

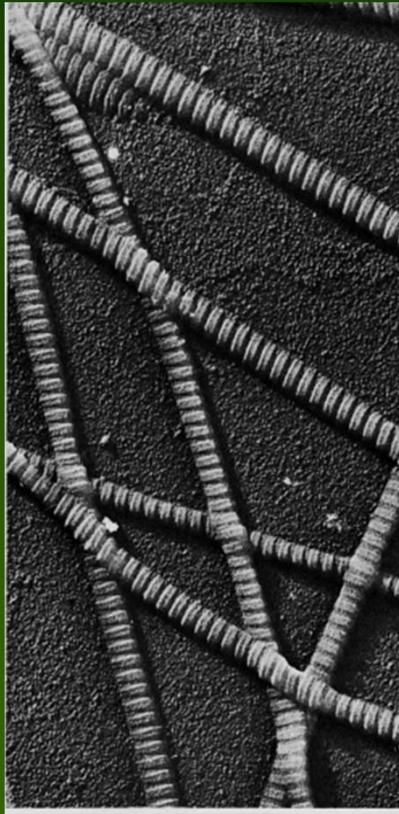
Fibrous proteins

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Overview

- Fibrous proteins form part of the extracellular matrix, also known as connective tissue.
- They are highly elongated molecules whose secondary structures are their dominant motif.
- Characteristic combination of a.a. into regular, secondary structure that provides their specific mechanical properties
- There is increased interest on these proteins due to their role in several normal as well as pathological processes.
 - Cell adhesion, cancer metastasis, arthritis, osteogenesis imperfecta, wound healing, calcification.

Collagen



- The most abundant protein in mammals, 25% of the total weight
- Present in almost every tissue including skin, bone, cartilage, tendons, ligaments, blood vessels, teeth, gingiva
- Organizes into insoluble fibers of great tensile strength
- It is the major stress bearing component of the connective tissue
- Approximate mass of 285 kD and 3000 Å in length
- The majority of the collagen is synthesized by fibroblast

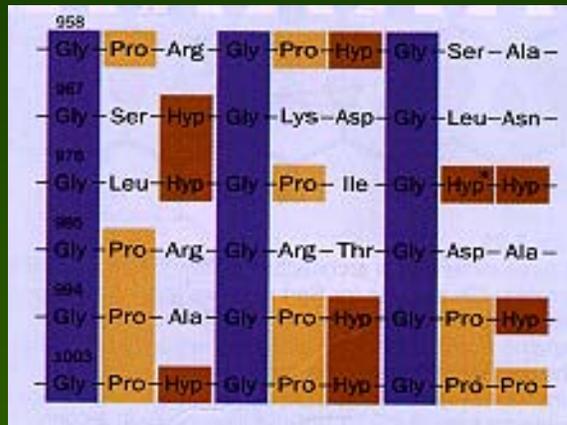
Collagen types and genes

| Types | Genes | Tissue | Chain composition |
|-------|---------------------------------|---|----------------------------------|
| I | <i>COL1A1, COL1A2</i> | Most connective tissues, including bone | $[\alpha 1(I)]_2[\alpha 2(I)]$ |
| II | <i>COL2A1</i> | Cartilage, vitreous humor | $[\alpha 1(II)]_3$ |
| III | <i>COL3A1</i> | Extensible connective tissues like skin, lungs, vascular system | $[\alpha 1(III)]_3$ |
| IV | <i>COL4A1-COL4A6</i> | Basement membranes | $[\alpha 1(IV)]_2[\alpha 2(IV)]$ |
| V | <i>COL5A1-COL5A3</i> | Minor component in tissues containing collagen I | |
| VI | <i>COL6A1-COL6A3</i> | Most connective tissues | |
| VII | <i>COL7A1</i> | Anchoring fibrils | |
| VIII | <i>COL8A1-COL8A2</i> | Endothelium, other tissues | |
| IX | <i>COL9A1-COL9A3</i> | Tissues containing collagen II | |
| X | <i>COL10A1</i> | Hypertrophic cartilage | |
| XI | <i>COL11A1, COL11A2, COL2A1</i> | Tissues containing collagen II | |
| XII | <i>COL12A1</i> | Tissues containing collagen I | |
| XIII | <i>COL13A1</i> | Many tissues | |
| XIV | <i>COL14A1</i> | Tissues containing collagen I | |
| XV | <i>COL15A1</i> | Many tissues | |
| XVI | <i>COL16A1</i> | Many tissues | |
| XVII | <i>COL17A1</i> | Skin hemidesmosomes | |
| XVIII | <i>COL18A1</i> | Many tissues | |
| XIX | <i>COL19A1</i> | Basement membranes | |

Collagen classification by structure formed

| Type | Class |
|------------------------|---|
| I, II, III, V, AND XI | Fibril-forming |
| IV, VIII, X | Network-like |
| IX, XII, XIV, XVI, XIX | Fibril-associated collagens with interrupted triple helix |
| VI | Beaded filaments |
| VII | Anchoring filaments |
| XIII, XVII | Transmembrane domain |
| XV, XVIII | Others |

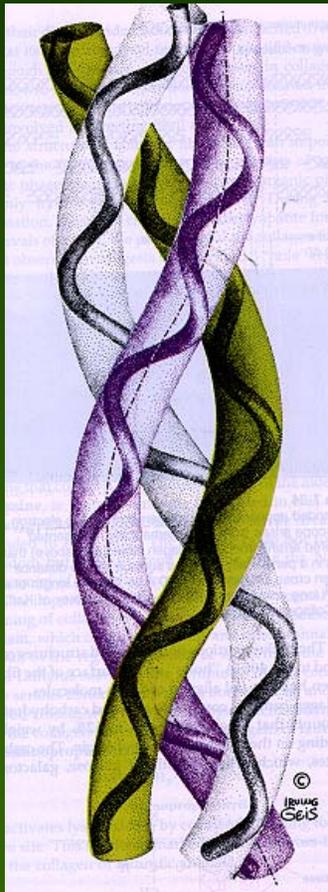
Collagen structure



■ Primary structure

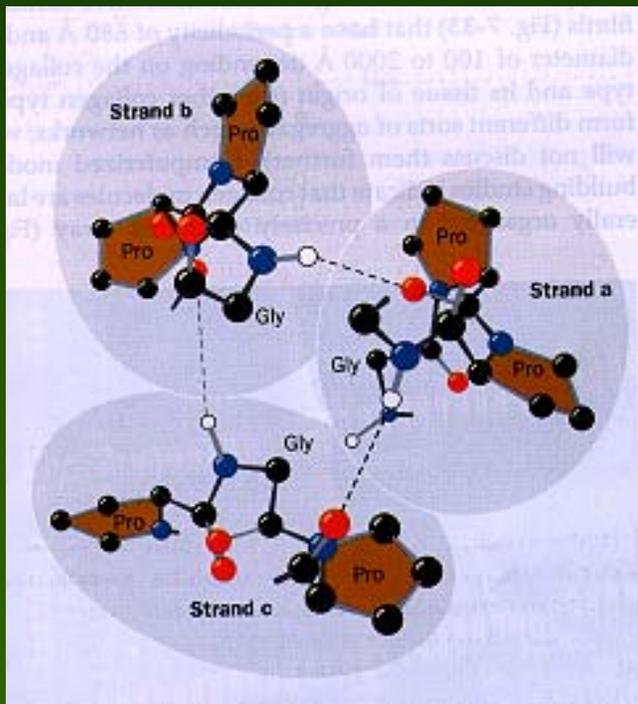
- Characteristic presence of glycine every third residue
- Repeating unit of Gly-X-Y where ~10% of the X positions are proline and ~10% of the Y position are hydroxyproline or hydroxylysine

