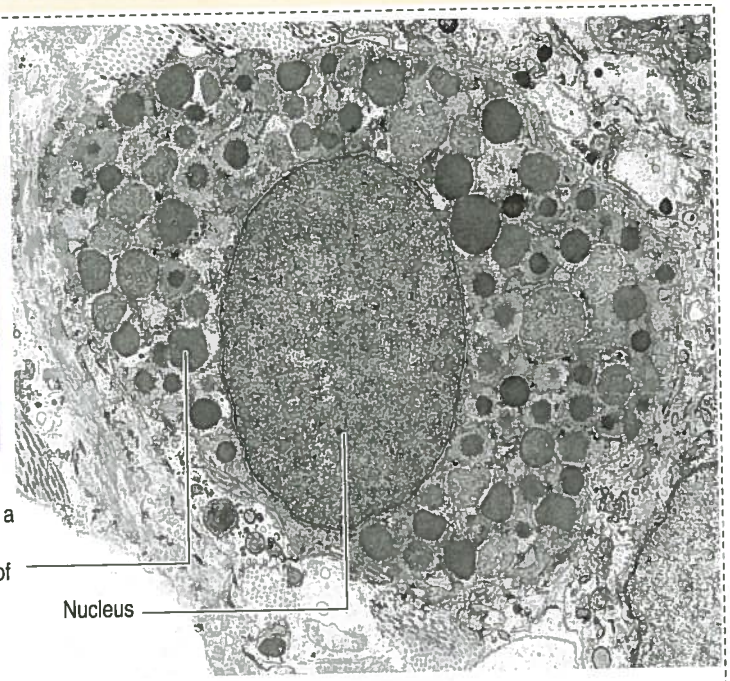
Mast cells in the connective tissue of skin release leukotrienes that induce increased vascular permeability associated with **urticaria** (Latin *urtica*, stinging nettle), a transient swelling in the dermis of the skin.

Figure 4-9. Mast cell



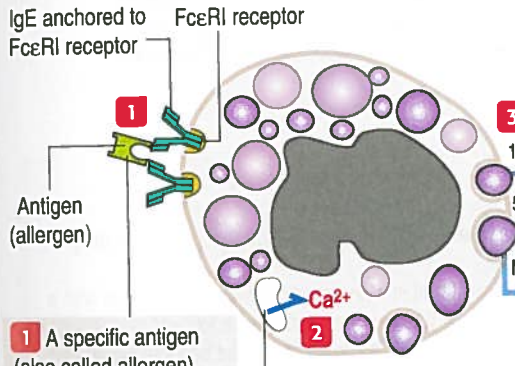
Mast cell with metachromatic granules in the cytoplasm

Blood vessel



Electron micrograph of a mast cell with cytoplasmic granules of different density

Nucleus



1 A specific antigen (also called allergen) bridges two adjacent IgE receptor molecules anchored to the FcεRI receptor

2 Cytosolic calcium is mobilized

3 Granule and lipid mediators and cytokines are released

Granule mediators	Histamine | Heparin | Tryptase | Chymase
Lipid mediators	Leukotriene C_4 | Prostaglandin D_2
Cytokines	Tumor necrosis factor-α | Interleukins (IL)-4, IL-5, IL-6, and IL-13

Nonactivated mast cells contain abundant granules storing **histamine, proteases**, and **proteoglycans**.

Histamine is formed by decarboxylation of histidine.

Proteoglycans contribute to the packaging and storage of histamine and proteases (mainly tryptase and chymase).

Tryptase is a unique **marker of mast cells**. It is not present in basophils.

After activation—binding of a specific antigen to two adjacent IgE receptors—mast cells:

1. Release histamine, proteases, and proteoglycans.
2. Synthesize mediators derived from **arachidonic acid** through the **cyclooxygenase** and **lipoxygenase** pathways.

Cyclooxygenase (**prostaglandin D_2**) and lipoxygenase (**leukotriene C_4**) metabolites are **not present in granules**. These metabolites are important inflammatory mediators.

Pour en savoir plus!

Plasma cells

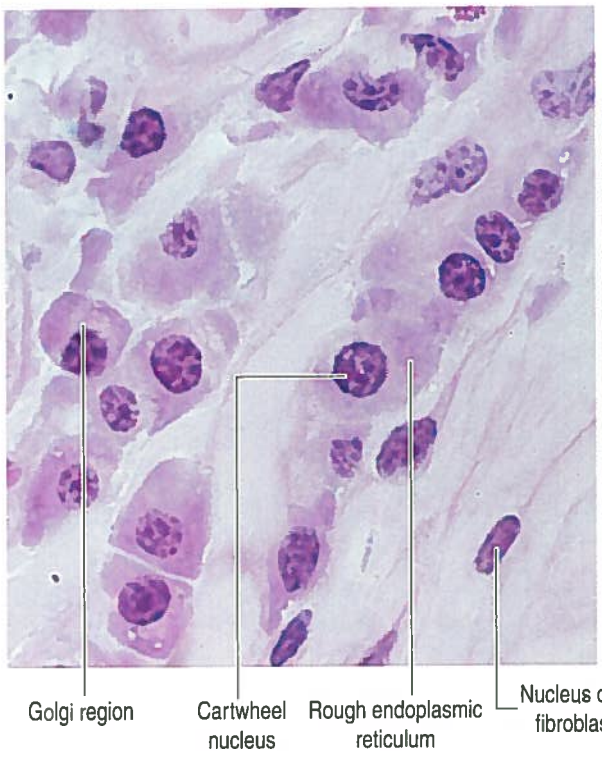
The plasma cell, which derives from the differentiation of **B lymphocytes** (also called **B cells**), synthesizes and secretes a single class of immunoglobulin (Figure 4-10). We discuss in Chapter 10, Immune-Lymphatic System, details of the origin of plasma cells.

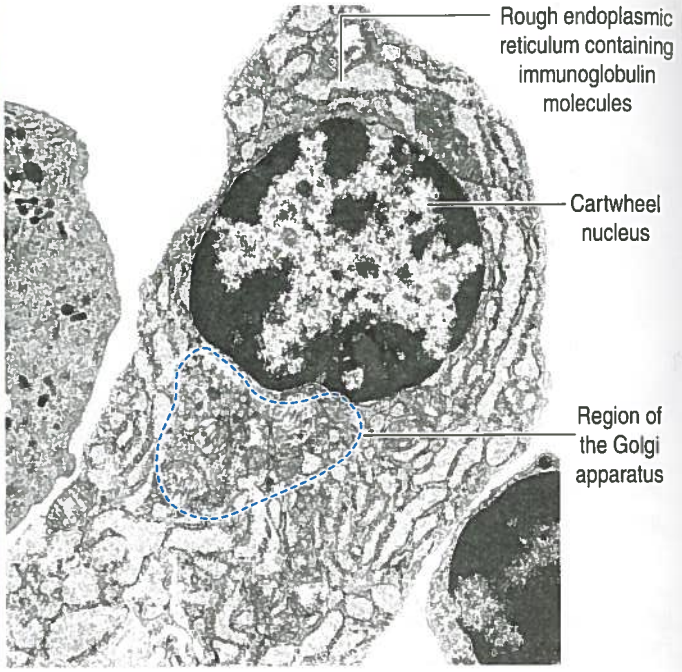
Immunoglobulins are glycoproteins, and therefore plasma cells have the three structural characteristics of cells active in protein synthesis and secretion:

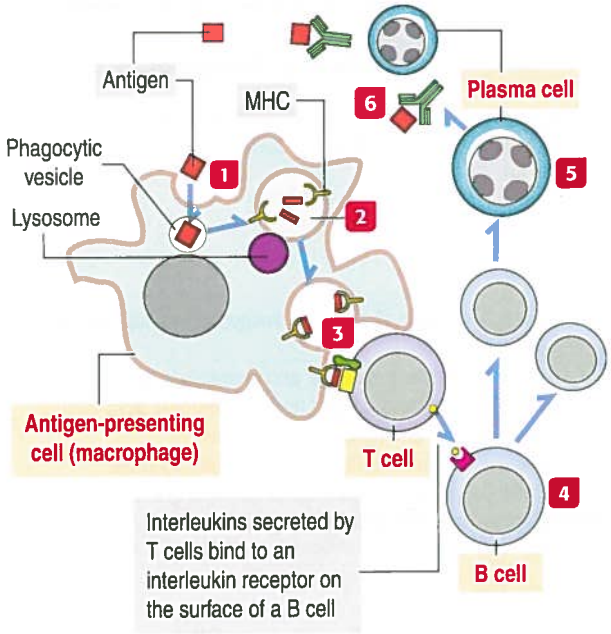
1. A well-developed **rough endoplasmic reticulum**
2. An extensive **Golgi apparatus**
3. A prominent **nucleolus**

At the light microscopic level, most of the cytoplasm of a plasma cell is basophilic because of the large amount of ribosomes associated with the endoplasmic

Figure 4-10. Plasma cell







Origin of a plasma cell

1 An antigen is taken up by a macrophage (antigen-presenting cell).
2 The antigen is stored in a phagocytic vesicle, which fuses with a lysosome to become a **phagosome**. Within an acidic pH microenvironment, lysosomal hydrolytic enzymes become active and break down the antigen into small peptides. Small peptides bind to **MHC molecules** inserted in the membrane of the phagosome.
3 The phagosome fuses with the plasma membrane and **the peptide-MHC is exposed to T cells**, which bind to the antigenic peptide and secrete cytokines or interleukins.
4 Interleukins bind to adjacent **B cells**, which are induced to divide by mitosis to increase their cell number.
5 B cells differentiate into immunoglobulin-secreting plasma cells.
6 Specific **immunoglobulins bind to free antigen** in the extracellular space to neutralize the damaging effect.

A more detailed analysis of the antigen-presenting cell and T cell–B cell interaction are discussed in Chapter 10, Immune-Lymphatic System.

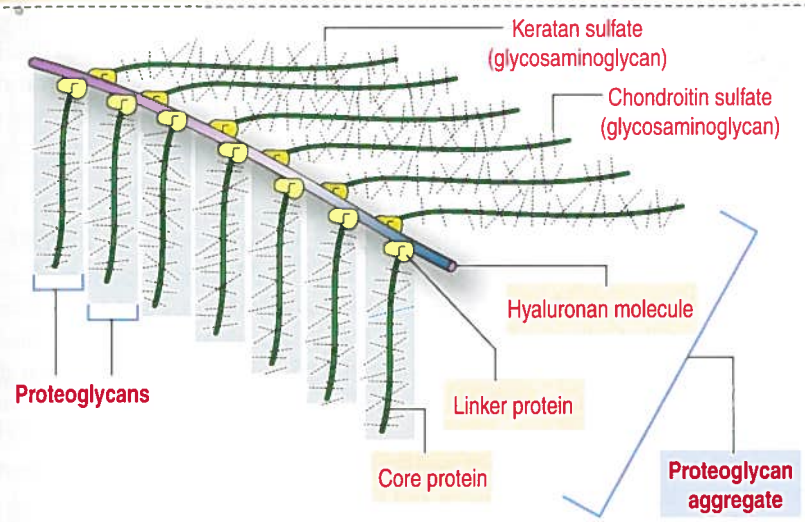
reticulum. A clear area near the nucleus is slightly **acidophilic** and represents the Golgi apparatus. The nucleus has a characteristic cartwheel configuration created by the particular distribution of heterochromatin.

Extracellular matrix

The ECM is a combination of **collagens**, **noncollagenous glycoproteins**, and **proteoglycans** surrounding cells and fibers of the connective tissue.

Recall that the **basement membrane** contains several ECM components such as **laminin**, **fibronectin**, various types of **collagen**, and **heparan sulfate proteoglycan**. In addition, epithelial and nonepithelial cells have receptors for

Figure 4-11. Proteoglycan aggregate



Proteoglycans are extracellular protein complexes of glycosaminoglycans

Proteoglycan aggregates are formed by:

1. An axial **hyaluronan molecule**
2. **Core proteins** attached to the hyaluronan molecule by a **linker protein**
3. **Glycosaminoglycans** attached to a core protein

Several chains of glycosaminoglycans bound to the core protein form a proteoglycan.

The molecular mass of a proteoglycan is about 10^8 kd.

ECM constituents. An example is the family of **integrins** with binding affinity for laminin and fibronectin. Integrins interact with the cytoskeleton, strengthening cell interactions with the ECM by establishing focal contacts or modifying cell shape or adhesion.

Several noncollagenous glycoproteins of the ECM mediate interactions with cells and regulate the assembly of ECM components. Noncollagenous glycoproteins have a widespread distribution in several connective tissues, although cartilage and bone contain specific types of noncollagenous glycoproteins. We study them later when we discuss the processes of **chondrogenesis** (formation of cartilage) and **osteogenesis** (bone formation).

Proteoglycan aggregates (Figure 4-11) are the major components of the ECM. Each proteoglycan consists of **glycosaminoglycans (GAGs)**, proteins complexed with polysaccharides. GAGs are linear polymers of disaccharides with sulfate residues. GAGs control the biological functions of proteoglycans by establishing links with cell surface components, growth factors, and other ECM constituents.

Different types of GAGs are attached to a **core protein** to form a proteoglycan. The core protein, in turn, is linked to a **hyaluronan molecule** by a **linker protein**. The hyaluronan molecule is the axis of a **proteoglycan aggregate**. Proteoglycans are named according to the prevalent GAG (for example, **proteoglycan chondroitin sulfate, proteoglycan dermatan sulfate, proteoglycan heparan sulfate**).

The **embryonic connective tissue** of the umbilical cord (**Wharton's jelly**) is predominantly ECM material surrounding the two umbilical arteries and the single umbilical vein. Proteoglycans have extremely high charge density and, therefore, significant osmotic pressure. These attributes enable a connective tissue bed to resist compression because of the very high swelling capacity of these molecules. The umbilical blood vessels, crucial elements for fetal-maternal fluid, gas, and nutritional exchange, are surrounded by a proteoglycan-enriched type of connective tissue to provide resistance to compression.

Degradation of the extracellular matrix

The ECM can be degraded by **matrix metalloproteinases**, a family of zinc-dependent proteases **secreted as latent precursors (zymogens)** proteolytically activated in the ECM. The activity of matrix metalloproteinases in the extracellular space can be specifically inhibited by **tissue inhibitors of metalloproteinases (TIMPs)**.

The expression of matrix metalloproteinase genes is regulated by cytokines, growth factors, and cell contact with the ECM.

The degradation of the ECM occurs normally during the development, growth, and repair of tissues. However, excessive degradation of the ECM is observed in several pathologic conditions such as rheumatoid arthritis, osteoarthritis, and diseases of the skin. Tumor invasion, metastasis, and tumor angiogenesis require the participation of matrix metalloproteinases whose expression increases in association with tumorigenesis.

