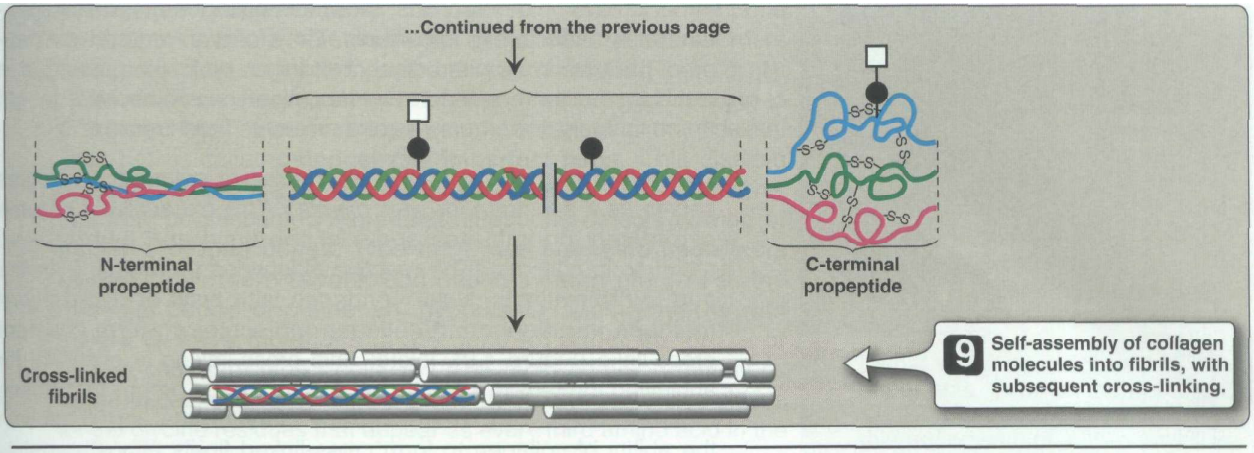


Figure 4.7
Formation of a collagen fibril. (*Continued from the previous page*)

2. **Hydroxylation:** The pro-α-chains are processed by a number of enzymic steps within the lumen of the RER while the polypeptides are still being synthesized (see Figure 4.7). Proline and lysine residues found in the Y-position of the –Gly–X–Y– sequence can be hydroxylated to form hydroxyproline and hydroxylysine residues. These hydroxylation reactions require molecular oxygen and the reducing agent vitamin C (ascorbic acid, see p. 375), without which the hydroxylating enzymes, *prolyl hydroxylase* and *lysyl hydroxylase,* are unable to function (see Figure 4.6). In the case of **ascorbic acid deficiency** (and, therefore, a lack of prolyl and lysyl hydroxylation), collagen fibers cannot be cross-linked (see below), greatly decreasing the tensile strength of the assembled fiber. One resulting deficiency disease is known as **scurvy.** Patients with ascorbic acid deficiency also often show bruises on the limbs as a result of subcutaneous extravascation of blood (**capillary fragility;** Figure 4.8).

3. **Glycosylation:** Some hydroxylysine residues are modified by glycosylation with glucose or glucosyl-galactose (see Figure 4.7).

4. **Assembly and secretion:** After hydroxylation and glycosylation, pro-α-chains form **procollagen,** a precursor of collagen that has a central region of triple helix flanked by the non-helical amino- and carboxyl-terminal extensions called **propeptides** (see Figure 4.7). The formation of procollagen begins with formation of interchain disulfide bonds between the C-terminal extensions of the pro-α-chains. This brings the three α-chains into an alignment favorable for helix formation. The procollagen molecules are translocated to the Golgi apparatus, where they are packaged in secretory vesicles. The vesicles fuse with the cell membrane, causing the release of procollagen molecules into the extracellular space.

5. **Extracellular cleavage of procollagen molecules:** After their release, the procollagen molecules are cleaved by *N-* and *C-procollagen peptidases,* which remove the terminal propeptides, releasing triple-helical collagen molecules.

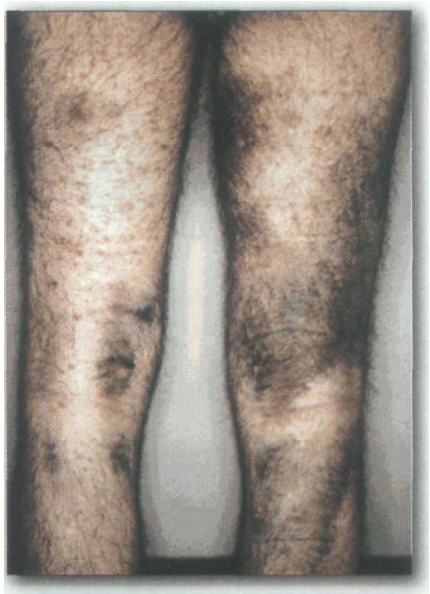


Figure 4.8
The legs of a 46-year-old man with scurvy.

