CONNECTIVE TISSUE FIBERS

- The 3 main types of connective tissue fibers are collagen, reticular and elastic.
- The collagen fibers are formed by protein collagen and elastic fibers are composed mainly of protein elastin.
- These fibers are distributed unequally among the different types of connective tissues.
- Actually, there are two systems of fibers-collagen system (consisting of collagen and reticular fibers) and elastic system (consisting of elastic, elaunin and oxytalan fibers).
- The connective tissue fibers are formed by proteins that polymerizes into elongated structures.

Collagen

- The collagens constitute a family of proteins selected during evolution for the execution of several functions.
- During the process of evolution of multicellular organisms, a family of structural proteins that were modified by environmental influences and the functional requirements of the animal organism develops to acquire varying degrees of rigidity, elasticity and strength. These proteins are collectively known as **collagen.**
- Collagen, in the form of elongated fibrils, is mostly found in fibrous tissues such as tendon, ligament and skin, and is also abundant in cornea, cartilage, bone, blood vessels, gut, and basal lamina.
- This is the most abundant protein in the human body, representing 30% of its dry weight.
- The collagen of vertebrates are produced by several cell types. The fibroblast is the most common cell which creates collagen.

According to structure and function, collagens can be classified in the following groups

Collagens that form long fibrils: The molecules of long fibril-forming collagens aggregate to form fibrils clearly visible in the electron microscope. These are collagen type I, II, III, V and XI. The collagen type I is most abundant and has a widespread distribution.

Fibril-associated Collagens: Fibril-associated collagens are short structures that bind collagen fibrils to one another and to other components of the extracellular matrix. They are collagens type IX, XII and XIV.

Collagens that form networks: Network forming collagen is type IV collagen whose molecules assemble in a meshwork that constitutes the structural components of the basal lamina.

Collagens that form anchoring fibrils: Anchoring collagen is type VII collagen present in the anchoring fibrils that bind collagen fibers to the basal lamina.

Collagen is composed of a triple helix, which generally consists of two identical chains (α1) and an additional chain that differs slightly in its chemical composition (α2). The amino acid composition of collagen is atypical for proteins, particularly with respect to its high hydroxyproline content. The most common motifs in the amino acid sequence of collagen are Glycine-Proline-X and Glycine-X-Hydroxyproline, where X is any amino acid other than glycine, proline or hydroxyproline.

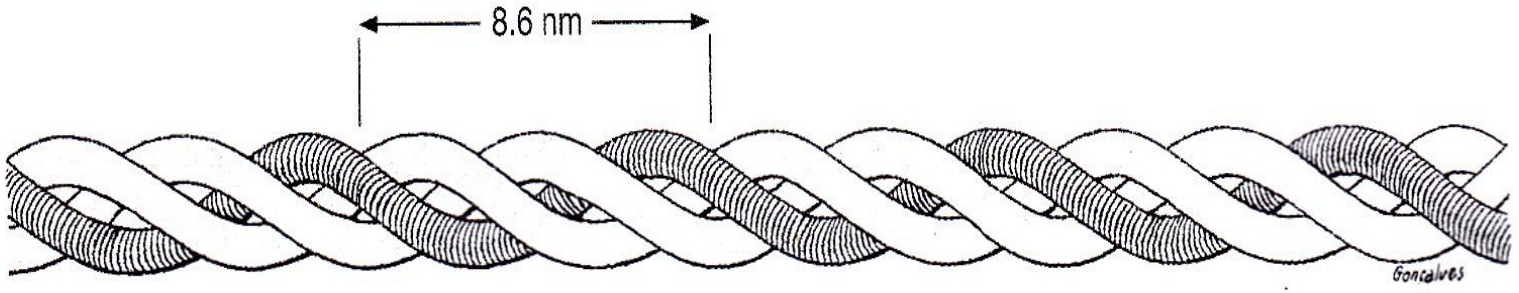


Figure 5–18. In the most abundant form of collagen, type I, each molecule (tropocollagen) is composed of two α1 and one α2 peptide chains, each with a molecular mass of approximately 100 kDa, intertwined in a right-handed helix and held together by hydrogen bonds and hydrophobic interactions. Each complete turn of the helix spans a distance of 8.6 nm. The length of each tropocollagen molecule is 280 nm, and its width is 1.5 nm.