Members of the family of matrix metalloproteinases include:

1. **Collagenases.** Collagenases 1, 2, and 3 can degrade types I, II, III, and V collagens. Collagenase 1 is synthesized by fibroblasts, chondrocytes (cartilage), keratinocytes (epidermis), monocytes and macrophages, hepatocytes (liver), and tumor cells. Collagenase 2 is stored in cytoplasmic granules of polymorphonuclear leukocytes and released in response to a stimulus. Collagenase 3 can degrade several collagens (types I, II, III, IV, IX, X, and XI), laminin and fibronectin, and other ECM components.
2. **Stromelysins** (1, 2, and metalloelastase), which degrade basement membrane components (type IV collagen and fibronectin) and elastin.
3. **Gelatinases A** and **B** can degrade type I collagen. Gelatinases are produced by alveolar macrophages.
4. **Membrane-type matrix metalloproteinases** are produced by tumor cells.

Matrix metalloproteinases are a target of therapeutic intervention to inhibit tumor invasion and metastasis. We come back to this topic in Chapter 23, Fertilization, Placentation, and Lactation, when we discuss the early stages of embryo implantation in the endometrial stroma or decidua.

Clinical significance: Molecular biology of tumor invasion

Invasion and metastasis are two important events of carcinoma (Greek *karkinoma*, from *karkinos*, crab, cancer + *oma*, tumor), a tumor derived from epithelial tissues. **Adenoma** is a structurally benign tumor of epithelial cell origin lacking invasive and metastatic properties. Malignant carcinomas may arise from benign adenomas. For example, a small benign adenoma or **polyp** of the colon can become an invasive carcinoma.

Sarcoma (Greek *sarx*, flesh + *oma*) is a tumor derived from the connective tissues (muscle, bone, cartilage) and mesodermal cells. For example, fibrosarcoma derives from fibroblasts and osteosarcoma originates from bone.

Invasion is defined by the **breakdown of the basement membrane** by tumor cells and implies the transition from precancer to cancer. **Metastasis** is the spread of tumor cells throughout the body through blood and lymphatic vessels, generally leading to death. Figure 4-12 illustrates and describes the initial events of tumor cell invasion.

Many carcinomas produce members of the matrix metalloproteinase family to digest various types of collagen as we have seen in the preceding section. Normal tissues produce tissue inhibitors of metalloproteinases that are neutralized by carcinoma cells. Tumors that behave aggressively are capable of overpowering the protease inhibitors.

One critical event during metastasis is angiogenesis, the development of blood vessels. Blood vessels supply oxygen and nutrients required for tumor growth. Angiogenesis is stimulated by tumor cells, in particular the proliferation of capillary endothelial cells forming new capillaries in the tumoral growth. In Chapter 12, Cardiovascular System, we discuss the mechanism of action and targets of endostatin and angiostatin, two new proteins that inhibit angiogenesis.

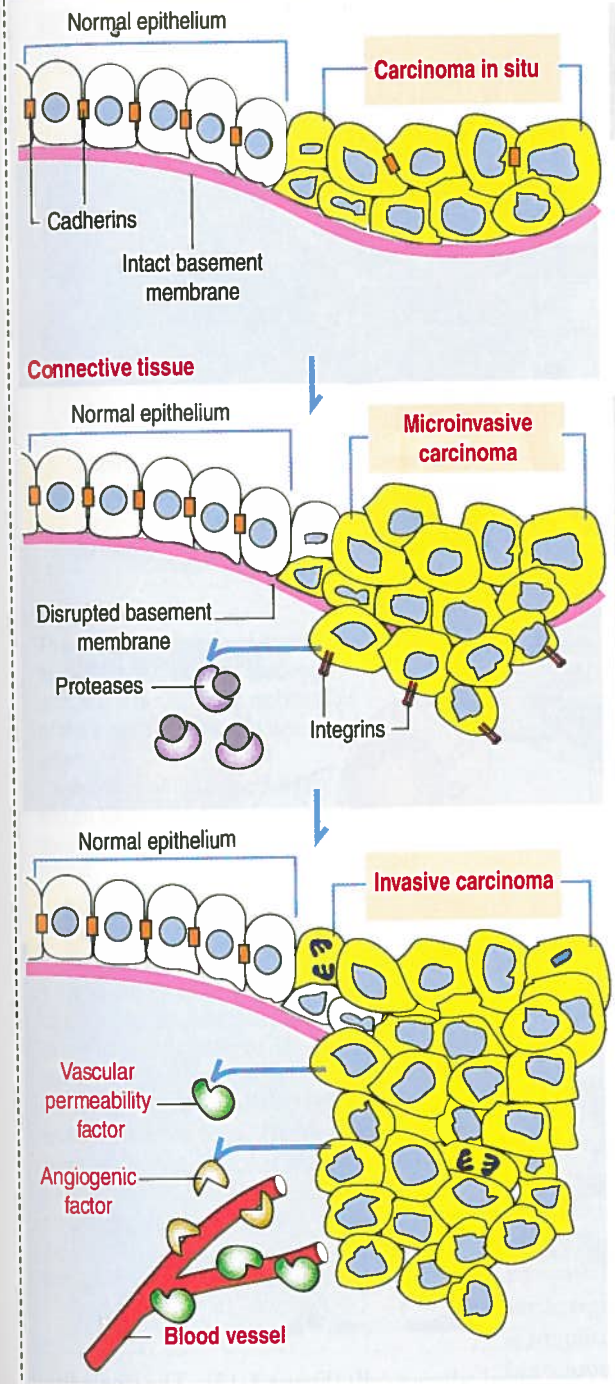
ADIPOSE TISSUE OR FAT

There are two classes of adipose tissue:

1. **White fat**, the major **reserve of long-term energy**

Pour en savoir plus!

Figure 4-12. Tumor invasion and metastasis



Tumor cells have not invaded the basement membrane and remain confined within the epithelial layer. This stage is known as **carcinoma in situ**.

The expression of cell adhesion molecules, such as **cadherins**, decreases. This decrease weakens the cohesive nature of the intraepithelial tumor cells, and **microinvasion** starts when the basement membrane breaks down.

Collagenase IV, released by invading tumor cells, dissolves the basement membrane and allows tumor cells to invade the subjacent connective tissue. Other proteases, such as **plasminogen activator**, **collagenases I**, **II**, and **III**, **cathepsins**, and **hyaluronidase**, destroy noncollagenous glycoproteins and proteoglycans, enabling further advancement of tumor cells into the destroyed connective tissue.

Invading tumor cells overexpress **integrins** (laminin and fibronectin receptors) to facilitate cell attachment and progression in the connective tissue. Tumor cells generally invade along pathways that provide low resistance, such as connective tissue.

As tumor cells start their **invasive phase**, they secrete:

1. **Autocrine motility factors** (to direct the motion of the advancing tumor cells).
2. **Vascular permeability factors** (to enable plasma proteins and nutritional factors to accumulate).
3. **Angiogenic factor** (to increase the vascularity and nutritional support of the growing tumor). See Chapter 12, Cardiovascular System, for a discussion of tumor angiogenesis.

Because newly formed blood vessels are connected with the general circulation, tumor cells can rapidly enter the blood vessels and disseminate to distant tissues. This event is known as **metastasis**.

2. **Brown fat**, which serves primarily to **dissipate energy** instead of storing energy.

Similar to fibroblasts, the primitive **preadipocyte** derives from a mesenchymal cell precursor. Preadipocytes can follow two cell differentiation pathways: one pathway results in the formation of white fat; the other generates brown fat. Adipogenesis occurs during both the prenatal and postnatal states of the individual and is reduced as age increases.

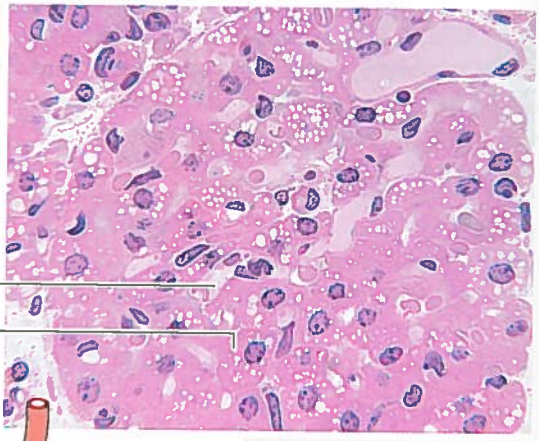
Under the influence of **insulin**—bound to **insulin-like growth factor-1 (IGF-1) receptor**—preadipocytes synthesize **lipoprotein lipase** and begin to accumulate fat in small droplets. Small droplets fuse to form a single large lipid-storage droplet, a characteristic of mature unilocular (Latin *unus*, single; *loculus*,

Pour en savoir plus!

Figure 4-13. Adipogenesis

Multilocular adipocyte (brown fat)

Aggregates of epithelial-like **multilocular, mitochondria-rich adipocytes surrounded by abundant blood vessels** are characteristic of brown fat. The main function of brown fat is to dissipate energy instead of storing it as does white fat. Heat is generated by uncoupling the production of ATP from the movement of H^+ across the inner mitochondrial membrane down the concentration gradient. **Uncoupling protein-1 (UCP-1)** activates uncoupling.



Mesenchymal cells give rise to **preadipocytes** that differentiate into **brown adipose tissue** (multilocular) and **white adipose tissue** (unilocular). **Insulin** binding to **insulin-like growth factor-1 (IGF-1) receptor** stimulates lipoprotein lipase blood-to-cell fat transfer. The primary fat formation in the fetus is brown fat. In the adult, multiple lipid droplets coalesce to form a single fat droplet (secondary fat formation).

Primary fat formation (fetus)

Multilocular adipocyte
Preadipocyte
Blood vessel
Lipoprotein lipase
Brown adipose tissue
Insulin
IGF-1 receptor

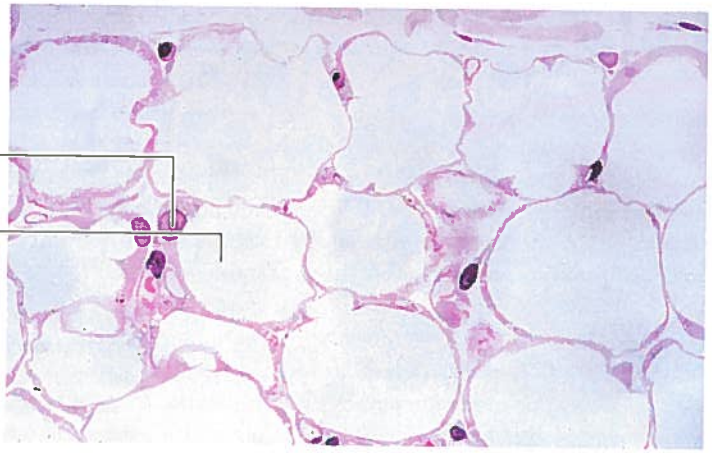
Mitochondrial biogenesis and expression of **UCP-1 protein** are key features of thermogenesis by brown fat.

White adipose tissue

Mesenchymal cell
Preadipocyte
Unilocular adipocyte

Secondary fat formation (adult)

Fibroblast



Unilocular adipocyte (white fat)

A **single large fat inclusion**, resulting from coalescing multiple lipid droplets, pushes the nucleus to an eccentric position. Fat in this preparation is unstained.

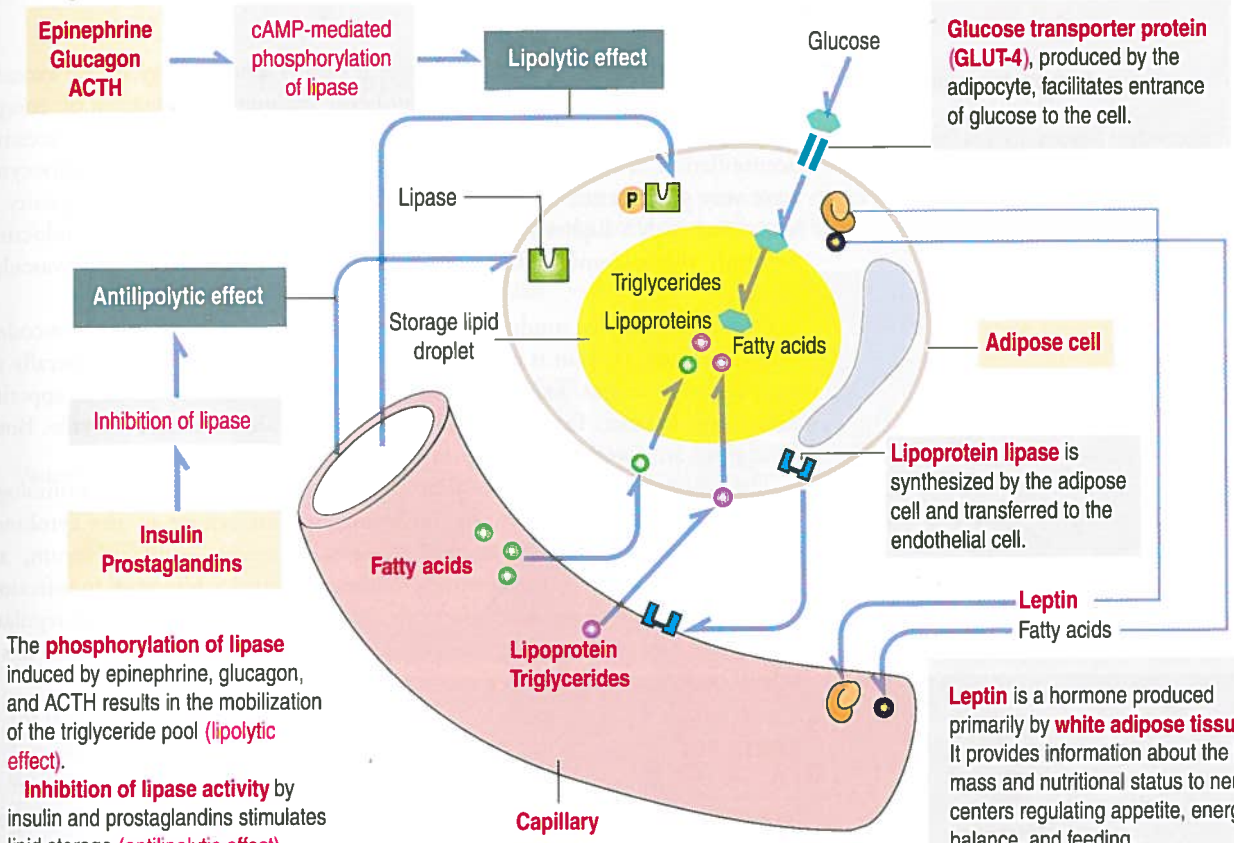
small place) **adipocytes** (also called **adipose cell**) (Figure 4-13). The single lipid-storage droplet pushes the nucleus to an eccentric position and the adipocyte assumes a "signet-ring" appearance. **In histologic sections, capillaries appear as single structures that may contain blood cell elements, whereas adipocytes form aggregates.**

Lipid droplets contain about 95% triglycerides rich in carotene, a lipid-soluble pigment that gives the so-called white fat a yellowish color. **Each lipid droplet is in direct contact with the cytosol and is not surrounded by a cytomembrane.** Therefore, lipid droplets can be classified as cell **inclusions** (see Box 4-E).