Collagen structure



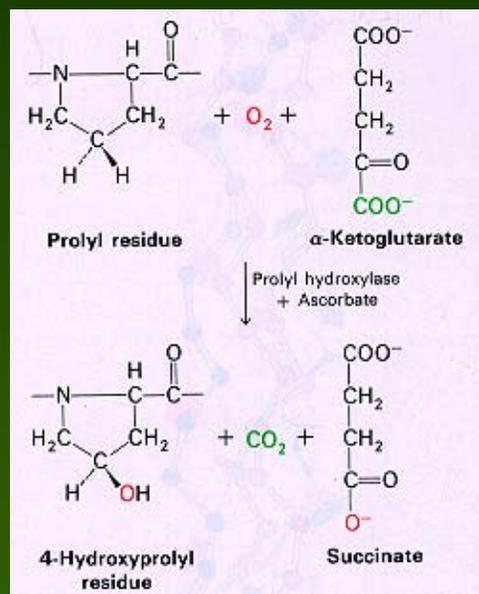
- Secondary structure
 - Each polypeptide forms a left handed helix called α -chain with three residues per turn.
 - Three α -chains are twisted together into a right handed superhelix, a triple-helical structure.
 - The presence of Gly every three residues allows close approximation of the three chains without interference of a side chain.
 - Each collagen type is characterized by a specific combination of three α -chains.

Collagen structure



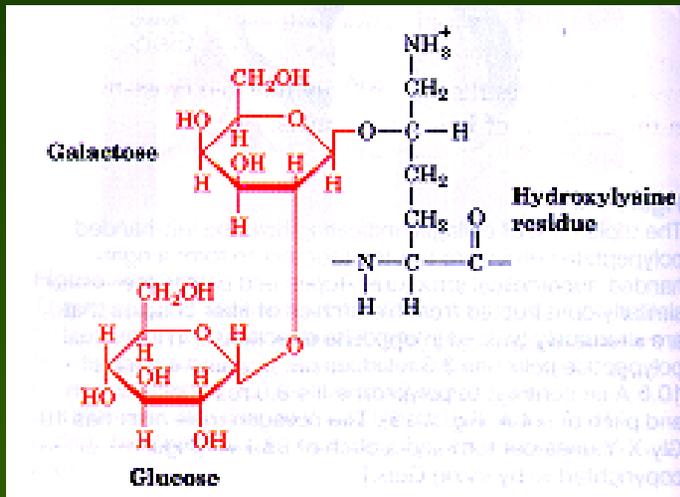
- Stabilization of the triple helix
 - Many side chains are oriented to the outside.
 - Bulky side chains like in Pro produce steric repulsion that together with hydrogen bonds help stabilize the triple helix.

Posttranslational modifications of collagen



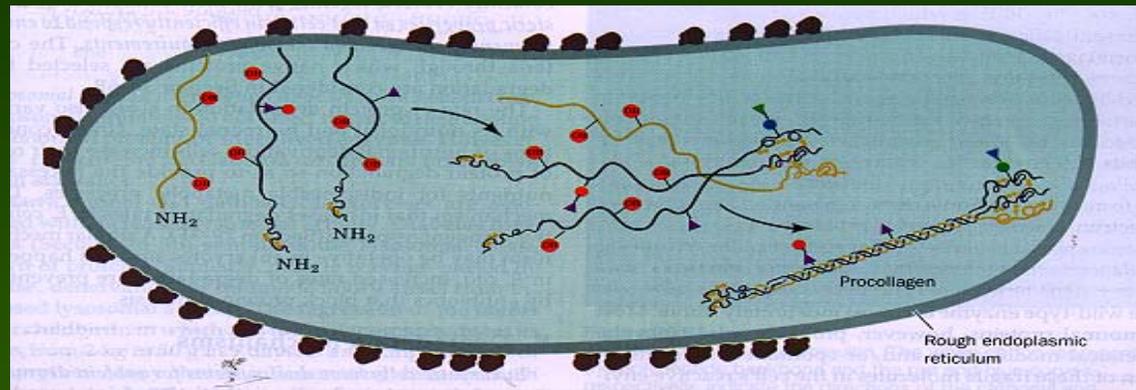
- Hydroxylation of Pro and Lys residues
 - Highly specific reaction for Pro or Lys incorporated in the chain, not on free a.a.
 - Catabolized by prolyl or lysyl hydroxylase
 - 4-Hyp always in the Y position
 - 3-Hyp always in the X position
 - 5-Hyl always in the Y position
- Deficiencies in hydroxylation result in defective synthesis of collagen that leads to diseases like scurvy.

Posttranslational modifications of collagen



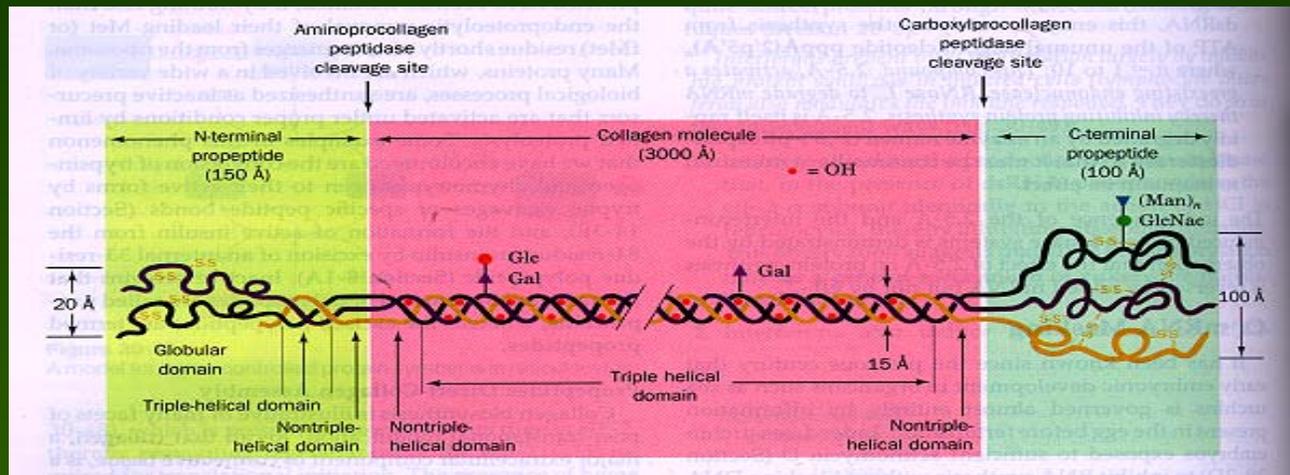
- Glycosylation of hydroxylysine
 - Covalent binding of galactose or glucose-galactose disaccharide to the hydroxyl group of Hyl.
 - Catabolized by galactosyl transferase and glucosyl transferase.
 - The role of these sugar units is not fully understood.
 - ☞ “We suggest that the role of glycosylation, a posttranslational modification, of specific hydroxylysine residues is to prevent their oxidative deamination to aldehydes, thereby precluding formation of complex stable crosslinks. Complex crosslinks would decrease the rate of collagen turnover.” (PNAS 1982-12 vol. 79 no. 24 7684-7688)
 - The number of sugar units depends on the tissue.