Collagen synthesis

It is an activity, originally believed to be restricted to the fibroblasts, chondroblasts, osteoblasts and odontoblasts, has now been shown to be widespread, with many cell types producing this protein.

The principal amino acids that make this protein are glycine (33.5%), proline (12%) and hydroxyproline (10%).

Collagen contains 2 amino acids that are characteristic of this protein: hydroxyproline and hydroxylysine.

The protein unit that polymerizes to form collagen fibrils is the elongated molecule called **tropocollagen** which measures 280 nm in length and 1.5 nm in width. Tropocollagen consists of 3 subunit polypeptide chains intertwined in a triple helix.

In collagen types I, II and III, tropocollagen molecules aggregate into microfibrillar subunits that are packed together to form fibrils. Hydrogen bonds and hydrophobic interactions are important in the aggregation and packing of these units. In the subsequent step, the structure is reinforced by the formation of covalent cross-links, a process catalyzed by the activity of the enzyme **lysyl oxidase.**

Collagen fibrils are thin, elongated structures that have a variable diameter (ranging from 20 to 90 nm) and can be several micrometers in length. They have transverse striations with a characteristic periodicity of 64 nm.

The dark bands retain more of the Pb-based stain used in electron microscope studies, because their more numerous free chemical groups react more intensely with the Pb solution than do the light bands.

In collagen type I and III, these fibrils associate to form fibers. In collagen type I, the fibers can associate to form bundles.

Biosynthesis of Collagen Type I

(because collagen type I is widely distributed in the body, its synthesis has been thoroughly studied)

Polypeptide α chains are assembled on polyribosomes bound to RER membranes and injected into the cisternae as **preprocollagen** molecules. The signal peptide clipped off, forming **procollagen.**

Hydroxylation of proline and lysine occurs after these amino acids are incorporated into polypeptride chains. Hydroxylation begins after the peptide chains has reached a certain minimum length and is still bound to ribosomes. The two enzymes involved are **peptidyl proline hydroxylase** and **peptidyl lysine hydroxylase.**

Glycosylation of hydroxylysine occurs after its hydroxylation. Different collagen types have different amounts of carbohydrate in the form of galactose or glycosylgalactose linked to hydroxylysine.

Each α chain is synthesized with an extra length of peptide called **registration peptide** on both amino- and carboxyl-terminal ends. Registration peptides probably ensures that the appropriate α chains (α1,α2) assemble in the correct position as a triple helix. In addition, the extra peptides make the resulting **procollagen molecule** soluble and prevent its premature intracellular assembly and precipitation as collagen fibrils. Procollagen is transported as such out of the cell to the extracellular environment.

Outside the cell, specific protease called **procollagen peptidase** removes the registration peptides. The altered protein, known as **tropocollagen**, is capable of assembling into polymeric collagen fibrils. The hydroxyproline residues contribute to the stability of the tropocollagen triple helix, forming hydrogen bonds between its polypeptide chains.

Collagen fibrils aggregate spontaneously to form fibers. Proteoglycans and structural glycoproteins play an important role in the aggregation of tropocollagen to form fibrils and in the formation of fibers from fibrils.

Fibrillar structure is reinforced by the formation of covalent cross-links between tropocollagen molecules. This process is catalysed by the action of enzyme **lysyl oxidase**, which also acts in the extracellular space.