The **main function of white fat is storage of energy.** Unlike brown fat, white fat responds slightly to cold and acts as an **insulator.** The blood supply to white fat, mainly capillaries, is not as extensive as in brown fat. Adipose tissue also

Pour en savoir plus!

Figure 4-14. Regulation of adipocyte function



Box 4-E | Fat in histologic sections

- Fat is usually dissolved by solvents (xylene) used during paraffin embedding. Only the nucleus and a narrow cytoplasmic rim, surrounding a central empty space, can be visualized.
- Fat that is fixed and stained with osmium tetroxide appears brown. This reagent is also used for the visualization of lipid-rich myelin in nerves (see Chapter 8, Nervous Tissue).
- Alcoholic solutions of fat-soluble dyes (such as Sudan III or Sudan black) can also be used for the detection of fat in frozen sections.

insulates the body against heat loss, fills spaces, and cushions certain anatomic parts, behaving as a shock-absorber in the soles of the feet, around the kidneys, and in the orbit around the eye. Most adipose tissues form at sites where loose connective tissue is present, such as the subcutaneous layer—or hypodermis—of the skin.

The **storage of lipids** by mature adipocytes are regulated by **insulin** and **prostaglandins**. The **breakdown and release of lipids** is regulated by **epinephrine**, **glucagon**, and **adrenocorticotropic hormone (ACTH)** (Figure 4-14). Adipose tissue is innervated by the sympathetic nervous system.

Preadipocytes can differentiate into mature **multilocular** (Latin *multus*, many; *loculus*, small place) **adipocytes of brown fat** in the fetus and newborn. Brown fat is found in the neck, shoulders, back, and the perirenal and para-aortic regions of the body. Brown fat is mostly lost during childhood. Brown fat is supplied by abundant blood vessels and sympathetic adrenergic nerve fibers. Lipochrome pigment and abundant mitochondria, rich in cytochromes, give this type of fat a brownish color.

As stated initially, the main function of brown fat is to dissipate energy in the form of heat (thermogenesis) in cold environments as a protective mechanism in the newborn. Thermogenesis by brown fat cells has two requirements (see Figure 4-13):

1. Mitochondrial biogenesis
2. The expression of the transporter **uncoupling protein-1 (UCP-1)**

As we briefly mentioned in Chapter 2, Epithelial Glands, in our discussion on UCP transporters in mitochondria, UCP-1 dissipates the proton gradient established across the inner mitochondrial membrane when electrons pass along the electron-transport chain. Thermogenesis takes place because UCP-1 allows

the reentry of protons down their concentration gradient into the mitochondrial matrix and uncouples respiration from ATP production.

Clinical significance: Obesity

Obesity is a disorder of energy balance. It occurs when energy intake exceeds energy expenditure. Protection against obesity without consideration of energy intake results in an increase in circulating levels of triglycerides, and excessive accumulation of fat in liver (**steatosis**). The metabolic activities of adipocytes have very significant clinical consequences. An increase in visceral adiposity is associated with a higher risk of insulin resistance (see Chapter 19, Endocrine System), **dyslipidemia** (alteration in blood fat levels), and cardiovascular disease.

One of the secreted products of adipocytes is **leptin**, a 16-kd protein encoded by the ***ob* gene**. Leptin is released into the circulation and acts peripherally to regulate body weight. Leptin acts on hypothalamic targets involved in appetite and energy balance. Leptin-deficient mice (*ob/ob*) are obese and infertile. Both conditions are reversible with leptin administration.

The leptin receptor in hypothalamic target cells shares sequence homology with cytokine receptors. During inflammation, the release of the cytokines **interleukin-1** and **tumor necrosis factor–α** increases leptin in serum, an indication that leptin interacts with cytokines to influence responses to infection and inflammatory reactions. Infections, injury, and inflammation up-regulate leptin gene expression and serum protein levels. As we discuss later, leptin has a role in bone formation.

CARTILAGE

Like the fibroblast and the adipocytes, the **chondroblast** derives from a mesenchymal cell. Chondroblasts contain lipids and glycogen, a well-developed RER (basophilic cytoplasm), and Golgi apparatus. The proliferation of chondroblasts results in growth of the cartilage.

Similar to typical connective tissue, the **cartilage consists of cells and ECM surrounded by the perichondrium.** The perichondrium is formed by a layer of undifferentiated cells that can differentiate into chondroblasts.

In contrast to typical connective tissue, the cartilage is **avascular** and cells receive nutrients by diffusion through the ECM. At all ages, chondrocytes have significant nutritional requirements. Although they rarely divide in the adult cartilage, they continuously synthesize molecules to replace a constantly turned-over ECM, in particular, proteoglycans (Figure 4-15; see Box 4-F).

Growth of cartilage (chondrogenesis)

Cartilage grows by two mechanisms (Figures 4-16 and 4-17):

1. By **interstitial growth** (from chondrocytes **within the cartilage**; see Figure 4-16).

2. By **appositional growth** (from undifferentiated cells **at the surface of the cartilage or perichondrium**; see Figure 4-17).

During chondrogenesis, chondroblasts produce and deposit **type II collagen** fibers and ECM (**hyaluronic acid** and **GAGs**, mainly chondroitin sulfate and keratan sulfate) until chondroblasts are separated and trapped within spaces in the matrix called **lacunae** (Latin *lacuna*, small lake). The cells are then called **chondrocytes**. The space between the chondrocyte and the wall of the lacuna seen in histologic preparations is an artifact of fixation.

The matrix in close contact with each chondrocyte forms a bluish (with hematoxylin and eosin), metachromatic (see Box 4-D), or PAS-positive basket-like structure called the **territorial matrix**.

Each cluster of chondrocytes (known as an **isogenous group**) enveloped by the territorial matrix is separated by a wide but pale **interterritorial matrix**.

Box 4-F | Cartilage repair after injury

- Cartilage has a modest repair capacity. Cartilage injuries frequently result in the formation of **repair cartilage** from the perichondrium.
- This repair cartilage contains undifferentiated cells with a potential to differentiate into chondrocytes that synthesize components of the cartilage matrix.
- The repair cartilage has a matrix composition intermediate between hyaline and fibrous cartilage (for example, it contains both types I and II collagen).

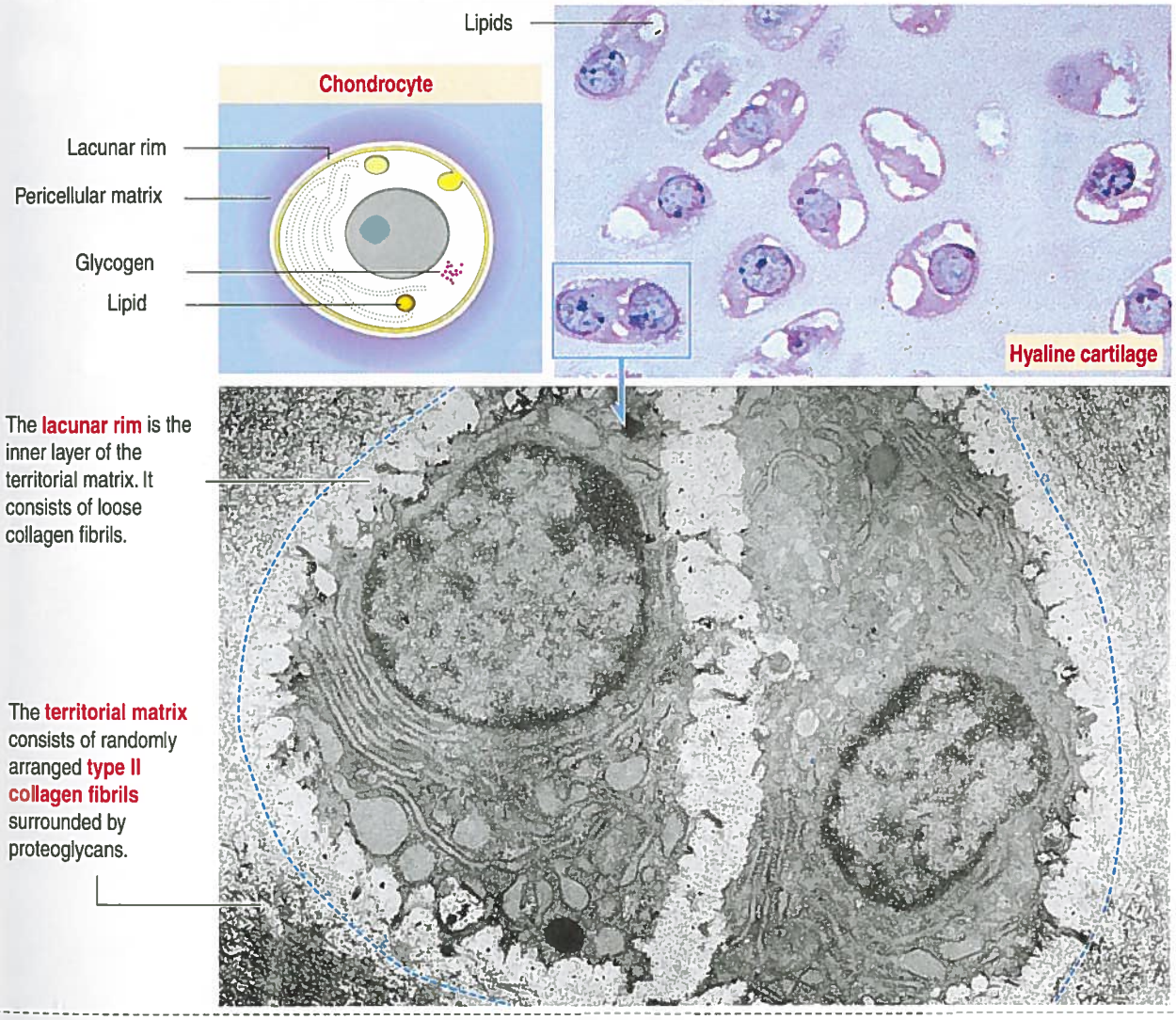
Figure 4-15. Chondrocytes and the surrounding matrix

Chondrocytes

The cells that produce the cartilage matrix are called **chondroblasts** or **chondrocytes**, depending on the relative maturity of the cells.

Chondrocytes occupy small cavities in the extracellular matrix called **lacunae**. Two chondrocytes may occupy a single lacuna.

The extracellular matrix is compartmentalized. A **pericellular matrix** (visible with special staining) is circumscribed by a moderately stained **territorial matrix** and a less intensely stained **interterritorial matrix**.



Types of cartilage

There are three major types of cartilage (Figure 4-18):

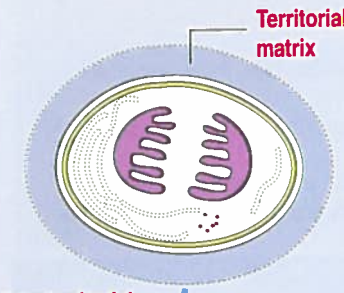
1. **Hyaline cartilage**
2. **Elastic cartilage**
3. **Fibrocartilage**

Hyaline cartilage is the most widespread cartilage in humans. Its name derives from the clear appearance of the matrix (Greek *hyalos*, glass).

In the fetus, hyaline cartilage forms most of the skeleton before it is reabsorbed and replaced by bone by a process known as **endochondral ossification**.

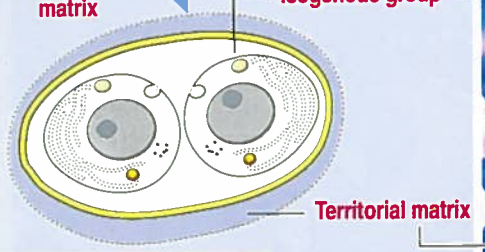
In adults, hyaline cartilage persists as the nasal, laryngeal, tracheobronchial, and costal cartilage. **The articular surface of synovial joints** (knees, shoulders) **is hyaline cartilage and does not participate in endochondral ossification.**

Figure 4-16. Chondrogenesis: Interstitial growth

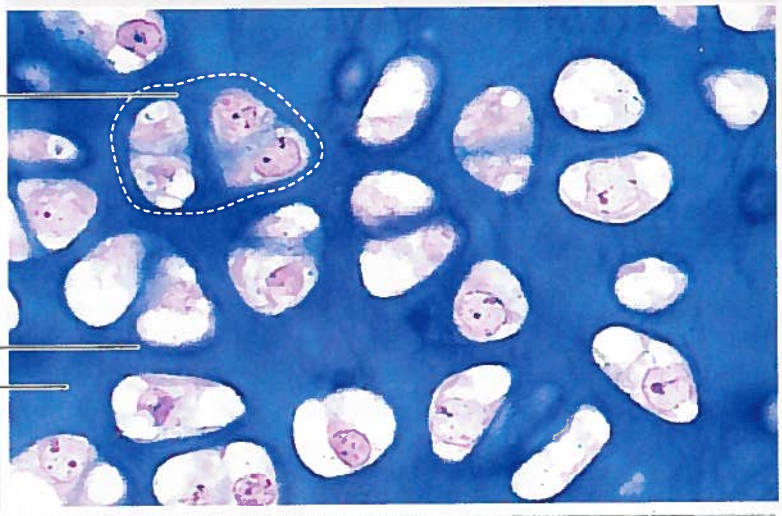


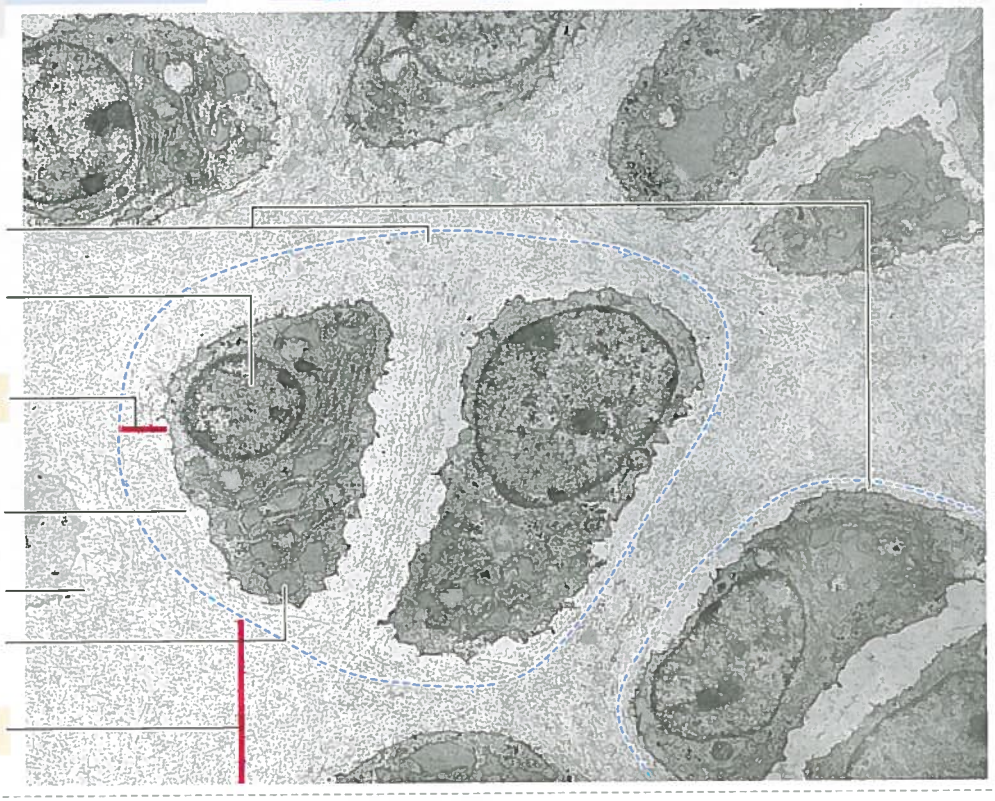
During embryogenesis, mesenchymal cells aggregate and differentiate into chondroblasts, which form **centers of chondrogenesis**. A center of chondrogenesis consists of chondroblasts surrounded by extracellular matrix. Chondroblasts divide by mitosis, and the daughter cells remain within the same space or lacuna forming an **isogenous cell group**. The isogenous group is surrounded by **territorial matrix**. A wider **interterritorial matrix** surrounds the territorial matrix.