Biosynthesis of collagen



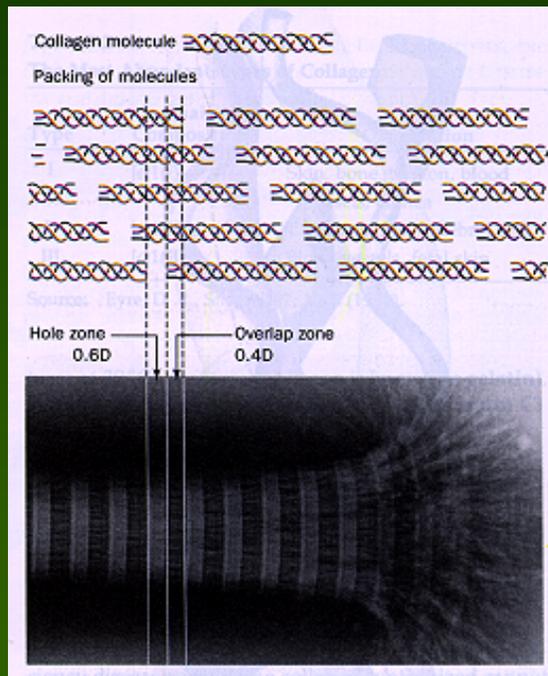
- Preprocollagen-nascent polypeptide with signal sequence for secretion allows the ribosome to attach to the ER.
- Signal peptide is rapidly cleaved inside the ER by signal peptidase.
- Posttranslational modifications of hydroxylation and glycosylation take place in the nascent chain called pro- α -chain in the ER.
- Cysteine residues in the C-terminal participate in the formation of intra- and inter-chain disulfide bonds that bring the chains together to form the triple helix conformation of procollagen.
- The procollagen molecules are then packed and secreted in vesicles.

Extracellular processing of procollagen

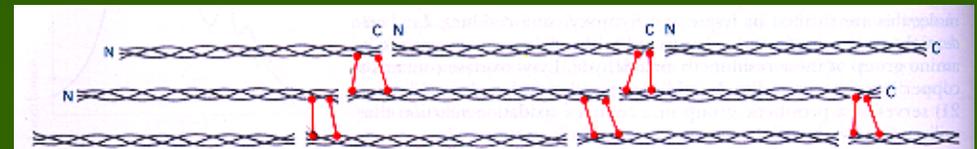


- On the extracellular space the aminopropeptides are cleaved first by aminoprocollagen peptidase and later the carboxypropeptides are cleaved by carboxyprocollagen peptidase.
- The triple helix collagen molecule is referred now as tropocollagen.
- Tropocollagen molecules assemble spontaneously into collagen fibrils.

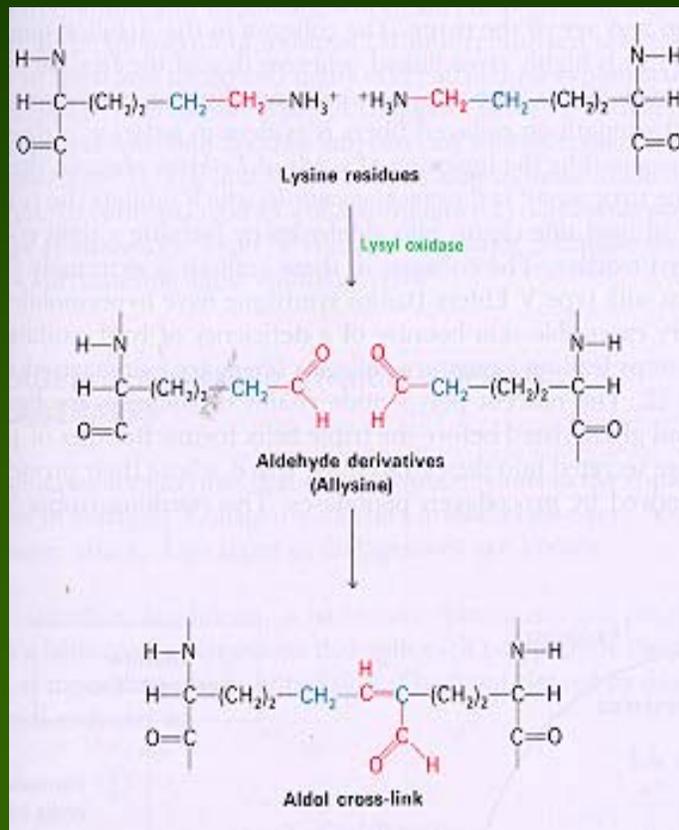
Assembly of collagen fibrils



- Tropocollagen associates with other molecules in a staggered fashion overlapping ~ 75% of the adjacent molecule
- The collagen fibers are strengthened by cross-linking of lysine and hydroxylysine residues at the amino and carboxy ends.

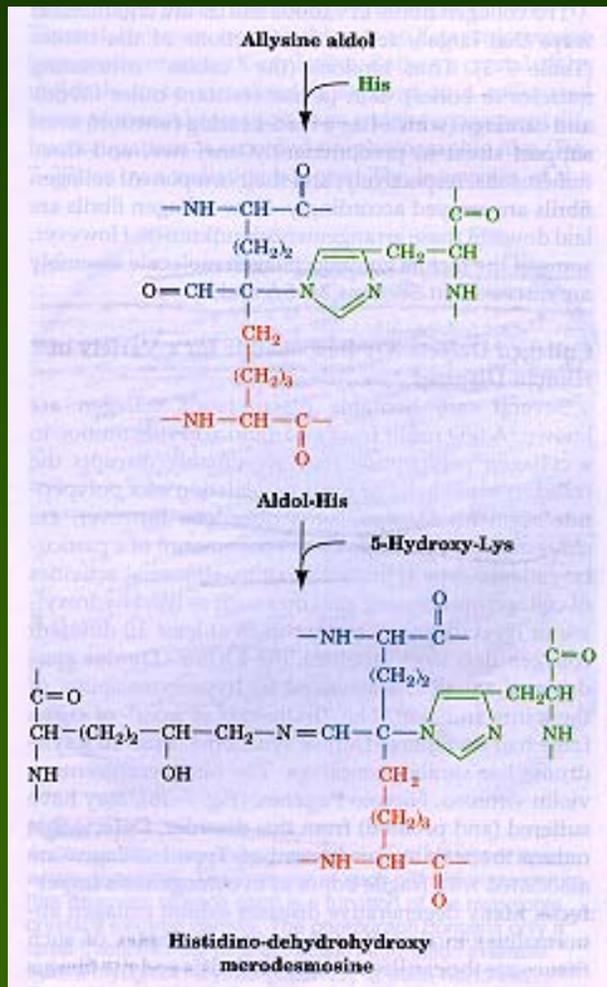


Cross-linking of tropocollagen



- Reaction is initiated by lysyl oxidase which oxidatively deaminates some Lys and Hyl into aldehydes (allysine and hydroxyallysine).
- The allysine and/or hydroxyallysine aldehyde group form an aldol cross-link by an aldol condensation reaction (allysine aldol)