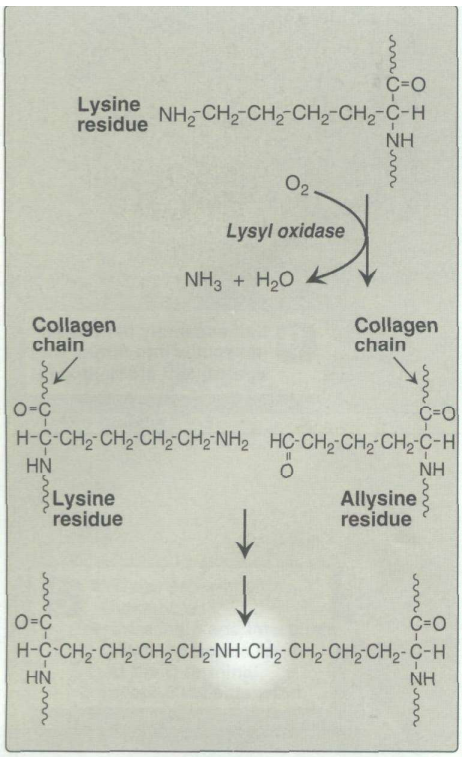


Figure 4.9
Formation of cross-links in collagen.

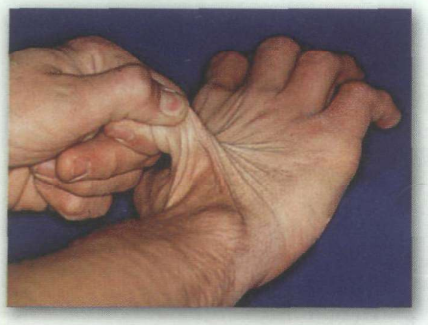


Figure 4.10
Stretchy skin of Ehlers-Danlos syndrome.

6. **Formation of collagen fibrils:** Individual collagen molecules spontaneously associate to form fibrils. They form an ordered, overlapping, parallel array, with adjacent collagen molecules arranged in a staggered pattern, each overlapping its neighbor by a length approximately three-quarters of a molecule (see Figure 4.7).

7. **Cross-link formation:** The fibrillar array of collagen molecules serves as a substrate for *lysyl oxidase.* This extracellular enzyme oxidatively deaminates some of the lysyl and hydroxylysyl residues in collagen. The reactive aldehydes that result **(allysine** and **hydroxyallysine)** can condense with lysyl or hydroxylysyl residues in neighboring collagen molecules to form covalent cross-links (Figure 4.9). [Note: This cross-linking is essential for achieving the tensile strength necessary for the proper functioning of connective tissue. Therefore, any mutation that interferes with the ability of collagen to form cross-linked fibrils almost certainly affects the stability of the collagen.]

D. Degradation of collagen

Normal collagens are highly stable molecules, having half-lives as long as several months. However, connective tissue is dynamic and is constantly being remodeled, often in response to growth or injury of the tissue. Breakdown of collagen fibrils is dependent on the proteolytic action of **collagenases,** which are part of a large family of matrix metalloproteinases. For type I collagen, the cleavage site is specific, generating three-quarter and one-quarter length fragments. These fragments are further degraded by other matrix proteinases to their constituent amino acids.

E. Collagen diseases

Defects in any one of the many steps in collagen fiber synthesis can result in a genetic disease involving an inability of collagen to form fibers properly and, thus, provide tissues with the needed tensile strength normally provided by collagen. More than 1000 mutations have been identified in 22 genes coding for twelve of the collagen types. The following are examples of diseases that are the result of defective collagen synthesis.

1. **Ehlers-Danlos syndrome (EDS):** This disorder is a heterogeneous group of generalized connective tissue disorders that result from inheritable defects in the metabolism of fibrillar collagen molecules. EDS can result from a deficiency of collagen-processing enzymes (for example, **lysyl-hydroxylase deficiency** or **procollagen peptidase deficiency),** or from mutations in the amino acid sequences of collagen types I, III, or V. The most clinically important mutations are found in the gene for type III collagen. Collagen containing mutant chains is not secreted, and is either degraded or accumulated to high levels in intracellular compartments. Because collagen type III is an important component of the arteries, potentially lethal vascular problems occur. [Note: Although collagen type III is only a minor component of the collagen fibrils in the skin, for unknown reasons, EDS patients also show defects in collagen type I fibrils. This results in stretchy skin and loose joints (Figure 4.10).]