This growth process, known as **interstitial growth** of the cartilage, is very active during **endochondral ossification** (see Chapter 5, Osteogenesis).



After cell division, daughter cells remain within the same space or lacuna, forming an **isogenous group** (Greek *isos*, equal; *genos*, family, kind)





Articular surfaces are not lined by an epithelium.

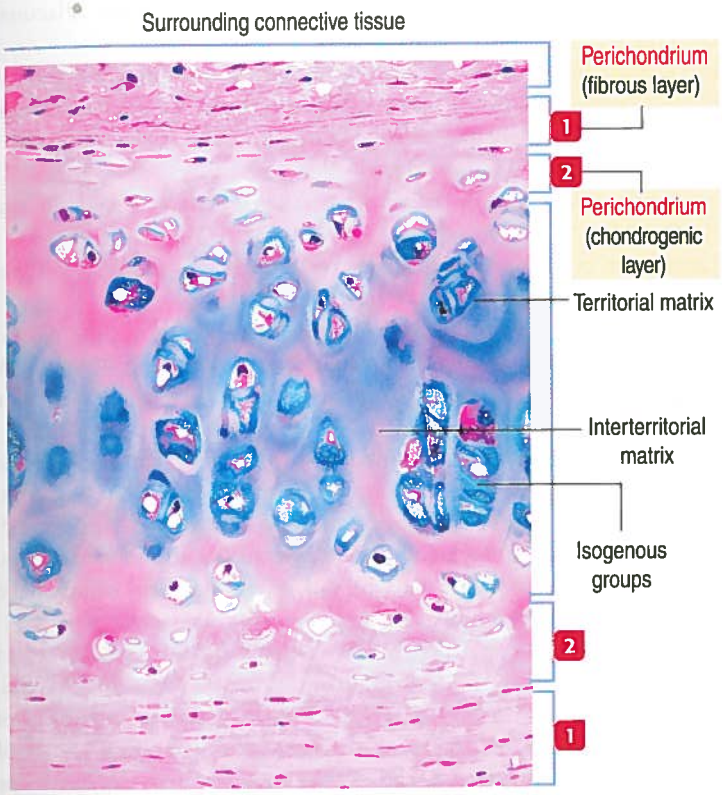
The hyaline cartilage contains:

1. **Cells** (chondrocytes)
2. **Fibers** (**type II collagen** synthesized by chondrocytes)
3. **ECM** (also synthesized by chondrocytes)

Chondrocytes have the structural characteristics of a protein-secreting cell

Pour en savoir plus!

Figure 4-17. Chondrogenesis: Appositional growth



1 The **outermost cells** of the developing cartilage are spindle-shaped and clustered in a regular fibrous layer called **perichondrium**, a transitional zone between cartilage and the surrounding general connective tissue.

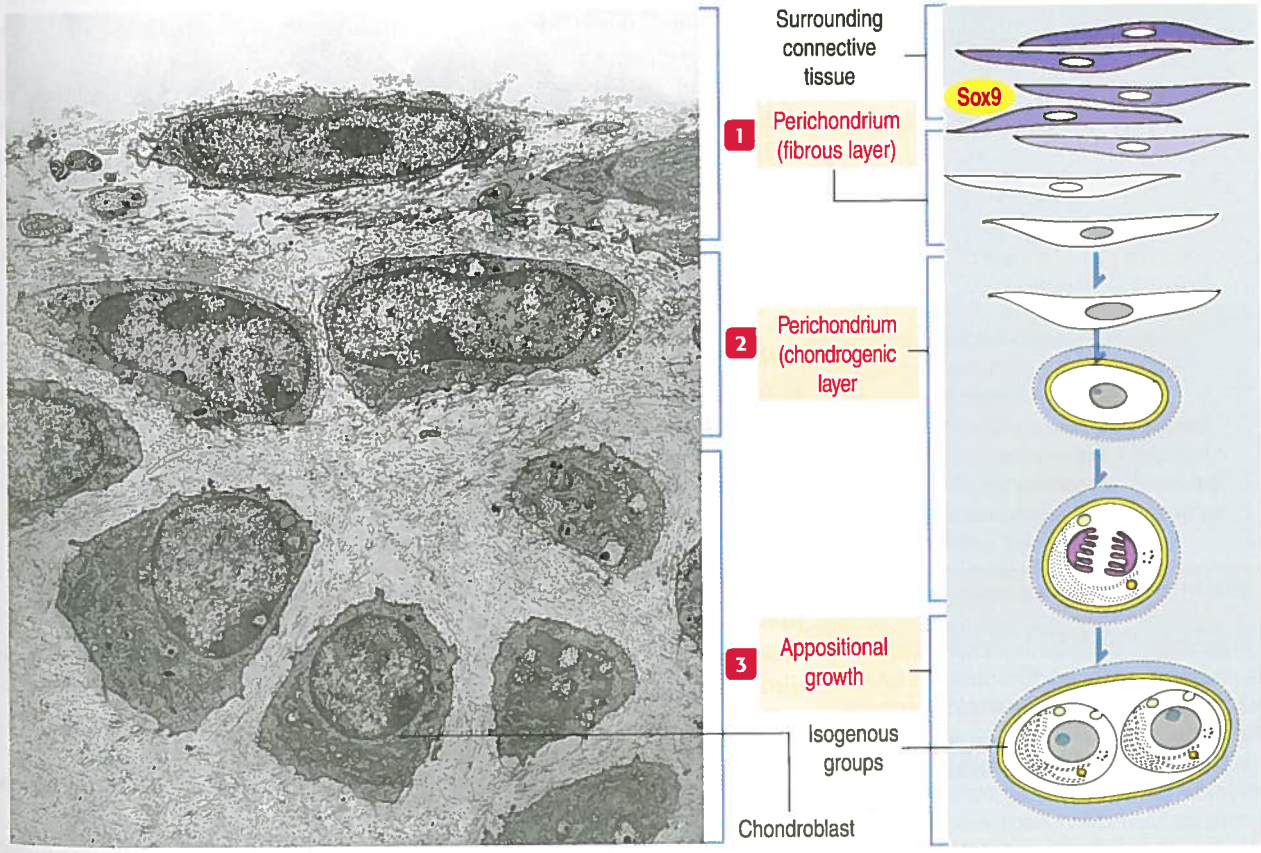
2 The **inner cells of the perichondrium**, the **chondrogenic layer**, differentiate into **chondroblasts**, which synthesize and secrete **type II collagen** precursors and other extracellular matrix components.

By this mechanism, new layers of cells and extracellular matrix are added to the surface of the cartilage by the process of **appositional growth**, and the overall size of the cartilage increases. This process increases the size of the initial **anlagen** (German *anlagen*, plan, outline) of the future skeleton.

A mutation in the gene expressing the **transcription factor Sox9** causes **campomelic dysplasia** in humans consisting in bowing and angulation of long bones, hypoplasia of the pelvic and scapular bones, abnormalities of the vertebral column, a decrease in the number of ribs, and craniofacial abnormalities. **Sox9 controls the expression of type II collagen and the proteoglycan aggrecan.**

Sox9-null chondrogenic cells remain in the perichondrium and do not differentiate into chondrocytes. Other members of the Sox family participate in chondrogenesis.

Sox9 participates in male sex determination (see Chapter 21, Sperm Transport and Maturation).



Box 4-G | Cartilage repair after injury

- The specialized extracellular matrix of hyaline cartilage has a dual role:
  1. It acts like a **shock absorber**, because of its stiffness and elasticity.
  2. It provides a **lubricated surface for movable joints**.

  The lubrication fluid (hyaluronic acid, immunoglobulins, lysosomal enzymes, collagenase in particular, and glycoproteins) is produced by the **synovial lining of the capsule of the joint**.
- The analysis of the **synovial fluid** is valuable in the diagnosis of joint disease.

(well-developed RER and Golgi apparatus, and large nucleolus) and store lipids and glycogen in the cytoplasm. Chondrocytes are coated by a pericellular matrix, surrounded by the territorial and interterritorial matrices, respectively. A lacunar rim separates the cell from the territorial matrix.

The surface of hyaline cartilage is covered by the **perichondrium**, a fibrocellular layer that is continuous with the periosteal cover of the bone and that blends into the surrounding connective tissue. **Articular cartilage lacks a perichondrium.**

The perichondrium consists of two layers:

1. An **outer fibrous layer**, which contains bundles of type I collagen and elastin.
2. An **inner layer**, called the **chondrogenic layer**, formed by flat chondrocytes aligned tangentially to the margin of the cartilage.

The ECM contains hyaluronic acid, proteoglycans (rich in the GAGs chondroitin sulfate and keratan sulfate), and a high water content (70% to 80% of its weight). **Aggrecan** is a large proteoglycan characteristic of cartilage (see Boxes 4-G and 4-H).

The **transcription factor Sox9** is required for expression of cartilage-specific ECM components such as type II collagen and the proteoglycan aggrecan. Sox9 activates the expression of collagen by the *COL2A1* gene. A lack of Sox9 expression prevents the chondrogenic layer to differentiate into chondrocytes. Mutations in the *Sox9* gene cause the rare and severe dwarfism **campomelic dysplasia** (Figure 4-17).

The structure of the **elastic cartilage** is similar to that of hyaline cartilage except that the ECM contains abundant **elastic fibers** synthesized by chondrocytes. Elastic cartilage is found in the auricle of the external ear, a major portion of the epiglottis, and some of the laryngeal cartilages. The specialized matrix of the cartilage has remarkable flexibility and the ability to regain its original shape after deformation.

Unlike hyaline cartilage, **fibrocartilage** is opaque, the matrix contains **type I collagen fibers**, the **ECM has a low concentration of proteoglycans and water**, and **it lacks a perichondrium.**

Fibrocartilage has great tensile strength and forms part of the intervertebral disk, pubic symphysis, and sites of insertion of tendon and ligament into bone.

The fibrocartilage is sometimes difficult to distinguish from dense regular connective tissue of some regions of tendons and ligaments. Fibrocartilage is distinguished by **characteristic chondrocytes within lacunae, forming short columns** (in contrast to flattened fibroblasts or fibrocytes lacking lacunae, surrounded by the dense connective tissue and ECM).

BONE

Bone is a rigid inflexible connective tissue in which the ECM has become impregnated with salts of calcium and phosphate by a process called mineralization. Bone is highly vascularized and metabolically very active.

The functions of bone are:

1. **Support and protection for the body and its organs**
2. **A reservoir for calcium and phosphate ions**

Box 4-H | How chondrocytes survive

- In **cartilage**, chondroblasts and chondrocytes are sustained by diffusion of nutrients and metabolites through **the aqueous phase of the extracellular matrix**.
- In **bone**, deposits of calcium salts in the matrix prevent the diffusion of soluble solutes, which thus must be transported from blood vessels to osteocytes through **canaliculi** (see Bone).

Macroscopic structure of mature bone

Based on its gross appearance (Figure 4-19), two forms of bone are distinguished:

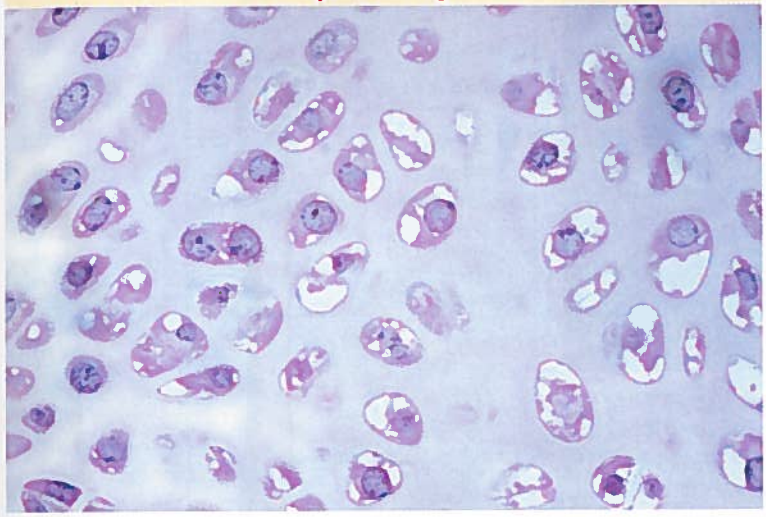
1. Compact or dense bone
2. Spongy, trabecular or cancellous bone

Compact bone appears as a solid mass. Spongy bone consists of a network of bony spicules or trabeculae delimiting spaces occupied by the bone marrow.