Cross-linking of tropocollagen

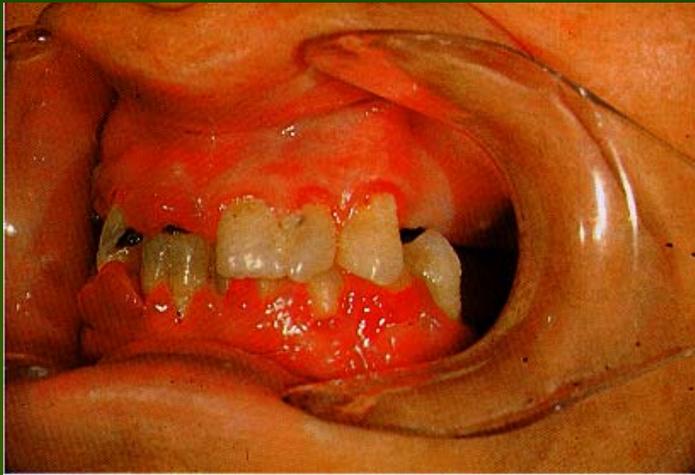


- Further cross-linking can be achieved by His reacting with allysine aldol followed by a reaction with Hyl to cross-link four chains.
- Cross-linking is important to achieving the tensile strength needed for function of the molecule.

Collagen degradation

- Collagen fibrils are relatively metabolically stable
- Breakdown increases during starvation and inflammatory processes.
- Collagenases degrade collagen by specific peptide cleavage.
- Two types of collagenase
 - Bacterial- *Clostridium histolyticum*, cause gas gangrene, destroys the connective tissue barriers.
 - Tissue collagenase-matrix metalloproteinases, for normal processes of growth and remodeling as well as repair after injury.
 - Collagenase activity is regulated by specific inhibitory proteins (TIMP-1 and 2, α -1-antitrypsin).
- Collagenase cleave collagen into smaller fragments that are further degraded to its constituent a.a. by phagocytosis and lysosomal enzymes action.

Diseases associated with abnormalities in collagen synthesis



- Scurvy-deficiency of ascorbic acid (Vit C).
 - Impaired function of prolyl and lysyl hydroxylases.
 - Decreased formation of hydrogen bonds and cross-linking
 - Decreased tensile strength
 - Symptoms-bleeding gums, loose teeth, subcutaneous hemorrhage, poor wound healing

Diseases associated with abnormalities in collagen synthesis

| Gene or Enzyme | Disease ² |
|--------------------------|--|
| COL1A1, COL1A2 | Osteogenesis imperfecta, type I ³ (MIM 1566200) Osteoporosis ⁴ (MIM 166710) Ehlers-Danlos syndrome type VII autosomal dominant (130060) |
| COL2A1 | Severe chondrodysplasias Osteoarthritis ⁴ (MIM 120140) |
| COL3A1 | Ehlers-Danlos syndrome type IV (MIM 130050) |
| COL4A3–COL4A6 | Alport syndrome (including both autosomal and X-linked forms) (MIM 104200) |
| COL7A1 | Epidermolysis bullosa, dystrophic (MIM 131750) |
| COL10A1 | Schmid metaphysial chondrodysplasia (MIM 156500) |
| Lysyl hydroxylase | Ehlers-Danlos syndrome type VI (MIM 225400) |
| Procollagen N-proteinase | Ehlers-Danlos syndrome type VII autosomal recessive (MIM 225410) |
| Lysyl hydroxylase | Menkes disease ⁵ (MIM 309400) |

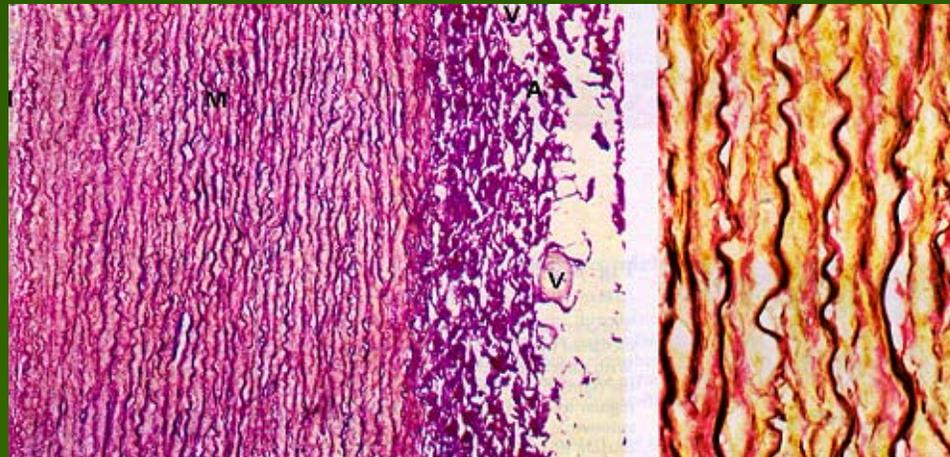
- Osteogenesis imperfecta-brittle bone syndrome
 - mutations in the collagen genes prevent stable triple helix formation and leads to unfolding of protein at body temp.
 - Four types, type II usually lethal, type III also high rate of mortality
 - Bone fragility with frequent fractures, blue sclera, opalescent dentin, hearing loss, hump-backs

Diseases associated with abnormalities in collagen synthesis

- Ehlers-Danlos syndrome
 - Inherited disorder of connective tissue due to defects in the collagen molecule
 - 11 types described, type I (severe) and type II (mild) are the most common
 - Type IV is the most serious due to rupture of large vessels secondary to abnormalities in the collagen type III molecule
 - The defects in collagen could be secondary to defective removal of propeptides by aminoprocollagen peptidase (type VII), deficiency of lysyl hydroxylase (type VI), or mutations in type I collagen genes (VII)
 - Common clinical findings include hypermobility of joints, hyperextensibility of skin, abnormal tissue fragility
 - Periodontal disease and hyperextensibility of the tongue can be present