2. **Osteogenesis imperfecta (OI):** This disease, known as **brittle bone syndrome,** is also a heterogeneous group of inherited disorders distinguished by bones that easily bend and fracture (Figure 4.11). Retarded wound healing and a rotated and twisted spine leading to a "humped-back" appearance are common features of the disease. Type I OI is called **osteogenesis imperfect tarda.** This disease presents in early infancy with fractures secondary to minor trauma, and may be suspected if prenatal ultrasound detects bowing or fractures of long bones. Type II OI, **osteogenesis imperfecta congenita,** is more severe, and patients die in utero or in the neonatal period of pulmonary hypoplasia. Most patients with severe OI have mutations in the gene for either the pro1– or pro2–α-chains of type I collagen. The most common mutations cause the substitution of single amino acids with bulky side chains for the glycine residues that appear as every third amino acid in the triple helix. The structurally abnormal pro–α-chains can prevent folding of the protein into a triple-helical conformation.

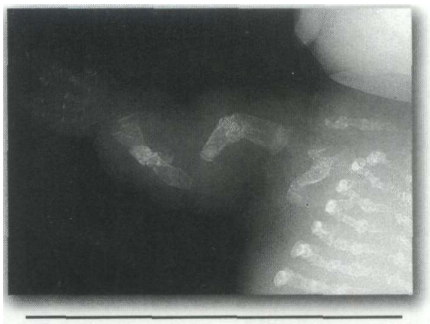


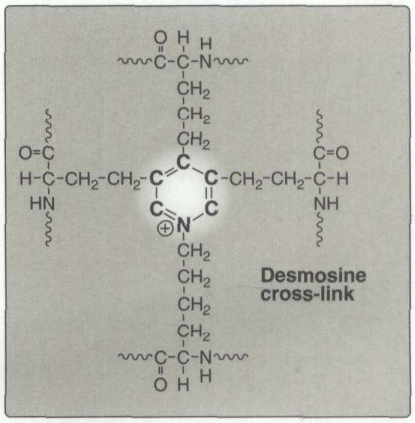
Figure 4.11
Lethal form of osteogenesis imperfecta in which the fractures appear in utero, as revealed by this radiograph of a stillborn fetus.

III. ELASTIN

In contrast to collagen, which forms fibers that are tough and have high tensile strength, elastin is a **connective tissue protein** with rubber-like properties. Elastic fibers composed of elastin and glycoprotein microfibrils are found in the lungs, the walls of large arteries, and elastic ligaments. They can be stretched to several times their normal length, but recoil to their original shape when the stretching force is relaxed.

A. Structure of elastin

Elastin is an insoluble protein polymer synthesized from a precursor, **tropoelastin,** which is a linear polypeptide composed of about 700 amino acids that are primarily small and nonpolar (for example, glycine, alanine, and valine, see p. 2). Elastin is also rich in proline and lysine, but contains only a little hydroxyproline and no hydroxylysine. Tropoelastin is secreted by the cell into the extracellular space. There it interacts with specific glycoprotein microfibrils, such as **fibrillin,** which function as a scaffold onto which tropoelastin is deposited. [Note: Mutations in the fibrillin gene are responsible for **Marfan's syndrome.**] Some of the lysyl side chains of the tropoelastin polypeptides are oxidatively deaminated by *lysyl oxidase,* forming allysine residues. Three of the allysyl side chains plus one unaltered lysyl side chain from the same or neighboring polypeptides form a desmosine cross-link (Figure 4.12). This produces **elastin**—an extensively interconnected, rubbery network that can stretch and bend in any direction when stressed, giving connective tissue elasticity (Figure 4.13).



3 allysines + 1 lys

Figure 4.12
Desmosine cross-link in elastin.

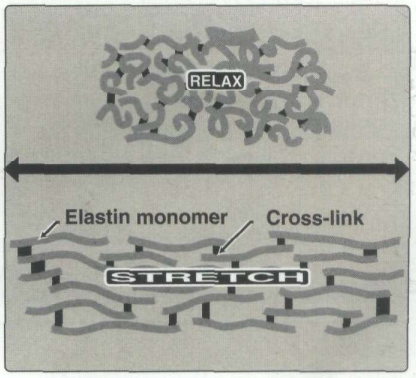


Figure 4.13
Elastin fibers in relaxed and stretched conformations.