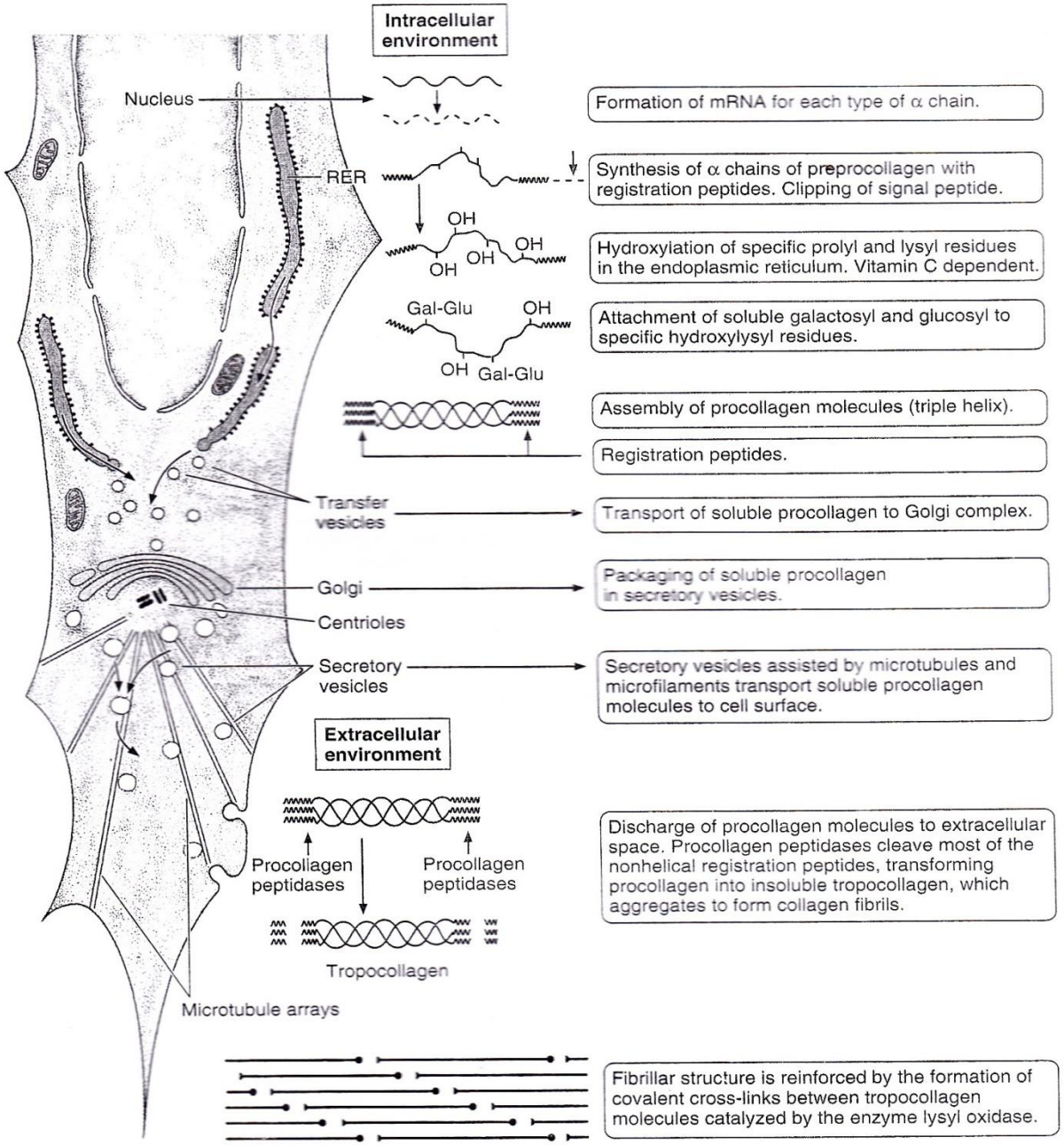