In long bones, such as the femur, the **shaft** or **diaphysis** consists of compact bone forming a hollow cylinder with a central marrow space, called the **medullary**

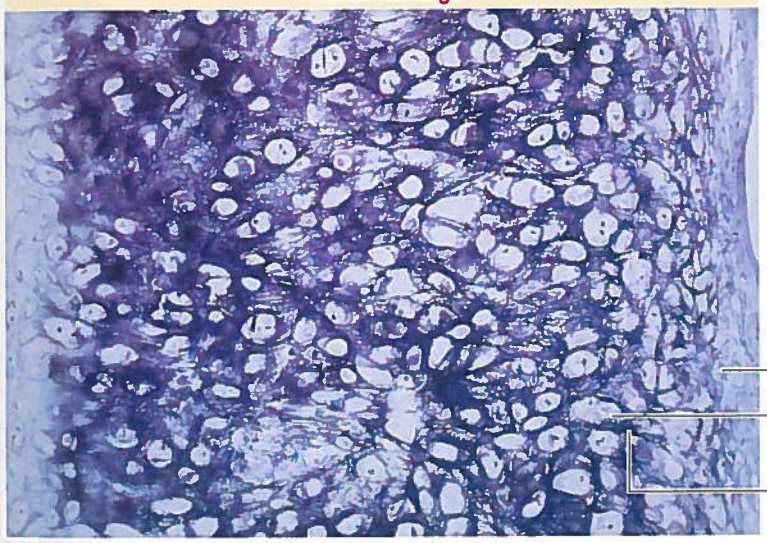
Figure 4-18. Types of cartilage

Hyaline cartilage



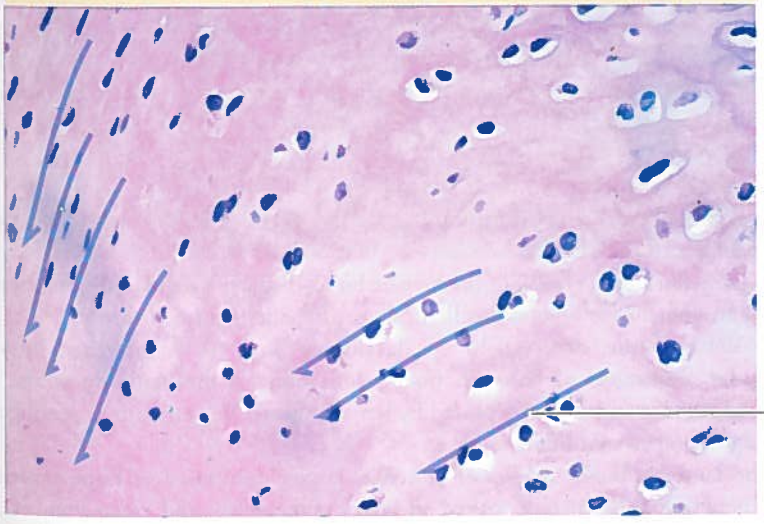
Hyaline cartilage has the following features:
It is **avascular**.
It is surrounded by **perichondrium** (except in articular cartilage). The perichondrium has an **outer fibrous layer**, an **inner chondrogenic layer**, and **blood vessels**.
It consists of chondrocytes surrounded by territorial and interterritorial matrices containing **type II collagen** interacting with proteoglycans.
It occurs in the **temporary skeleton of the embryo**, **articular cartilage**, and the **cartilage of the respiratory tract** (nose, larynx, trachea, and bronchi) and costal cartilages.

Elastic cartilage



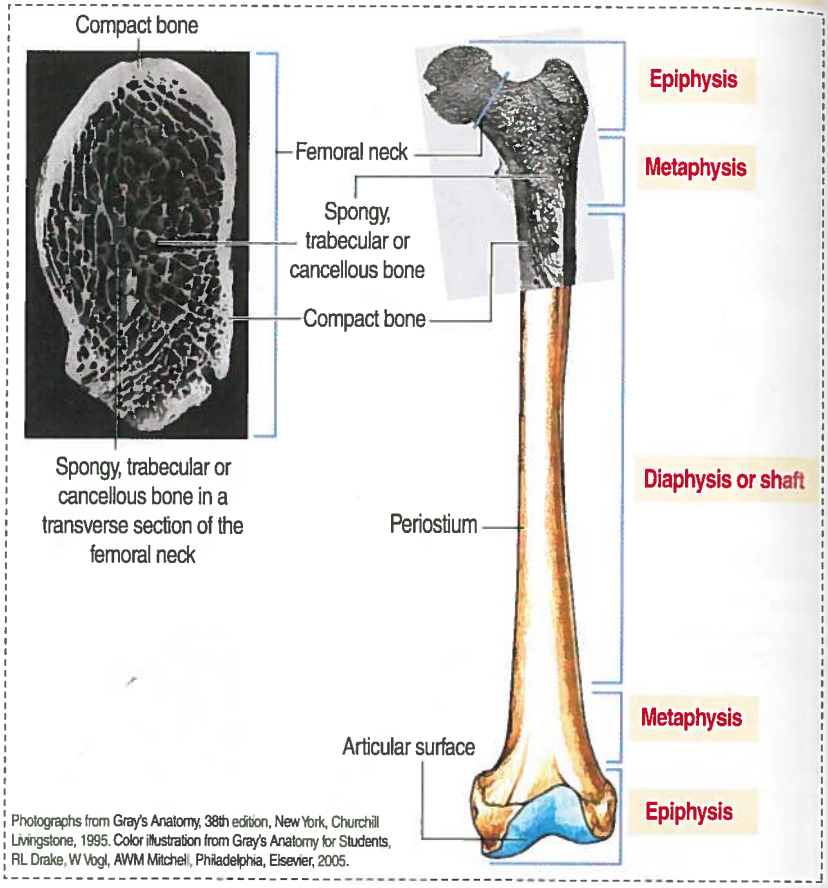
Elastic cartilage has the following features:
It is **avascular**.
It is surrounded by **perichondrium**.
It consists of chondrocytes surrounded by territorial and interterritorial matrices containing **type II collagen** interacting with proteoglycans and **elastic fibers**, which can be stained by **orcein** for light microscopy.
It occurs in the **external ear**, **epiglottis**, and **auditory tube**.

Fibrocartilage



Fibrocartilage has the following features:
It is generally **avascular**.
It **lacks a perichondrium**.
It consists of **chondrocytes** and **fibroblasts** surrounded by **type I collagen** and a less rigid extracellular matrix. Fibrocartilage is considered an intermediate tissue between hyaline cartilage and dense connective tissue.
It predominates in the **intervertebral disks**, **articular disks of the knee**, **mandible**, **sternoclavicular joints**, and **pubic symphysis**.

Figure 4-19. General architecture of a long bone



Photographs from Gray's Anatomy, 38th edition, New York, Churchill Livingstone, 1995. Color illustration from Gray's Anatomy for Students, RL Drake, W Vogl, AWM Mitchell, Philadelphia, Elsevier, 2005.

or **marrow cavity**.

The ends of the long bones, called **epiphyses**, consist of spongy bone covered by a thin layer of compact bone. In the growing individual, epiphyses are separated from the diaphysis by a cartilaginous **epiphyseal plate**, connected to the diaphysis by spongy bone. A tapering transitional region, called the **metaphysis**, connects the epiphysis and the diaphysis. Both the epiphyseal plate and adjacent spongy bone represent the growth zone, responsible for the increase in length of the growing bone.

The **articular surfaces**, at the ends of the long bones, are covered by hyaline cartilage, the **articular cartilage**. Except on the articular surfaces and at the insertion sites of tendons and ligaments, most bones are surrounded by the **periosteum**, a layer of specialized connective tissue with osteogenic potential.

The marrow cavity of the diaphysis and the spaces within spongy bone are lined by **endosteum**, also with osteogenic potential.

Microscopic structure of mature bone

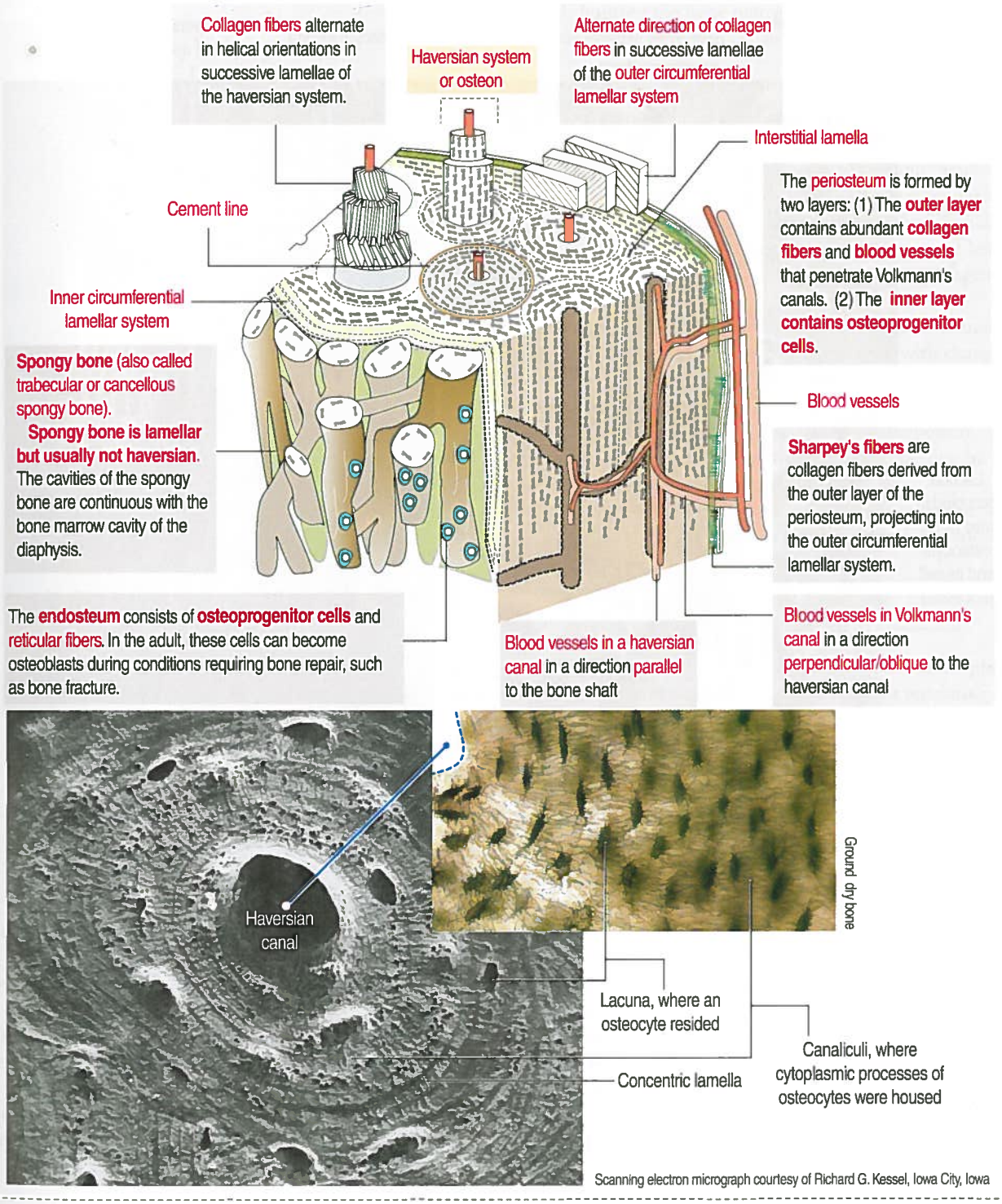
Two types of bone are identified on the basis of the microscopic organization of the collagen fibers:

1. **Lamellar bone**, typical of the mature bone, displays a **regular alignment of collagen fibers**. This bone is mechanically strong and is formed slowly.

2. **Woven bone**, observed in the developing bone, is characterized by an **irregular alignment of collagen fibers**. This bone is mechanically weak, is formed rapidly, and is then replaced by lamellar bone. Woven bone is produced during the repair of a bone fracture.

The **lamellar bone** consists of **lamellae**, largely composed of **bone matrix**, a mineralized substance deposited in layers or lamellae, and osteocytes, each one occupying a cavity or **lacuna** with radiating and branching **canaliculi** that

Figure 4-20. Haversian system or osteon

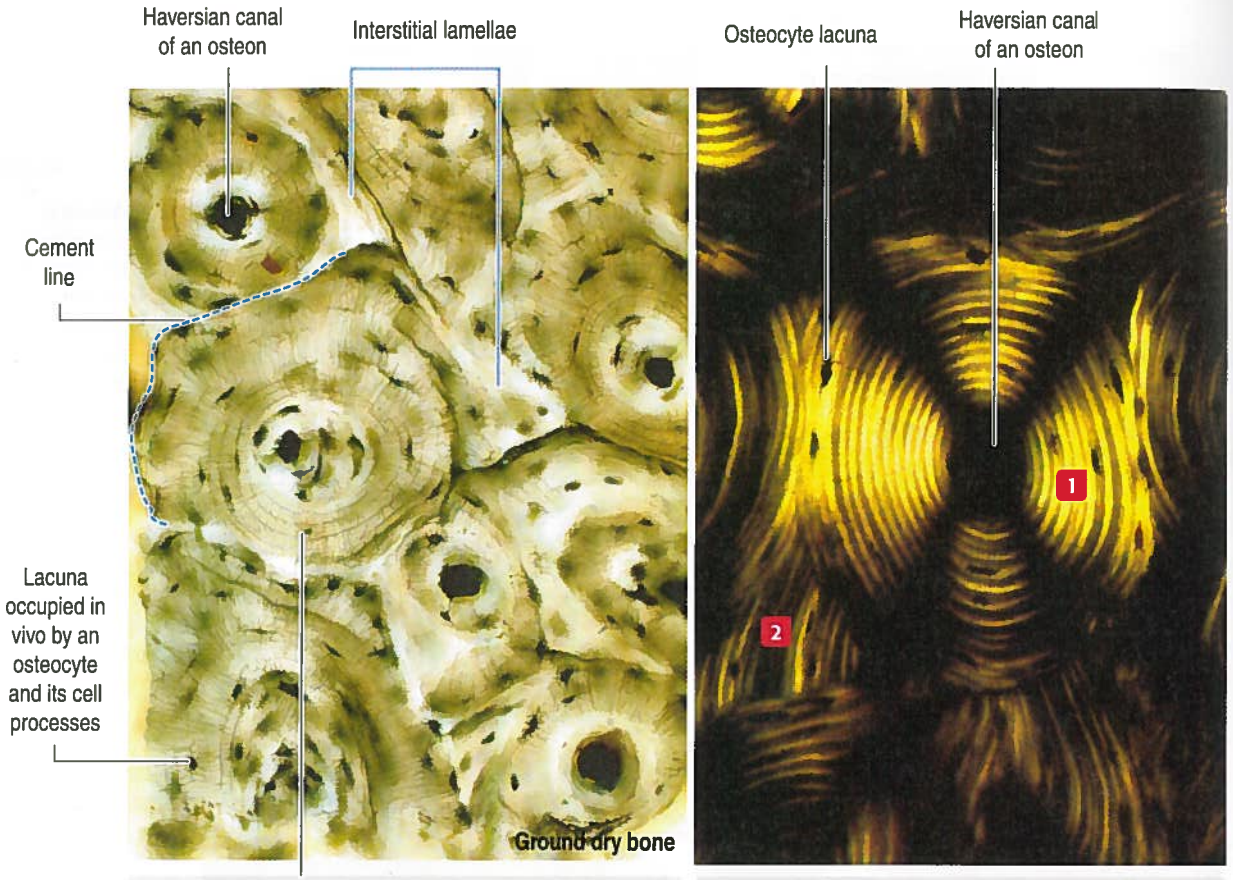


Scanning electron micrograph courtesy of Richard G. Kessel, Iowa City, Iowa

penetrate the lamellae of adjacent lacunae. The lamellar bone displays four distinct patterns (Figure 4-20):

1. The **osteons** or **haversian systems**, formed by concentrically arranged lamellae around a longitudinal vascular channel.
2. The **interstitial lamellae**, observed between osteons and separated from them by a thin layer known as the **cement line**.
3. The **outer circumferential lamellae**, visualized at the external surface of the compact bone under the periosteum.