B. Role of α_1-antitrypsin in elastin degradation

1. **α_1-Antitrypsin:** Blood and other body fluids contain a protein, α_1 -antitrypsin (**α_1-AT,** currently also called **α_1-antiproteinase**), that inhibits a number of proteolytic enzymes (also called proteases or proteinases) that hydrolyze and destroy proteins. [Note: The inhibitor was originally named α_1-antitrypsin because it inhibits the activity of *trypsin* (a proteolytic enzyme synthesized as trypsinogen

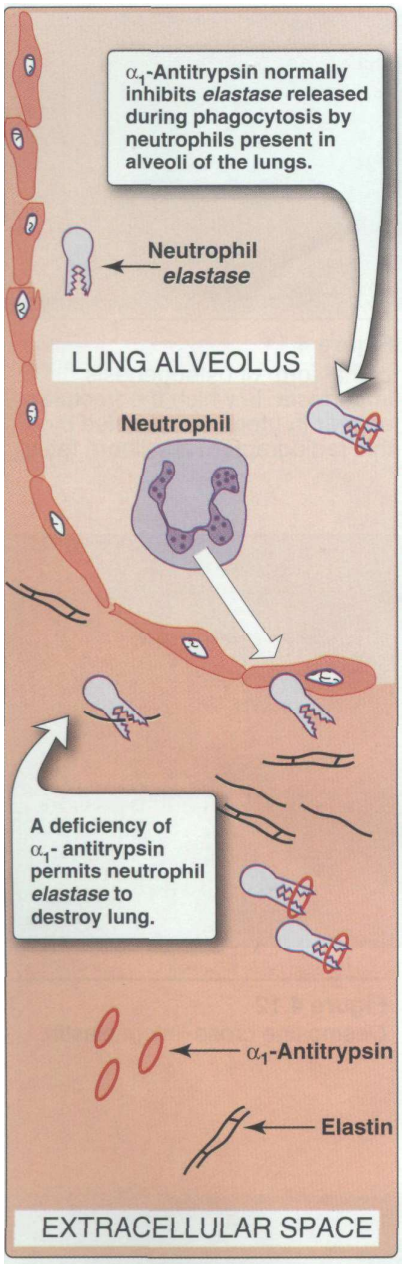


Figure 4.14
Destruction of alveolar tissue by *elastase* released from neutrophils.

by the pancreas, see p. 246).] α_1-AT comprises more than ninety percent of the α_1-globulin fraction of normal plasma. α_1-AT has the important physiologic role of inhibiting neutrophil *elastase*—a powerful protease that is released into the extracellular space, and degrades elastin of alveolar walls, as well as other structural proteins in a variety of tissues (Figure 4.14). Most of the α_1-AT found in plasma is synthesized and secreted by the liver. The remainder is synthesized by several tissues, including monocytes and alveolar macrophages, which may be important in the prevention of local tissue injury by *elastase.*

2. **Role of α_1-AT in the lungs:** In the normal lung, the alveoli are chronically exposed to low levels of neutrophil *elastase* released from activated and degenerating neutrophils. This proteolytic activity can destroy the elastin in alveolar walls if unopposed by the inhibitory action of α_1-AT, the most important inhibitor of neutrophil *elastase* (see Figure 4.14). Because lung tissue cannot regenerate, **emphysema** results from the destruction of the connective tissue of alveolar walls.

3. **Emphysema resulting from α_1-AT deficiency:** In the United States, approximately two to five percent of patients with emphysema are predisposed to the disease by inherited defects in ai-AT. A number of different mutations in the α_1-AT gene are known to cause a deficiency of this protein, but one single purine base mutation (GAG $\rightarrow$ AAG, resulting in the substitution of lysine for glutamic acid at position 342 of the protein) is clinically the most widespread. An individual must inherit two abnormal α_1-AT alleles to be at risk for the development of emphysema. In a heterozygote, with one normal and one defective gene, the levels of α_1-AT are sufficient to protect the alveoli from damage. [Note: A specific α_1-AT methionine is required for the binding of the inhibitor to its target proteases. Smoking causes the oxidation and subsequent inactivation of that methionine residue, thereby rendering the inhibitor powerless to neutralize elastase. Smokers with α_1-AT deficiency, therefore, have a considerably elevated rate of lung destruction and a poorer survival rate than nonsmokers with the deficiency.] The deficiency of *elastase* inhibitor can be reversed by weekly intravenous administration of α_1-AT. The α_1-AT diffuses from the blood into the lung, where it reaches therapeutic levels in the fluid surrounding the lung epithelial cells.