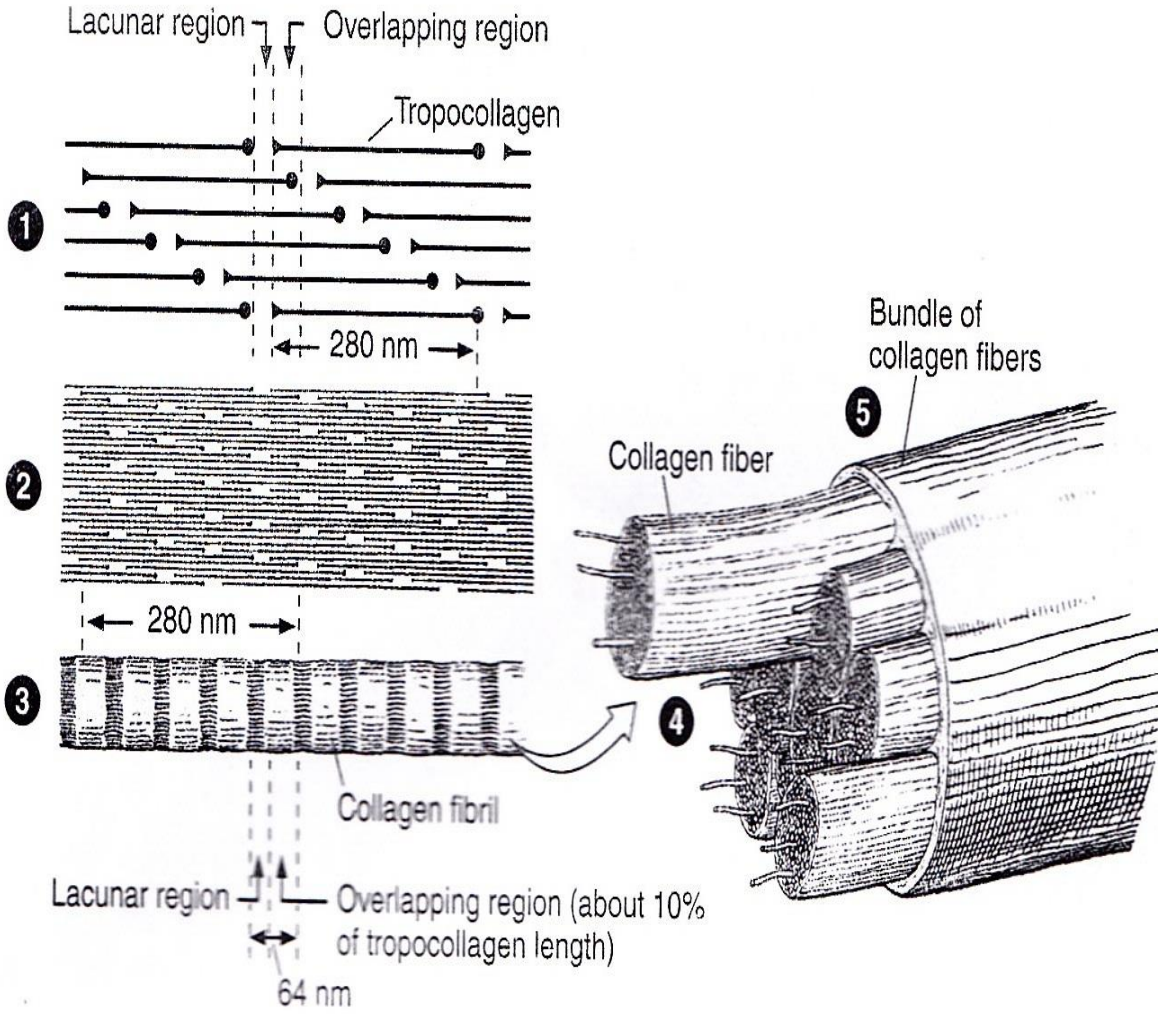