Elastin

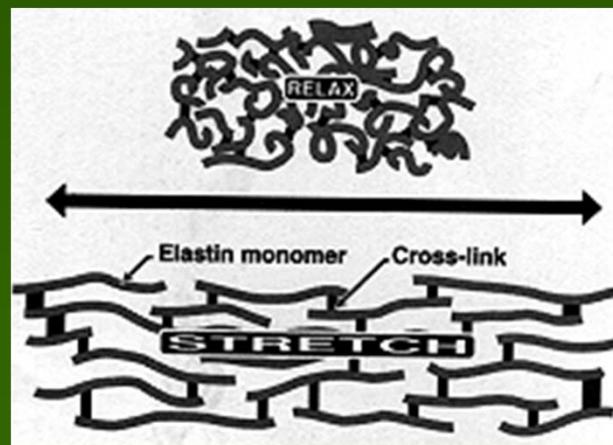
- Connective tissue protein responsible for properties of extensibility and elastic recoil in tissues
- Present in large amounts in lungs, large arterial vessels, elastic ligaments
- Smaller amounts also found in skin, ear cartilage, eustachian tube
- Encoded by one gene
- Synthesized as soluble monomer called tropoelastin (70 kD) by fibroblast



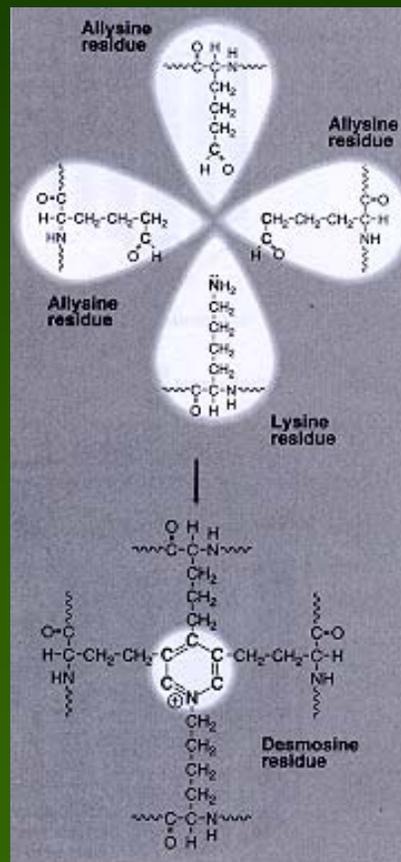
Elastin

■ Structure of elastin

- Amino acid composition
 - ☐ nonpolar small a.a. like Gly (30%), Ala + Val (30%)
 - ☐ Rich in Pro and Lys but only some Hyp and no Hyl
- 3-D conformation network of cross-linked polypeptides with irregular conformation (random coil) that can stretch several times its length and recoil back.



Elastin cross-link



- Unique cross-link of four lysine residues (up to four chains)
- Three Lys are converted to allysine by lysyl oxidase
- A desmosine cross-link is produced by condensation of the allysine residues with an unmodified Lys
- Cross-linked elastin is highly insoluble and very stable (low turn over rate)

Elastin

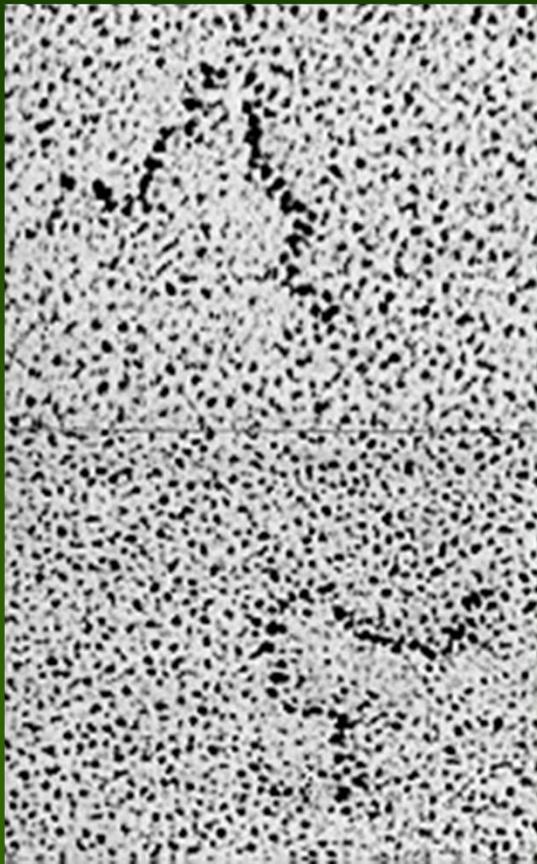
■ Degradation

- Elastase secreted by neutrophils degrades elastin
- Activity is regulated by α -1-antitrypsin, a protease inhibitor in plasma mostly synthesized by the liver and monocytes and lung macrophages
- Excessive degradation of elastin in the lung can produce emphysema
- 2% of patients with emphysema present excessive elastin degradation due to defects in α -1-antitrypsin
- Treatment is injection of α -1-antitrypsin

Differences between collagen and elastin

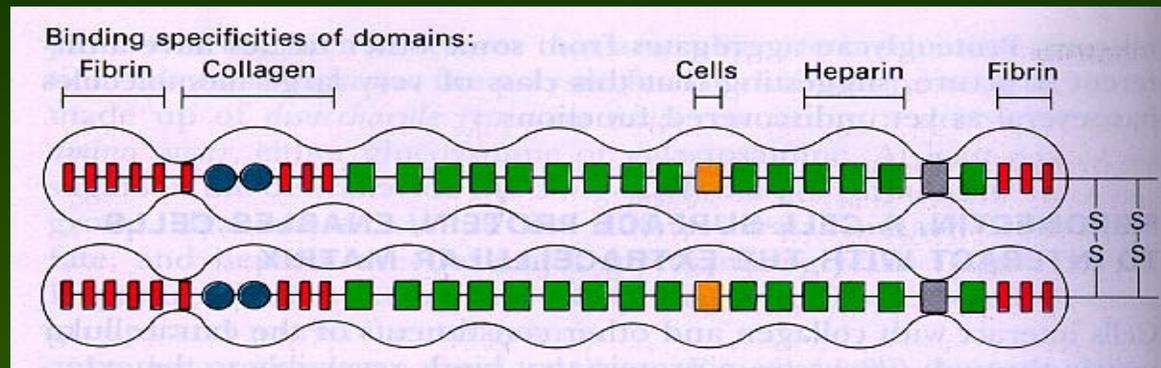
| | Collagen | Elastin |
|---|--|-------------------------------------|
| 1 | Many different genetic types | One genetic type |
| 2 | Triple helix | Random coil |
| 3 | (Gly-X-Y) _n | No repeating structure |
| 4 | Presence of hydroxylysine | No hydroxylysine |
| 5 | Carbohydrate-containing | No carbohydrate |
| 6 | Intramolecular aldol cross-link | Intramolecular desmosine cross-link |
| 7 | Presence of propeptides during synthesis | No propeptides |