IV. CHAPTER SUMMARY

Collagen molecules contain an abundance of **proline, lysine,** and **glycine,** the latter occurring at every third position in the primary structure. Collagen also contains **hydroxyproline, hydroxy lysine, and glycosylated hydroxylysine,** each formed by posttranslational modification. Collagen molecules typically form **fibrils** containing a long, stiff, triple-stranded helical structure, in which three collagen polypeptide chains are wound around one another in a rope-like superhelix **triple helix.** Other types of collagen form mesh-like networks. **Elastin** is a connective tissue protein with rubber-like properties in tissues such as the lung. **α_1-Antitrypsin (ai-AT),** produced primarily by the liver but also by tissues such as monocytes and alveolar macrophages, prevents elastin degradation in the alveolar walls. A deficiency of α_1-AT can cause **emphysema.**

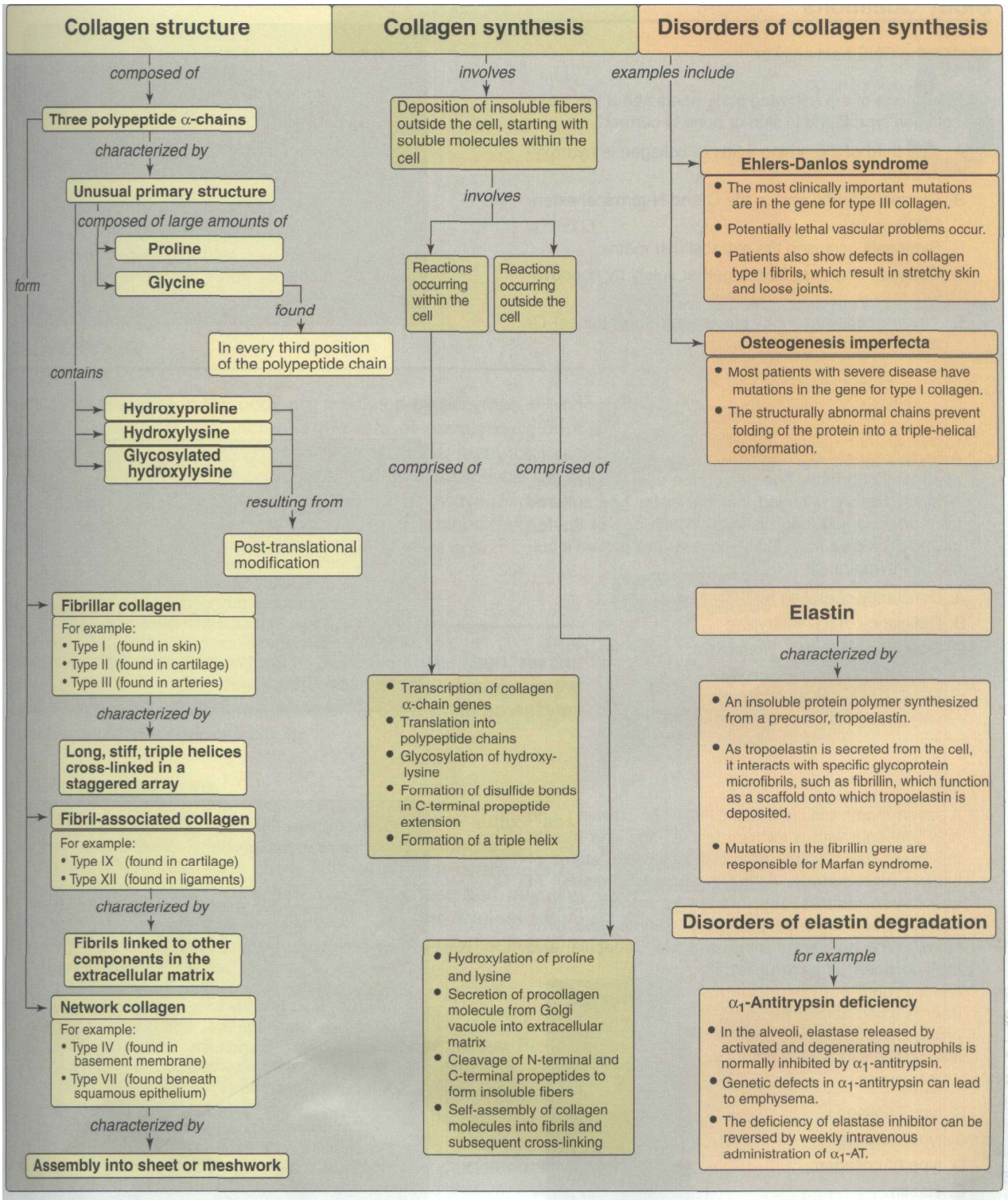


Figure 4.15
Key concept map for the fibrous proteins, collagen, and elastin.