Figure 4-21. Organization of compact bone: Osteon



Concentric array of lamellar bone
Osteocytes are concentrically arranged between lamellae.
Osteocytes of adjacent lamellae are interconnected by cell processes lodged in canaliculi.

Array of lamellar bone visualized by polarized light.
Note:
1 The concentric array of the lamellae.
2 The banding distribution of interstitial lamellae.

4. The **inner circumferential lamellae**, seen on the internal surface subjacent to the endosteum.

The **vascular channels** in compact bone have two orientations with respect to the lamellar structures:

1. The longitudinal capillaries and postcapillary venules, running in the center of the osteon within a space known as the **haversian canal** (Figures 4-20 to 4-22).

2. The haversian canals are connected with one another by transverse or oblique canals known as **Volkmann's canals**, containing blood vessels from the marrow and some from the periosteum.

Periosteum and endosteum

During embryonic and postnatal growth, the periosteum consists of an **inner layer of bone-forming cells (osteoblasts)** in direct contact with the bone. The inner layer is the **osteogenic layer**. In the adult, the periosteum contains inactive connective tissue cells that retain their osteogenic potential in case of bone injury and repair.

The **outer layer** is rich in blood vessels, some of them entering Volkmann's canals, and thick anchoring collagen fibers, called **Sharpey's fibers**, that penetrate

the outer circumferential lamellae deep in the bone (see Figure 4-20).

The **endosteum** consists of squamous cells and connective tissue fibers covering the spongy walls housing the bone marrow and extending into all the cavities of the bone, including the haversian canals.

Bone matrix

The **bone matrix** consists of organic (35%) and inorganic (65%) components. The organic bone matrix contains **type I collagen fibers** (90%); **proteoglycans**, enriched in **chondroitin sulfate**, **keratan sulfate**, and **hyaluronic acid**; and **noncollagenous proteins**. The **inorganic component of the bone** is represented predominantly by deposits of **calcium phosphate** with the crystalline characteristics of **hydroxyapatite**. The crystals are distributed along the length of collagen fibers through an assembly process assisted by noncollagenous proteins.

Type I collagen is the predominant protein of the bone matrix. In mature lamellar bone, collagen fibers have a highly ordered arrangement with changing orientations with respect to the axis of the haversian canal in successive concentric lamellae (see Figure 4-20).

Noncollagenous matrix proteins include **osteocalcin**, **osteopontin**, and **osteonectin**, synthesized by osteoblasts and with unique properties in the mineralization of bone.

Osteocalcin and osteopontin synthesis increases following stimulation with the active vitamin D metabolite, 1α,25-dihydroxycholecalciferol. Osteocalcin inhibits osteoblast function.

Osteonectin is not exclusively an osteoblast product and is present in tissues undergoing remodeling and morphogenesis.

Bone sialoprotein is also a bone matrix component.

As we later discuss in greater detail, **osteoprotegerin**, **RANKL**, and **macrophage colony-stimulating factor** are products of osteoblasts required for regulating the differentiation of osteoclasts.

Cellular components of bone

Actively growing bone contains cells of two different lineages:

1. The **osteoblast lineage**, which includes the osteoprogenitor cells and derived osteoblasts and osteocytes
2. The **osteoclast lineage**

Osteoprogenitor cells are of **mesenchymal origin** and have the properties of **stem cells**: the **potential for proliferation** and a **capacity to differentiate**. Osteoprogenitor cells give rise to osteoblasts by a regulatory mechanism involving growth and transcription factors and are present in the inner layer of the periosteum and the endosteum. Osteoprogenitor cells persist throughout postnatal life as bone-lining cells; they are reactivated in the adult during the repair of bone fractures and other injuries.

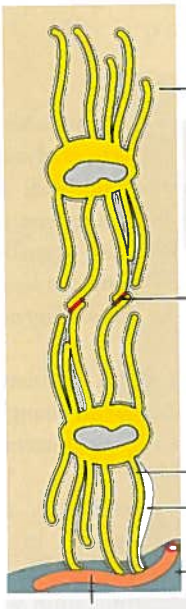
Osteoblasts differentiate into osteocytes after they are trapped inside lacunae within the mineralized matrix they produce. Their differentiation involves the participation of two transcription factors: Cbfa1/Runx2 and osterix (see Box 4-I).

The **osteoclast** lineage derives from the **monocyte-macrophage lineage** in the bone marrow.

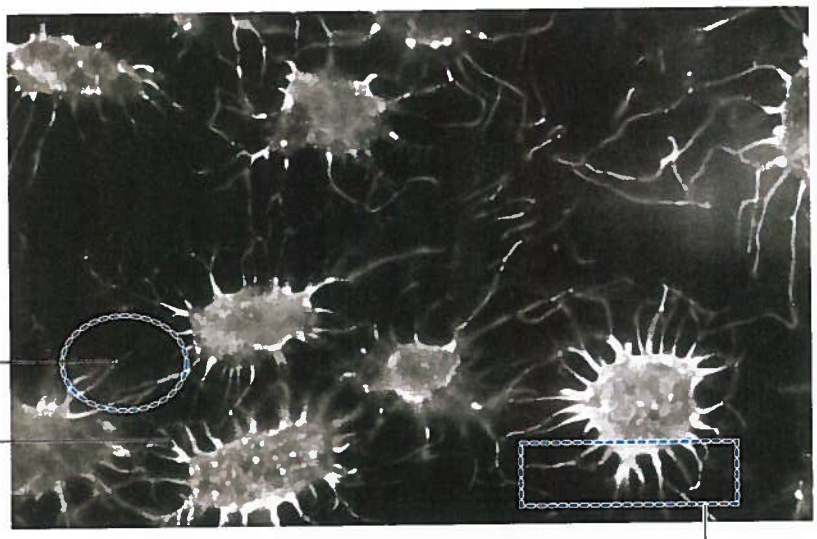
Osteoblasts and osteocytes

Osteoblasts are epithelial-like cells with cuboidal or columnar shapes, forming a monolayer covering all sites of active bone formation. Osteoblasts are highly polarized cells: they deposit **osteoid**, the **nonmineralized organic matrix of the bone**, along the osteoblast-bone interface. Osteoblasts initiate and control the subsequent mineralization of the osteoid.

Figure 4-22. Osteocytes are connected to each other by cell processes



Gap junctions are present at the contact points of osteocyte processes



Photograph from: Gray's Anatomy, 38th edition, New York, Churchill Livingstone, 1995.

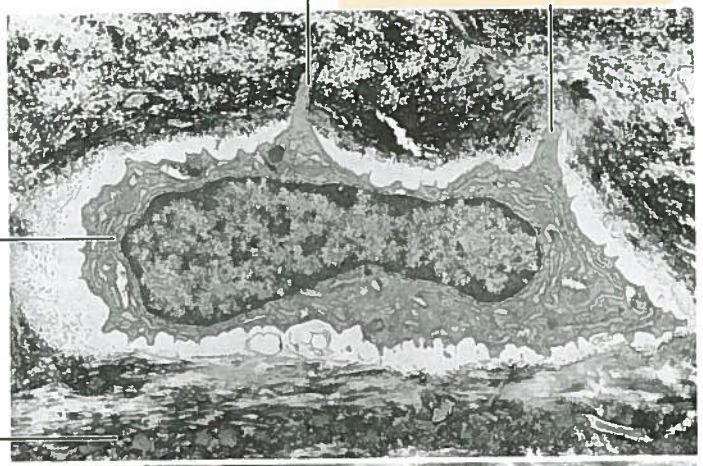
Cell processes are embedded within canaliculi, spaces surrounded by mineralized bone. Extracellular fluid within the lumen of the canaliculi transports molecules by passive diffusion.

A **blood vessel** within the haversian canal provides nutrients to osteocytes.

Nutrients are transported through a chain of cell processes away from the haversian canal, toward osteocytes located far from the canal.

The transport of the canalicular system is limited to a distance of about 100 µm.

Cell processes entering canaliculi

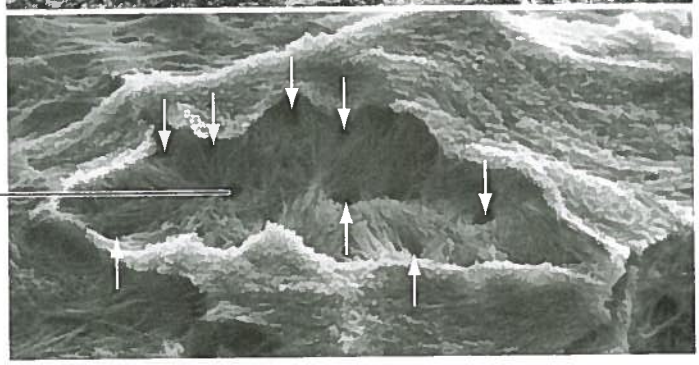


Electron micrograph courtesy from Patricia C. Cross, Stanford, California.

An **osteocyte**, trapped in the calcified matrix, occupies a space or lacuna.

Osteocytes are responsible for maintenance and turnover of the bone matrix.

Calcified bone matrix

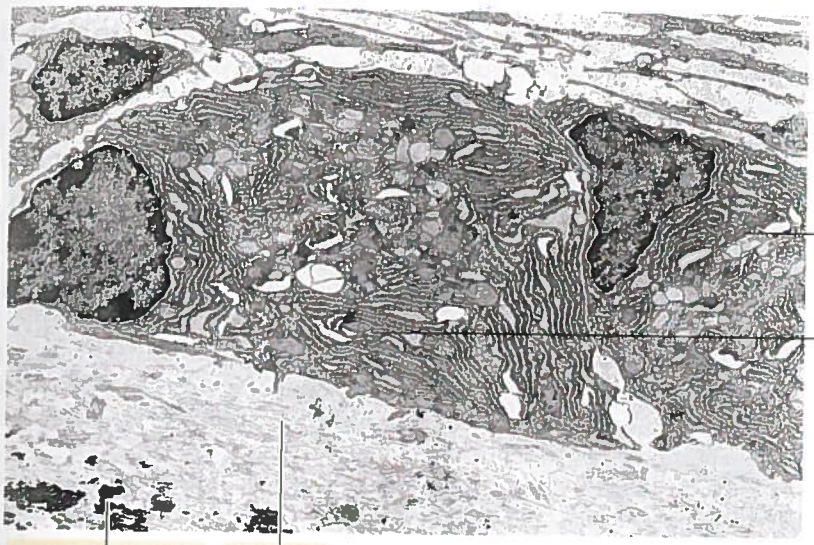


Scanning electron micrograph from: Gray's Anatomy, 38th edition, New York, Churchill Livingstone, 1995.

The wall of an **osteocyte lacuna** shows several openings of canaliculi (arrows) occupied in vivo by cell processes of an osteocyte housed in the space surrounded by calcified bone matrix.

In electron micrographs, osteoblasts display the typical features of cells actively engaged in protein synthesis, glycosylation, and secretion. Their specific products are **type I collagen, osteocalcin, osteopontin,** and **bone sialoprotein** (Figure 4-23). Osteoblasts give a strong cytochemical reaction for **alkaline phosphatase**

Figure 4-23. Function of the osteoblast



Electron micrograph courtesy of Patricia C. Cross, Stanford, California

Pour en savoir plus!

Osteoblasts derive from osteoprogenitor cells. Osteocytes are the most mature or terminally differentiated cells of the osteoblastic lineage.

Osteoblasts synthesize the organic matrix of bone, the **osteoid**, and control the mineralization of the matrix.

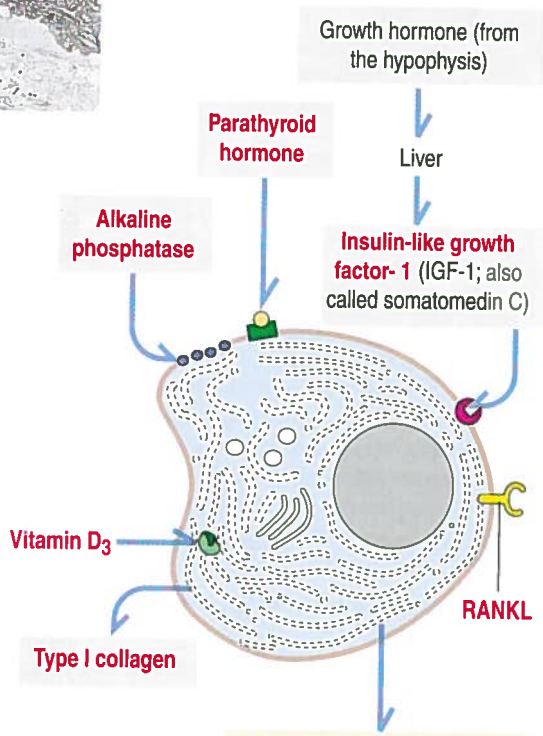
Alkaline phosphatase is an **ectoenzyme** (a cell surface protein) that hydrolyzes monophosphate esters at high pH. This enzyme disappears when the osteoblast ceases protein synthesis and becomes embedded in the mineralized bone matrix as an osteocyte.

Vitamin D_3 (1α,25-dihydroxycholecalciferol) regulates the expression of **osteocalcin**, a protein with high binding affinity for hydroxyapatite.

Growth hormone stimulates the production of **IGF-1** in hepatocytes. IGF-1 stimulates the growth of long bones at the level of the epiphyseal plates.

The major protein products of an osteoblast are:

1. **Type 1 collagen**. Osteoid consists of type I collagen and proteoglycans. As a typical protein-producing cell, the osteoblast has a well-developed rough endoplasmic reticulum.
2. Several **noncollagenous proteins**. They include: **RANKL**, the ligand for receptor for activation of nuclear factor kappa B (RANK)—present in osteoclast precursor cells; **osteocalcin**—required for bone mineralization; **osteopontin**—to mediate the formation of the sealing zone; **bone sialoprotein**—to mediate binding of osteoblasts to the extracellular matrix through integrins.



that disappears when the cells become embedded in the matrix as osteocytes. In addition, osteoblasts produce growth factors, in particular members of the **bone morphogenetic protein family**, with bone-inductive activities.

When bone formation is completed, osteoblasts flatten out and transform into osteocytes. Osteocytes are highly branched cells with their body occupying small spaces between lamellae, called **lacunae**. Small channels, the **canaliculi**, course through the lamellae and interconnect neighboring lacunae. Adjacent cell processes, found within canaliculi, are connected by **gap junctions** (see Figure 4-22). Nutrient materials diffuse from a neighboring blood vessel, within the haversian canal, through the canaliculi into the lacunae. As you can see, the dense network of osteocytes depends not only on intracellular communication

across gap junctions but also on the mobilization of nutrients and signaling molecules along the extracellular environment facilitated by canaliculi running from lacuna to lacuna.