Fibronectin



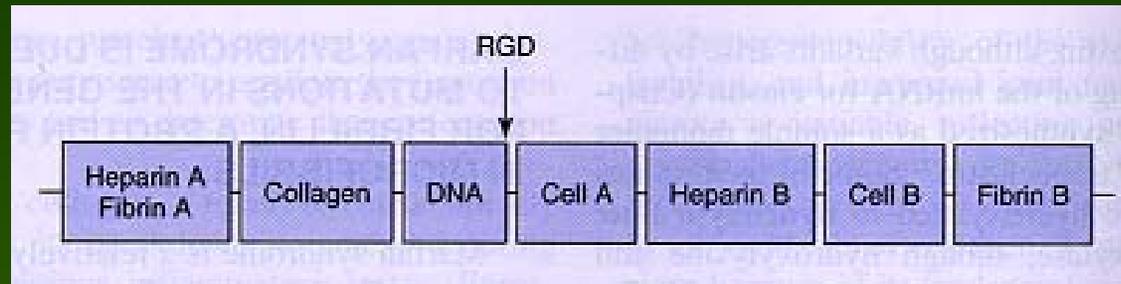
- Major glycoprotein of the extracellular matrix (ECM) but also present in soluble form in plasma
- Encoded by one gene but with multiple variation due to RNA processing
- Adhesive cell surface protein
- Important role in
 - Adhesion of cells to ECM
 - Migration of cells
 - Wound healing

Fibronectin structure



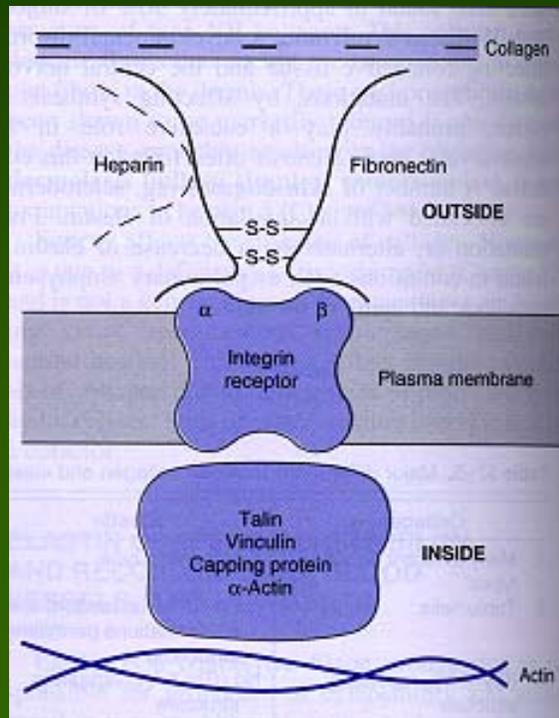
- Consist of two identical subunits each of ~230 kD
- Units are covalently linked by disulfide bonds near the carboxy terminal
- Contains three types of repeating motifs
 - Type I-red
 - Type II-blue
 - Type III-green

Fibronectin structure



- Repeating motifs are organized into 7 functional domains
 - Heparin binding, fibrin, collagen, DNA, and cell surface
- The cell binding function is mediated by a sequence of a.a. known as the RGD sequence (Arg-Gly-Asp)

Fibronectin

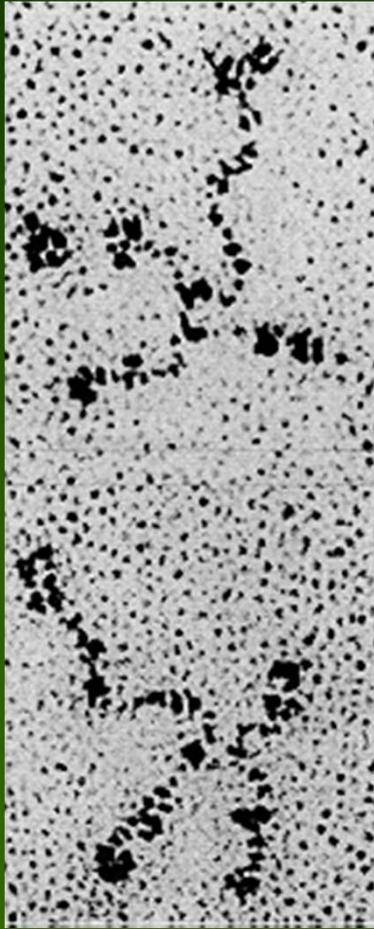


- RGD sequence allows fibronectin to bind to its receptor in the cell surface
- The receptor is member of the integrins family of cell adhesion molecules
- Binding to the cell surface can be inhibited by synthetic peptides containing RGD sequence
- The integrins interact with attachment proteins in the cell providing a route for the exterior of the cell to communicate with the interior and affect cell behavior

Important functions of fibronectin domains

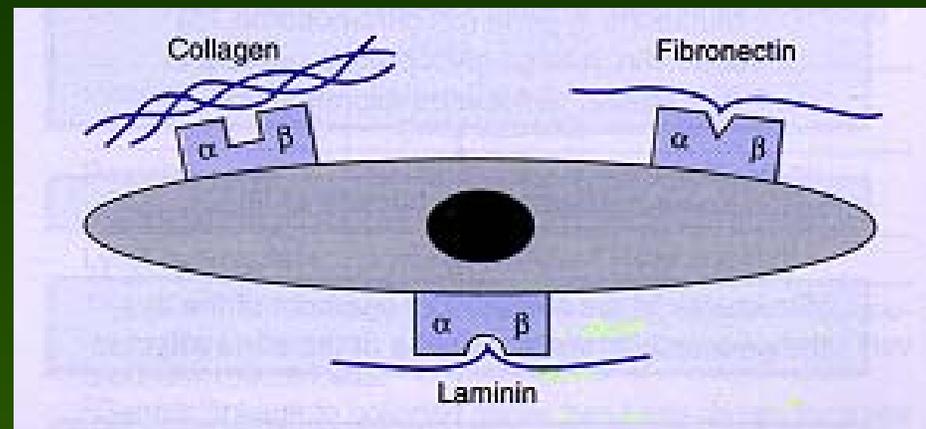
| Domain | Function |
|--------------|--|
| Heparin | Allows fibronectin to bind to proteoglycans of the ECM |
| Fibrin | Binding to fibrin in blood clots permits fibroblast to migrate into injured tissue to start the repair |
| Collagen | Help cells to bind to the ECM and to migrate through it by binding and releasing collagen |
| Cell surface | Cell adhesion to ECM and indirect interaction with the cell interior |
| DNA | Not characterized at this time |