Figure 5–21. Collagen synthesis. The assembly of the triple helix and the hydroxylation and glycosylation of procollagen molecules are simultaneous processes that begin as soon as the 3 chains cross the membrane of the rough endoplasmic reticulum (RER). Because collagen synthesis depends on the expression of several genes and on several post-translation events, many collagen diseases have been described.

Figure 5–19. Schematic drawing of an aggregate of collagen molecules (tropocollagen), fibrils, fibers, and bundles. There is a stepwise overlapping arrangement of rodlike tropocollagen subunits, each measuring 280 nm (**1**). This arrangement results in the production of alternating lacunar and overlapping regions (**2**) that cause the cross-striations characteristic of collagen fibrils and confer a 64-nm periodicity of dark and light bands when the fibril is observed in the electron microscope (**3**). Fibrils aggregate to form fibers (**4**), which aggregate to form bundles (**5**) routinely called collagen fibers. Collagen type III usually does not form bundles.



The other fibrillar collagen are probably formed according to the same pattern described for collagen type I with only minor differences.

The synthesis of collagen involves a cascade of unique post-translational biochemical modifications of the original procollagen polypeptide.

Collagen renewal is in general a very slow process. In some organs, such as tendons and ligaments, the collagen is very stable, whereas in others, as in periodontal ligament, the turnover of collagen is very high.

To be renewed, the collagen must first be degraded. The degradation is initiated by specific enzyme called **collagenase.** These enzymes cut the collagen molecule into two parts that are susceptible to further degradation by nonspecific proteases.

Fibers of Collagen Type I

Collagen fibers made up of collagen type I are the most numerous fibers in connective tissue. Although fresh collagen fibers are colorless strands, when they are present in great numbers the tissues in which they occur are white.

The orientation of the elongated tropocollagen molecules in collagen fibers makes them birefringent.

In many parts of the body, collagen fibers are organized in parallel to each other, forming **collagen bundles**.

Glycosylation of hydroxylysine in collagens and other proteins

The glycosylation of hydroxylysyl residues occurs as a post-translational event in collagen biosynthesis. The carbohydrates linked to hydroxylysyl residues are either a monosaccharide galactose or a disaccharide glucosylgalactose.

The formation of hydroxylysine linked carbohydrate units is catalyzed by two specific enzymes called **hydroxylysyl galactosyltransferase** and **galactosylhydroxylysyl glucosyltransferase**.

The extent of glycosylation of the hydroxylysyl residues and the ratio of galactosylhydroxylysine to glucosylgalactosylhydroxylysine vary considerably

among different collagen types and within the same collagen type from various sources.

Hydroxylysyl residues in the Y position of -Gly-X-Y- triplets in collagen type VI are fully glycosylated. The chains of collagen type II and IV are also highly glycosylated from 6% in α1(II) to almost 15% in α1(IV) whereas the chains of collagen type I and III contain very low levels of hydroxylysyl-linked carbohydrate, being only 0.5-1%.

The function of the hydroxylysyl-linked carbohydrate units remains poorly understood. It has been suggested that they may play a role in the control of the organization of the fibrils. Studies on fibrillar collagens have indicated that an increase content of glycosylated hydroxylysyl residues is associated with a decrease of fibril diameter both *in vivo* and *in vitro*.

Collagen Types

Collagen occurs in many places throughout the body. Over 90% of the collagen in the body, however, is of type one.

Type	Structure	Representative tissue	Main function
Collagen that forms fibrils			
I	300 nm molecules 67 nm banded fibrils	Skin, Tendon, Bone Dentin	Resistance to tension
II	300 nm molecules 67 nm banded fibrils	Cartilage, vitreous body	Resistance to pressure
III	67 nm banded fibrils	Skin,muscle,blood vessel,frequently in together with type I	structural maintenance expansible organs
V	390 nm molecules	Fetal tissue, skin Bone, placenta	Participates in type I collagen function
XI	300 nm molecules	Cartilage	Participates in type II collagen function

Type	Structure	Representative tissue	Main function
Fibril-associated collagen			
IX	200 nm molecules	Cartilage	Bound Glycosaminoglycans associated with type II collagen
XII	Large N-terminal domains	Embryonic tendon and skin	Interact with type I
XIV	Large N-terminal Domains	Fetal skin and tendon	----------
Collagen that forms achoring fibrils			
VII basal	450 nm	Epithelia	Anchors skin epidermal lamina to underlying stroma
Collagen that form networks			
IV	2D Cross-link Network	All basement membrane	Support of delicate structures

EXAMPLES OF CLINICAL DISORDERS RESULTING FROM DEFECTS IN COLLAGEN SYNTHESIS

DISORDER	DEFECT	SYMPTOMS
Ehlers-Danlos Type IV	Faulty transcription translation of type III	Aortic and/or intestinal rupture
Ehlers-Danlos Type VI	Faulty lysine hydroxylation	Augmented skin elasticity, rupture of eyeball
Ehlers-Danlos Type VII	Decrease in procollagen peptidase activity	Increased articular mobility, frequent luxation (dislocation)
Scurvy	Lack of Vit.C (cofactor for proline hydroxylase)	Ulceration of gums Hemorrhages
Osteogenesis Imperfecta	Change of one nucleotide in genes for collagen type I	Spontaneous fractures Cardiac insufficiency