The life of an osteocyte depends on this nutrient diffusion process and the life of the bone matrix depends on the osteocyte. Osteocytes can remain alive for years provided vascularization is continuous.

In compact bone, 4 to 20 lamellae are concentrically arranged around the haversian canal; they contain a blood vessel, either a capillary or a postcapillary venule.

Pour en savoir plus!

Clinical significance: Osteoblast to osteocyte differentiation

A pluripotent mesenchymal cell is the precursor of osteblasts as well as muscle cells, adipocytes, fibroblasts, and chondroblasts.

The differentiation of the osteoblast is controlled by growth and transcription factors. Several members of the **bone morphogenetic protein (BMP) family** and **transforming growth factor–β** can regulate the embryonic development and differentiation of the osteoblast.

Osteoblast-specific genes modulate the differentiation of the osteoblast progeny (Figure 4-24): ***Cbfa1/Runx2*** (a member of the core-binding factor family) encodes a **transcription factor** that induces the differentiation of osteoblasts and controls the expression of osteocalcin. Cbfa1/Runx2 is the earliest and most specific indicator of osteogenesis and its expression is induced by BMP7, followed by the expression of osteocalcin and osteopontin. **Osteocalcin** is a specific secretory protein expressed only in terminally differentiated osteoblasts under the control of Cbfa1/Runx2 (see Box 4-I).

Cbfa1/Runx2-deficient mice develop to term and have a skeleton consisting of cartilage. There is no indication of osteoblast differentiation or bone formation in these mice. In addition, Cbfa1/Runx2-deficient mice lack osteoclasts. As we will discuss soon, osteoblasts produce proteins that regulate the formation of osteoclasts.

Consistent with the skeletal observations in the Cbfa1/Runx2-deficient mice is a condition in humans known as **cleidocranial dysplasia** (CCD). CCD is characterized by hypoplastic clavicles, delayed ossification of sutures of certain skull bones, and mutations in the *Cbfa1/Runx2* gene.

Leptin, a peptide synthesized by **adipocytes** with binding affinity to its receptor in the hypothalamus, regulates bone formation by a central mechanism. Although details of the leptin-hypothalamic control mechanism are unknown, mice deficient in leptin or its receptor have a considerably higher bone mass than wild-type mice. In fact, patients with generalized **lipodystrophy** (absence of adipocytes and white fat) exhibit **osteosclerosis** (increased bone hardening) and accelerated bone growth.

Box 4-I | **How osteocytes differentiate**

- The osteoblast to osteocyte differentiation process requires the activation of two transcription factors: **Cbfa1/Runx2** (for core binding factor a1/runt homeodomain protein 2) and **osterix**.
- We have already seen that chondrogenesis involves the transcription factor **Sox9** (see Figure 4-17). We discuss in Chapter 5, Ossification, that Cbfa1/Runx2 controls the conversion of proliferating chondrocytes to hypertrophic chondrocytes, an event that is prevented by Sox9.
- The transcription factors Sox9, Cbfa1/Runx2, and osterix (the latter specific for osteoblast to osteocyte differentiation) play critical roles in the development of the skeleton.
- Mutations in genes encoding these transcription factors are the genetic basis of skeletal diseases. For example, a total lack of expression of the *Cbfa1/Runx2* gene determines that the entire skeleton consists only of cartilage.

Osteoclasts

Pour en savoir plus!

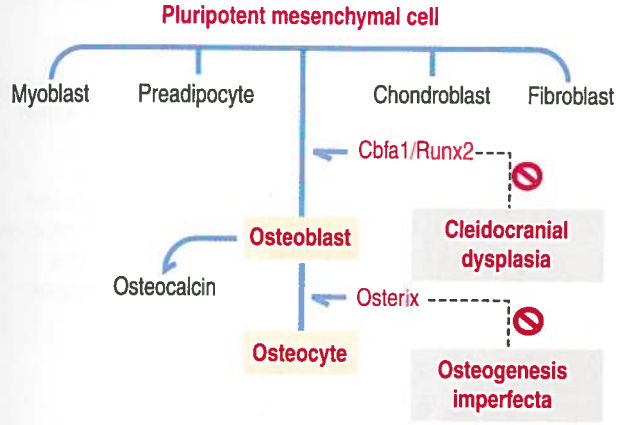
Osteoclasts do not belong to the osteoprogenitor cell lineage. Instead, osteoclasts derive from the **monocyte-macrophage progenitor cell lineage** in the bone marrow, which diverges into the **osteoclast progenitor pathway**.

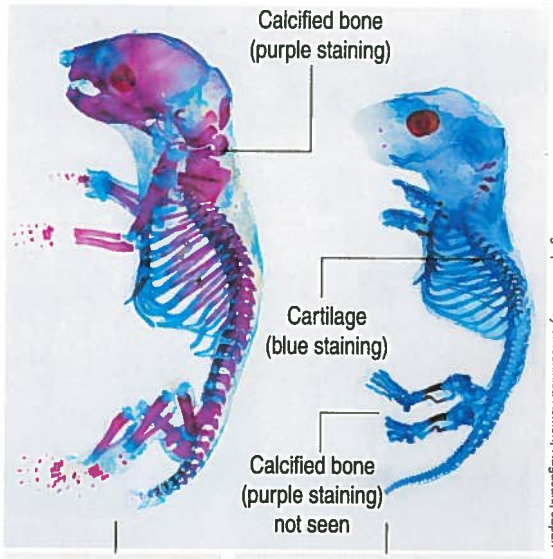
The osteoclast precursor cells are **monocytes**, which reach the bone through the blood circulation and fuse into multinucleated cells with as many as 30 nuclei to form osteoclasts by a process regulated by osteoblasts and stromal cells of the bone marrow.

After attachment to the target bone matrix, osteoclasts generate a secluded acidic environment required for bone resorption. Bone resorption involves first the dissolution of the inorganic components of the bone (**bone demineralization**) mediated by H^+-ATPase (adenosine triphosphatase) within an acidic environment, followed by enzymatic degradation of the organic matrix (type I collagen and noncollagenous proteins) by the protease cathepsin K.

Osteoclasts play an essential role in **bone remodeling and renewal**. This process

Figure 4-24. Osteoblast differentiation





Photograph courtesy of Toshihisa Komori, Nagasaki, Japan

Clinical significance: Transcriptional control of osteoblast differentiation

Osteoblasts derive from a pluripotent mesenchymal cell that gives rise to muscle cells, adipocytes, fibroblasts, and chondroblasts.

Two osteoblast specific genes control the differentiation of the osteoblast progeny: ***Cbfa1/Runx2*** (for core-binding factor family/runt homeodomain protein 2)—encoding a **transcription factor** that induces the differentiation of osteoblasts and controls the expression of osteocalcin, a specific secretory protein expressed only in terminally differentiated osteocytes.

Cbfa1/Runx2-deficient mice have a skeleton consisting of cartilage without any indication of osteoblast differentiation represented by bone formation and mineralization. In addition, because osteoblasts regulate the formation of osteoclasts, Cbfa1/Runx2-deficient mice lack osteoclasts.

Patients with **cleidocranial dysplasia** (hypoplastic clavicles and delayed ossification of sutures of certain skull bones) have a *Cbfa1/Runx2* type of gene mutation. Osterix-deficient patients have "brittle bone disease" (**osteogenesis imperfecta**).

involves removal of bone matrix at several sites, followed by its replacement with new bone by osteoblasts.

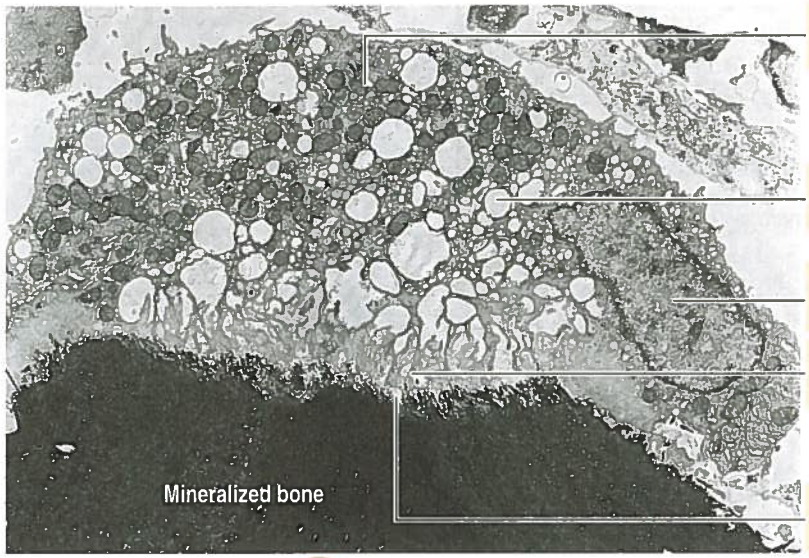
The osteoclast is a large (up to 100 μm in diameter) and highly polarized cell that occupies a shallow concavity called **Howship's lacuna** or the **subosteoclastic compartment** (Figures 4-25 and 4-26).

The cytoplasm of the osteoclast is very **rich in mitochondria** and **acidified vesicles**. The membrane of the acidified vesicles contains H^+-ATPase; mitochondria are the source of adenosine triphosphate (ATP) to drive the H^+-ATPase pumps required for the **acidification of the subosteoclastic compartment** for the subsequent **activation of the enzyme cathepsin K**. Cathepsin K breaks down the bone organic matrix following removal of the mineral component of bone. Figure 4-26 provides a step-by-step sequence of the activation of an osteoclast. We discuss in Chapter 15, Upper Digestive Segment, that the mechanism of production of HCl in the stomach is very similar to the acidification of Howship's lacuna.

The cell domain facing the lacuna has deep infoldings of the cell membrane, the **ruffled border**. When the cell is not active, the ruffled border disappears and the osteoclast enters into a resting phase. Around the circumference of the ruffled border—at the point where the cell membrane is closely applied to the bone just at the margins of the lacuna—**actin filaments** accumulate and participate, together with $\alpha_v\beta_3$ integrin, to form a **sealing zone**. The sealing zone seals off the bone resorption lacuna.

Osteoclasts are transiently active in response to a metabolic demand for the

Figure 4-25. Function of the osteoclast

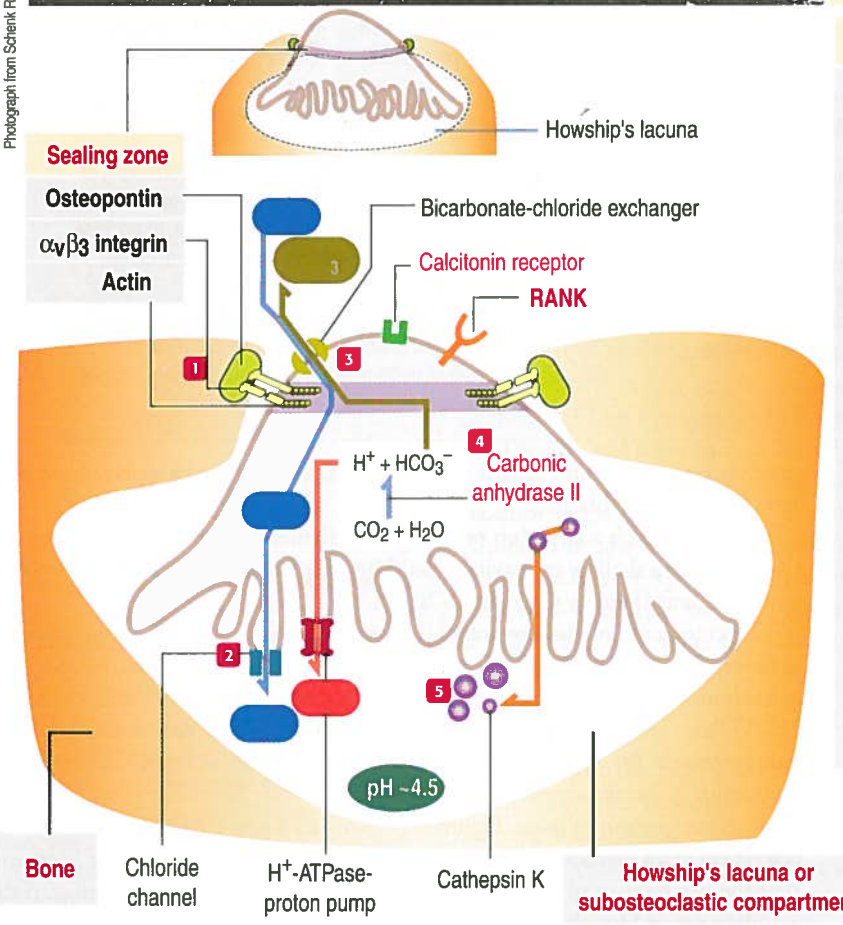


The osteoclast is a highly polarized cell associated with a shallow concavity, **Howship's lacuna** or the **subosteoclastic compartment**.

The active surface facing the lacuna displays a **ruffled border**.

Osteoclasts are multinucleated cells and contain abundant mitochondria and acidified vesicles (containing electrogenic H^+-ATPase).

Photograph from Schenk RK, Felix R, Hotsetter W: Connective Tissue and its Heritable Disorders. New York, Wiley-Liss, 1993.



Osteoclast

1. Around the circumference of the ruffled border, where the plasma membrane is closely applied to the bone, **actin filaments** accumulate to form a **sealing zone**, together with **$\alpha_v\beta_3$ integrin** and **osteopontin**.
2. A chloride channel prevents an excessive rise of intracellular pH.
3. Bicarbonate (HCO_3^-) is exchanged for chloride (Cl^-), which is then transported by the chloride channel (located in the ruffled membrane) to Howship's lacuna. A bicarbonate-chloride exchanger ensures the maintenance of cytoplasmic electroneutrality.
4. **Carbonic anhydrase II** generates protons (H^+) from CO_2 and H_2O. H^+ is released into Howship's lacuna by an **H^+-ATPase pump** to create an acidic environment (pH ~4.5) for solubilizing mineralized bone.
5. **Cathepsin K** is released into Howship's lacuna to degrade the exposed organic matrix (collagen and noncollagenous proteins) following solubilization of minerals by acidification.