Laminins



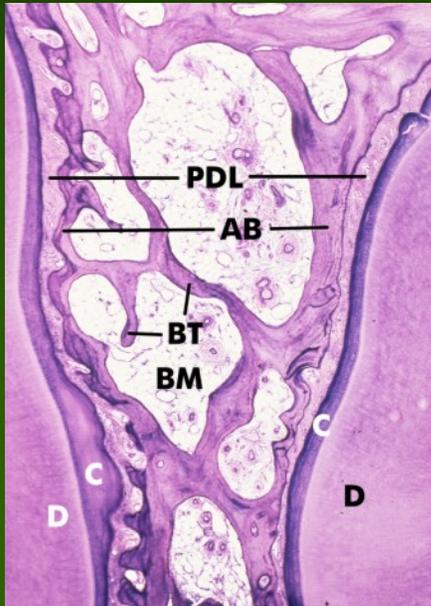
- Adhesive glycoprotein of the ECM
- High affinity for Type IV collagen (basement membrane)
- helps epithelial cells to bind to the underlying connective tissue

Laminin structure and function



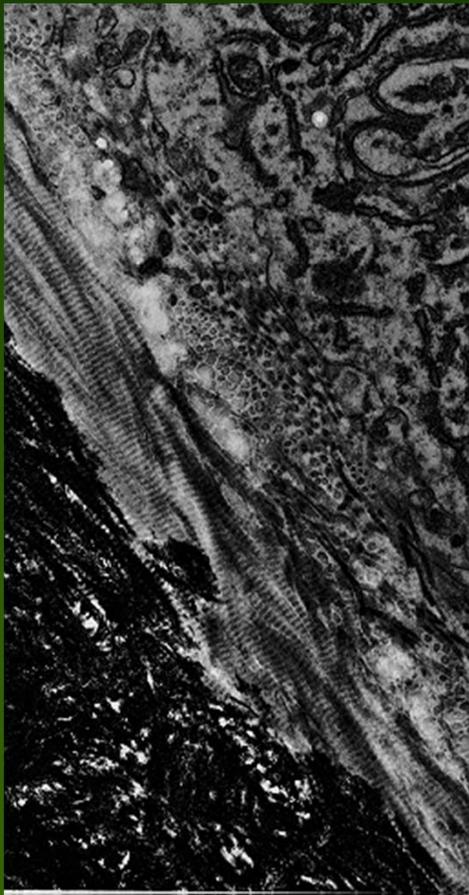
- Consist of three elongated polypeptide chain (A_1 , B_1 , B_2) linked together to form a cruciform shape (850 kD, 70 nm long)
- Like fibronectin, contains multiple domains with distinct binding functions
 - Collagen (type IV)
 - Heparin
 - Integrins
- As fibronectin, helps mediate cell adhesion to ECM, especially anchoring the epithelial cells in the basement membrane to the basal lamina

Calcified connective tissue



- Specialized connective tissue which has a role in structural support and mastication as well as calcium metabolism
- Four types of calcified tissue
 - Bone
 - Enamel
 - Dentin
 - Cementum
- The process of calcification varies from tissue to tissue and it is in part dependent of tissue specific ECM proteins

Bone tissue



- Composed of organic and inorganic material
 - Organic-ECM proteins, the most abundant being type I collagen
 - Inorganic-mainly crystals of hydroxyapatite
- Osteoblast is the main cell involved in the synthesis of bone
 - Synthesis of most of the proteins found in bone
 - Synthesis of growth factors and cytokines
- Osteoblast is responsible for the deposition of new bone matrix (osteoid) and its subsequent mineralization

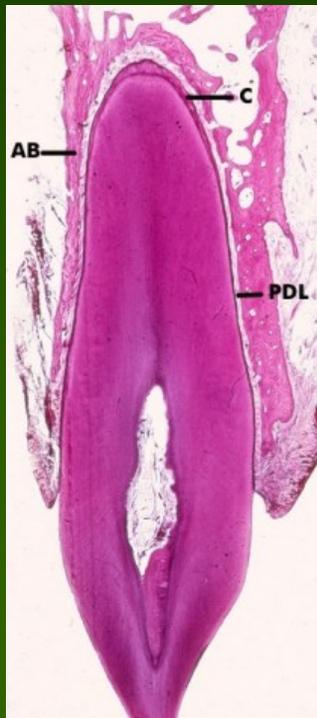
Mineralization of bone

- Deposition of bone matrix
- Control of mineralization by regulating the passage of calcium and phosphate ions across surface membrane
 - Alkaline phosphatase used to generate phosphate ions from organic phosphate
 - Matrix vesicles containing Ca^{2+} and PO_4
 - Nucleation centers in the gaps of tropocollagen molecule in the fibrils of type I collagen
 - Nucleation centers on matrix proteins like acidic phosphoprotein, bone sialoprotein
 - ▣ Contain stretches of a.a. Glu and Asp that bind calcium
- Maturation involves increased mineralization with removal of some of the organic matrix and water

Principal proteins of bone

| Proteins | Comments |
|---------------------------------------|--|
| Collagens Collagen type I | 90% of total bone protein |
| Noncollagen Plasma proteins | Mixture of various plasma proteins |
| Proteoglycans CS-PG III | Bone specific, found in calcification sites |
| Osteonectin (bone SPARC protein) | Secreted protein acidic and rich in Cys, has high affinity for Ca, HAP, and collagen type I |
| Osteocalcin | Bone specific, contains γ -carboxyglutamate that binds HAP |
| Bone sialoprotein | Bone specific, acidic phosphoprotein that binds calcium, may serve as nucleation site for mineralization |
| Bone morphogenic proteins | Secreted proteins with osteoinduction properties (BMP-2, BMP-7) |

Calcified tissue in oral cavity

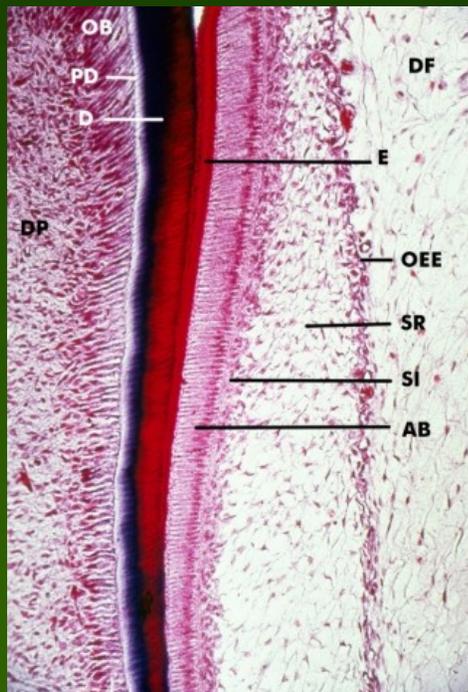


- In addition to bones, teeth are the other calcified tissue
- Teeth are formed by three different calcified tissue protecting the inner soft connective tissue containing the blood supply and nerve endings of the tooth
 - Enamel-the hardest tissue in the body, surrounds the clinical crown of teeth
 - Dentin-surrounds the pulp chamber, second to enamel in hardness, protects pulp chamber
 - Cementum-very similar to bone in composition, serves as anchorage of the tooth to the bone by insertion of periodontal ligament fibers

Matrix proteins role in teeth calcification

- The physical properties of each calcified tissue depends on the nature of its organic and inorganic matrices
- The hardness of the tissue results from a higher mineral content
- How the tissue gets calcified is a function of the tissue specific cells that form the calcified tissue and the tissue specific matrix proteins they secrete
 - Ameloblast-enamel
 - Odontoblast-dentin
 - Cementoblast-cementum

Dentin formation

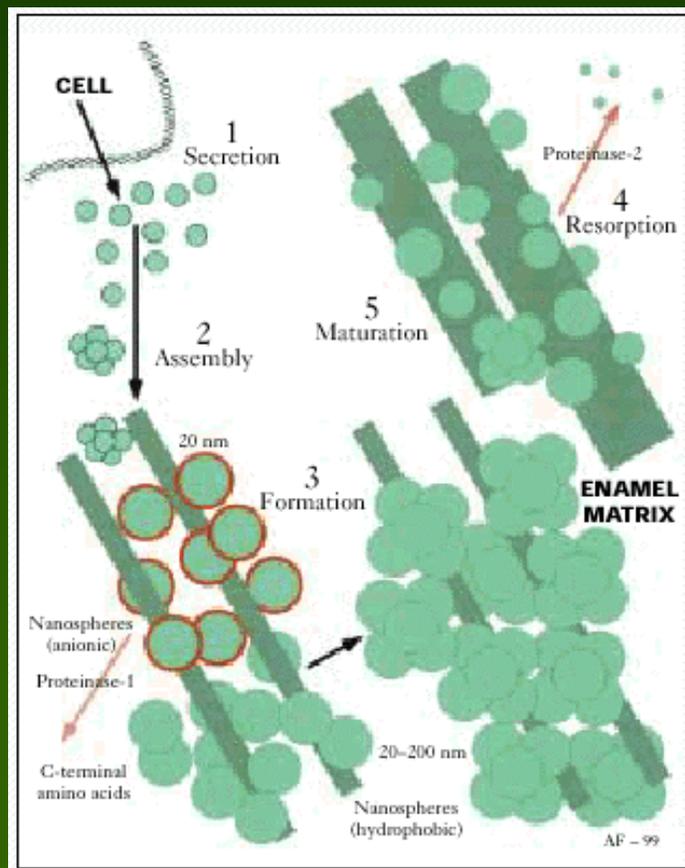


- Odontoblasts secrete an ECM which contain
 - type I collagen
 - Dentine phosphoproteins (DPP)
 - Dentine sialoprotein (DSP)
 - Osteocalcin
 - Osteonectin
 - Proteoglycans
- DPP and DSP are unique to dentine
 - DPP-highly anionic peptide (80% consist of phosphoserine and Asp)
 - High affinity for calcium, may regulate dentine mineralization
- The cell secretes matrix vesicles with Ca and alkaline phosphatase to produce the HAP crystal that grow on the matrix protein.

Enamel formation

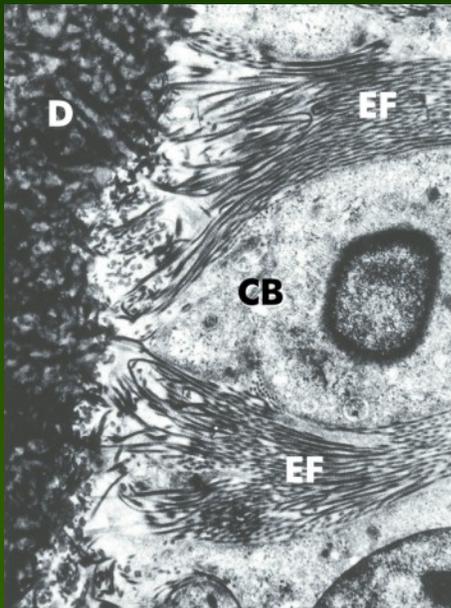
- Deposition of the dentin matrix and initiation of mineralization trigger the ameloblast to secrete enamel matrix proteins in direct contact with dentin (dentinoenamel junction)
- Both mineralized layers continue to be synthesized in opposite directions
- Ameloblasts produce two major group of proteins that appear to regulate enamel formation
 - Amelogenins-hydrophobic peptides rich in Pro, His, Gln, represent 90% of the total organic enamel matrix, localized to the intercrystal spaces, it is enzymatically removed with water as the enamel matures
 - Enamelins-anionic glycoproteins, rich in Glu, Asp, Ser, and Gly, represents ~10% of the organic matrix, localized to the surface of the HAP crystal and is not removed from HAP
- Collagen type I is also produced and secreted in the matrix
- Collagen and enamelins appear to function as the initial nucleation sites for mineralization and amelogenins seem to control the size and shape of the crystals.

Model of amelogenins role in enamel formation



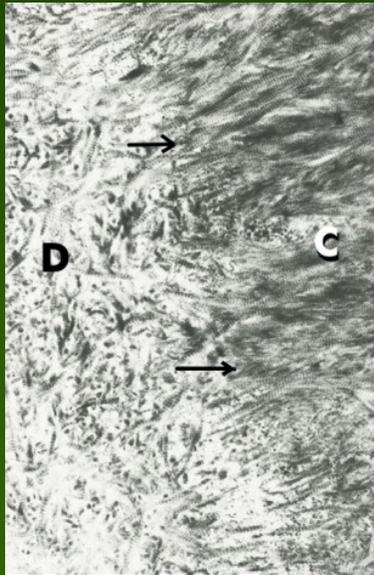
- Secretion of amelogenins to ECM
- Assembly of nanospheres with anionic charges on the surface
- Electrostatic interaction with forming crystals keeping a 20 nm space that prevent crystal-crystal fusion
- Enzymatic removal of the charged surface produce hydrophobic nanospheres that interact together and stabilizes the growing crystals
- Other enzymes degrade the nanosphere and the amelogenin fragments are resorbed by the cell (water is also removed)
- Crystals thicken and may fuse to produce mature enamel

Cementum formation



- It is not clear what triggers the formation of cementum
- It appears that enamel related proteins may induce cementoblasts to produce cementum but also is believed that multiple adhesion proteins (fibronectin, laminin, osteopontin, tenascin) may play a role
- Cementum is very similar to bone and the process of formation follows a similar pattern as in bone
 - Some of the ECM proteins involved in bone formation also participate in cementum formation

Cementum formation



- Proteins involved in the ECM of cementum
 - Type I collagen-nucleation site and anchorage of the tooth by PDL
 - Fibronectin-cell adhesion, collagen binding, bacterial adhesion
 - BSP-same as in bone
 - Osteopontin-phosphoprotein, may control adhesion and differentiation of precementoblasts
 - Laminin-adhesion
 - Osteonectin and osteocalcin-same as in bone
 - Proteoglycans

Difference in composition of mineralized tissue

| % by weight | Enamel | Dentin | Cementum | Bone |
|-------------|--------|--------|----------|------|
| Mineral | 95 | 70 | 61 | 45 |
| Organic | 1 | 20 | 27 | 30 |
| Water | 4 | 10 | 12 | 25 |