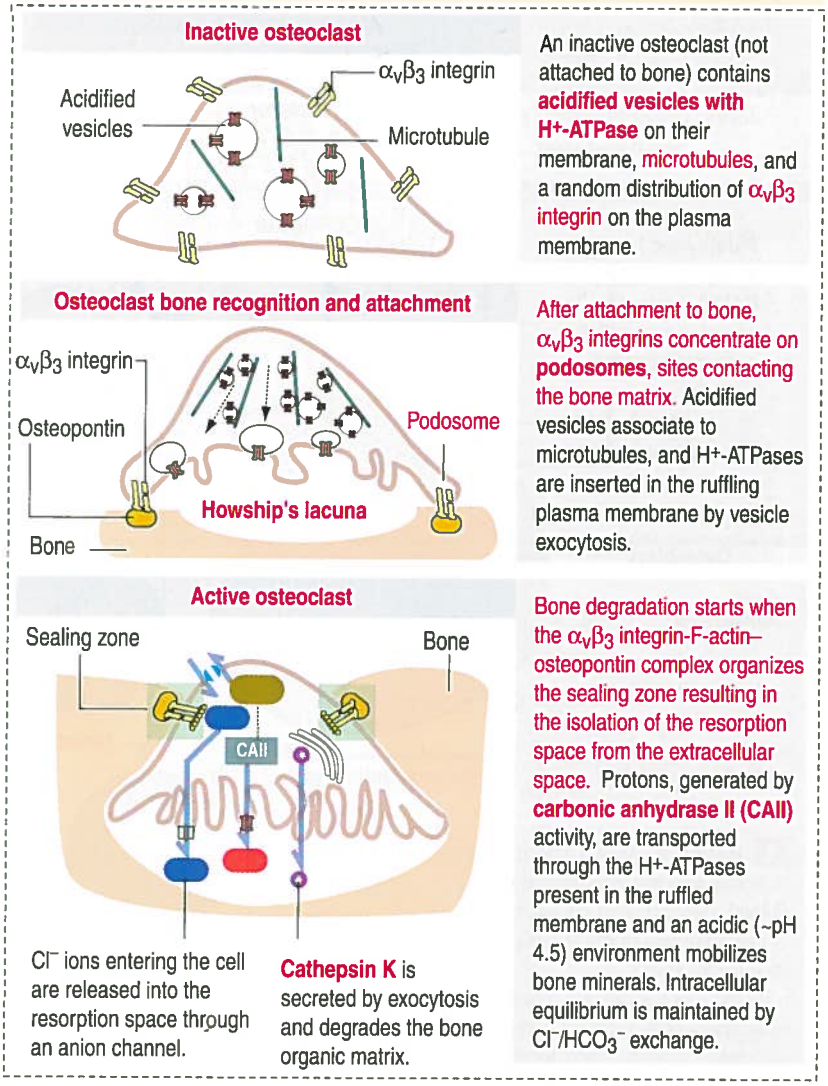
mobilization of calcium from bone into blood. Osteoclast activity is directly regulated by **calcitonin** (synthesized by neural crest derived parafollicular or C cells of the thyroid follicle), **vitamin D_3**, and regulatory molecules produced by osteoblasts and stromal cells of the bone marrow (see Osteoclastogenesis).

Pour en savoir plus!

Osteoclastogenesis (osteoclast differentiation)

Osteoclastogenesis is triggered by two relevant molecules produced by the osteoblast: (1) **macrophage colony-stimulating factor** (M-CSF), and (2) **nuclear**

Figure 4-26. Osteoclast differentiation



factor kappa B (NF-κB) ligand (RANKL).

The osteoclast precursor, the monocyte, responds to M-CSF, a secretory product of osteoblasts. M-CSF is required for the survival and proliferation of the osteoclast precursor (Figure 4-27). Its role was established by studies of the *op/op* mouse, which does not express M-CSF, lacks osteoclasts, and has an increase in bone mass (**osteopetrosis;** Greek *osteon*, bone; *petra*, stone; *osis*, condition). In humans, **osteopetrosis is characterized by high-density bone due to absent osteoclastic activity.** In long bones, this condition leads to the **occlusion of marrow spaces** and to **anemia.**

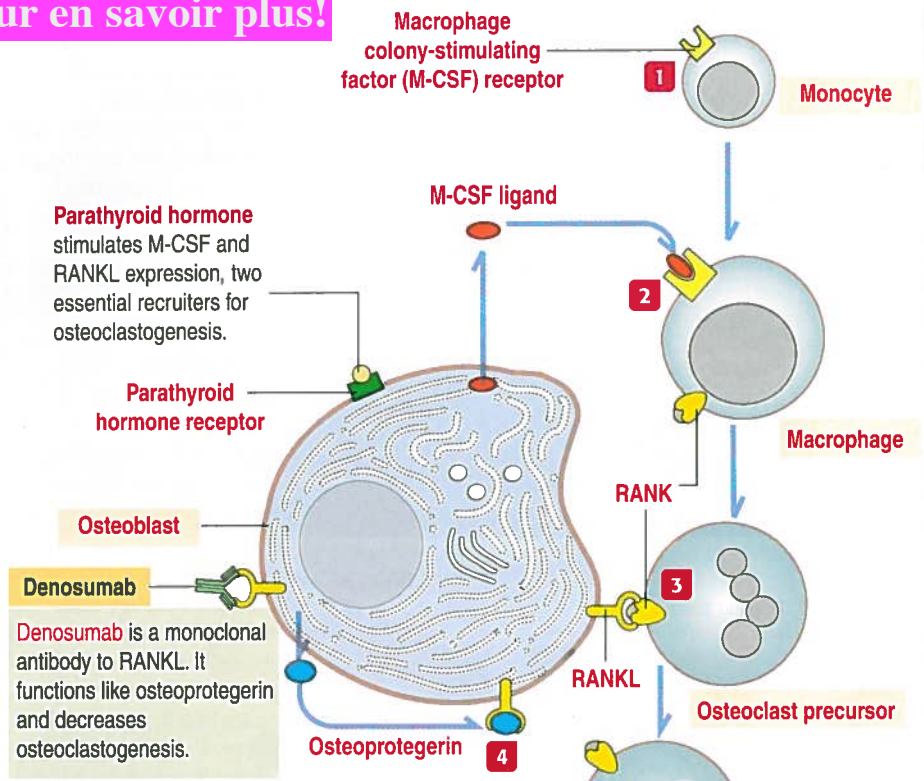
Both **osteoblasts** and **stromal cells of the bone marrow** produce **RANKL,** a member of the **tumor necrosis factor (TNF) superfamily.** RANKL binds to **RANK receptor** present on the surface of differentiating osteoclasts. RANKL binding leads to RANK trimerization and the recruitment of an adaptor molecule called **TRAF6** (for TNF receptor–associated factor 6).

TRAF6 stimulates a downstream signaling cascade, including the nuclear relocation of two transcription factors: **NF-κB** and **NFATc1 (for nuclear factor–activated T cells c1).** In the nucleus, these two transcription factors activate genes leading to osteoclast differentiation. We discuss in Chapter 3, Cell Signaling (see Figure 3-8), that NF-κB is a critical transcription factor heterodimer activated in response to inflammatory or immunologic signaling.

TRAF6 also interacts with c-Src to stimulate a pathway leading to cytoskeletal

Figure 4-27. Osteoblasts regulate osteoclastogenesis

Pour en savoir plus!



1 A **monocyte**, derived from bone marrow, reaches an area of bone formation and remodeling. A receptor for **M-CSF** is expressed on its surface.

2 The monocyte becomes a **macrophage**. **M-CSF ligand** binds to the M-CSF receptor and induces the expression of **RANK** (transmembrane receptor for activation of nuclear factor kappa B) for its **ligand (RANKL)** expressed on the surface of osteoblasts.

3 The osteoblast-expressed transmembrane protein ligand **RANKL** binds to the osteoclast RANK (receptor) and commits the cell to osteoclastogenesis.

The mononucleated monocyte becomes a multinucleated **osteoclast precursor**, which still cannot reabsorb bone.

4 RANKL-stimulated osteoclastogenesis is inhibited by the osteoblast-derived RANKL decoy protein **osteoprotegerin**. Osteoprotegerin blocks RANKL binding to its receptor RANK.

By this mechanism, the osteoblast (more specifically, osteoprotegerin) regulates the population of functional osteoclasts. Note that osteoblasts control osteoclast differentiation, not function.

5 A resting (nonfunctional) osteoclast uncouples from the osteoblast.

6 The maturation of osteoclasts is completed when the sealing zone and ruffled border appear. The formation of the sealing zone requires $\alpha_v\beta_3$ integrin.

reorganization and prevention of apoptosis. Figure 4-28 summarizes the relevant signaling steps following RANKL binding to RANK.

The interaction of the RANK receptor on osteoclast precursor cells with RANKL, exposed on the surface of osteoblasts, determines cell-cell contact required for further maturation of the osteoclast precursor.

Osteoblasts synthesize **osteoprotegerin**, a protein with high binding affinity for RANKL. Osteoprotegerin is a soluble "decoy" protein that binds to RANKL and prevents RANK-RANKL interaction. Consequently, **osteoprotegerin modulates the osteoclastogenic process.**

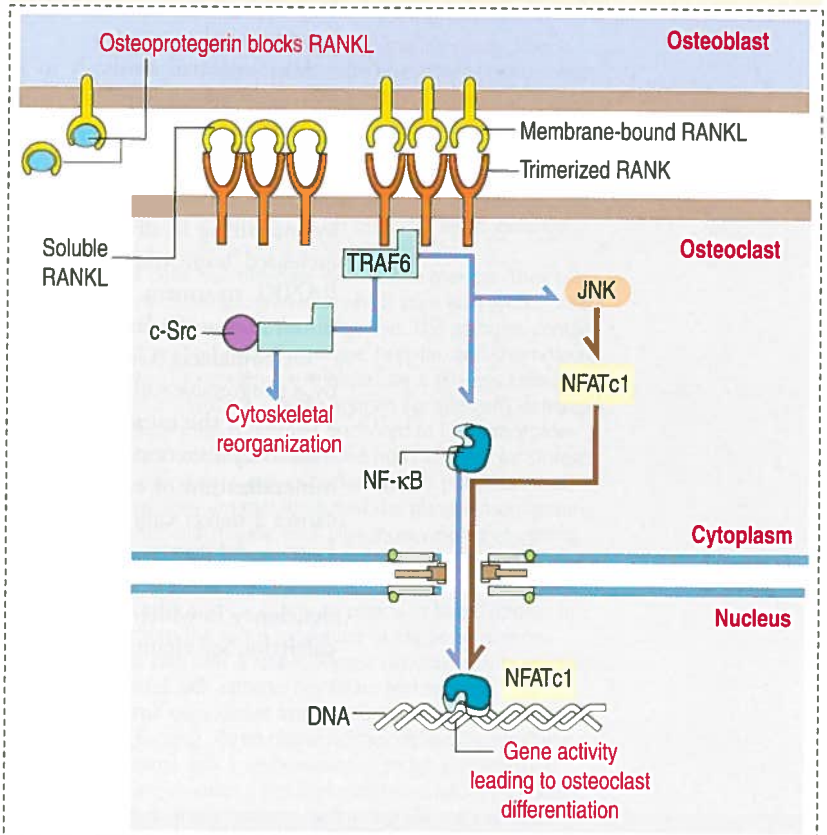
Parathyroid hormone stimulates the expression of osteoclastogenic RANKL. By this mechanism, the pool of RANKL increases relative to osteoprotegerin. An excess of parathyroid hormone enhances osteoclastogenesis (see Chapter 19, Endocrine System).

Denosumab-induced inhibition of RANKL in hyperparathyroidism prevents bone loss caused by excessive production of parathyroid hormone.

We mentioned that a lack of M-CSF in the *op/op* mutant mouse results in **osteopetrosis**. For comparison, **osteosclerosis** is an increase in bone mass due to an **increase in osteoblastic activity**.

Pour en savoir plus!

Figure 4-28. RANK-RANKL signaling



Binding of membrane-bound or soluble RANKL to RANK determines RANK trimerization followed by the recruitment of adaptor molecules, in particular **TRAF6** (for TNF [tumor necrosis factor] receptor–associated factors).

TRAF6 interacts with **c-Src** to stimulate a regulatory pathway of cytoskeletal reorganization and apoptosis.

TRAF6 regulates **JNK** (jun kinase)—leading to the nuclear translocation of the osteoclastogenic transcription factors **NFATc1** (for nuclear factor–activated T cells c1), and **NF-κB** (nuclear factor kappa B), to stimulate gene activity conducive to osteoclast differentiation.

Pour en savoir plus!

Clinical significance: Osteoporosis and osteomalacia

The realization that RANKL plays a major contribution in osteoclast development and in bone resorptive activity stimulated the development of pharmaceutical agents to arrest skeletal disorders. **Osteoporosis** (Greek *osteon*, bone; *poros*, pore; *osis*, condition) is defined as the loss of bone mass leading to bone fragility and susceptibility to fractures.

The major factor in osteoporosis is the deficiency of the sex steroid **estrogen** that occurs in postmenopausal women. In this condition, the amount of reabsorbed old bone—due to an **increase in the number of osteoclasts**—exceeds the amount of formed new bone. This accelerated turnover state can be reversed by estrogen therapy and calcium and vitamin D supplementation. Osteoporosis and osteoporotic fractures are also observed in men.

Osteoporosis is asymptomatic until it produces skeletal deformity and bone fractures (typically in the spine, hip, and wrist). The **vertebral bones** are predominantly **trabecular bone** surrounded by a thin rim of compact bone. Therefore, they may be crushed or may wedge anteriorly, resulting in pain and in a reduction in height. Elderly persons with osteoporosis are unlikely to have a hip fracture unless they fall.

The diagnosis of osteoporosis is made radiologically or, preferentially, by

measuring bone density by dual-energy x-ray absorptiometry (DEXA). DEXA measures photon absorption from an x-ray source to estimate the amount of bone mineral content.

A monoclonal antibody to RANKL, called **denosumab** (Amgen), functions like osteoprotegerin. The antibody has been administered subcutaneously every 3 months for 1 year in postmenopausal women with severe osteoporosis determined by low bone mineral density detected by DEXA. Denosumab mimics the function of osteoprotegerin and decreases bone resorption, as determined by measuring in urine and serum of bone-collagen degradation products and increased bone mineral density at 1 year. A concern with denosumab anti-RANKL treatment is the expression of RANKL-osteoprotegerin in cells of the immune system (dendritic cells and B and T cells).

Osteomalacia (Greek *osteon*, bone; *malakia*, softness) is a disease characterized by a progressive softening and bending of the bones. Softening occurs because of **a defect in the mineralization of the osteoid** due to lack of vitamin D or renal tubular dysfunction (see Chapter 14, Urinary System). In the young, a defect in **mineralization of cartilage** in the growth plate (see Chapter 5, Osteogenesis), causes a defect called **rickets (juvenile osteomalacia)**. Osteomalacia can result from a deficiency of vitamin D (for example, intestinal malabsorption) or heritable disorders of vitamin D activation (for example, **renal 1α-hydroxylase deficiency** in which **calciferol** is not converted to the active form of vitamin D, **calcitriol**; see vitamin D in Chapter 19, Endocrine System).

Résumé: Concept mapping | Connective Tissue