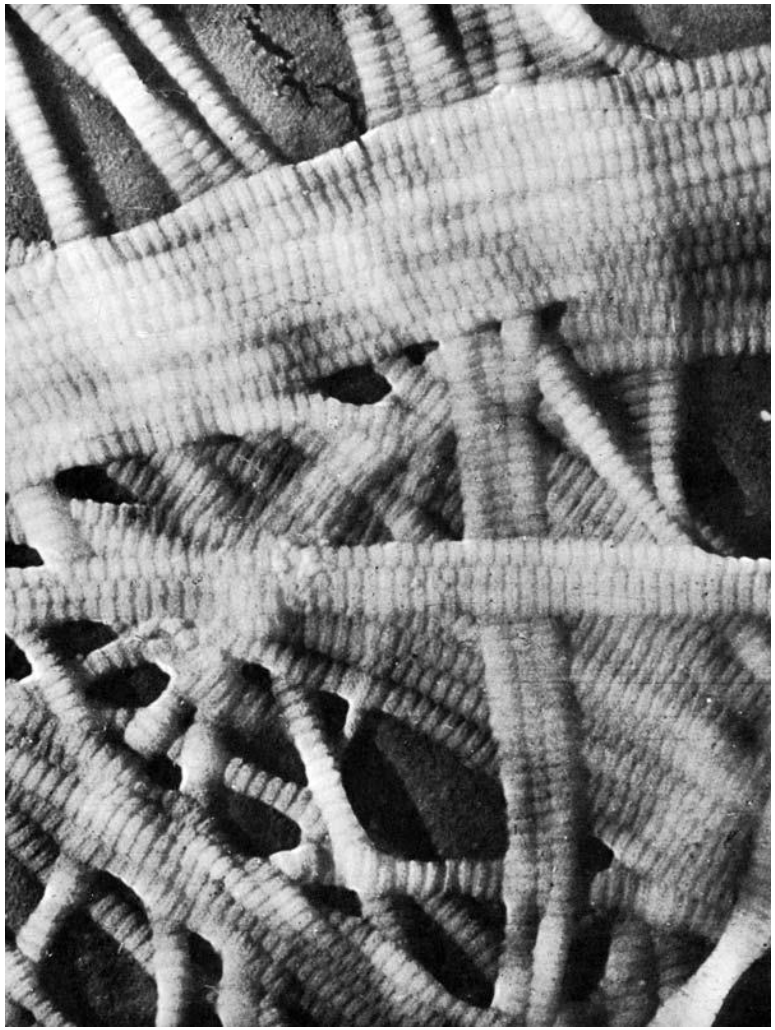
COLLAGENO

Fibre di collageno



Collageno

- Proteina (glicoproteina) più abbondante nel corpo umano (25% del totale)
- Costituente del **tessuto connettivo**, insieme a elastina, fibrillina e proteoglicani, tutti prodotti da fibroblasti, condroblasti, osteoblasti
- **Pelle 72%**
- **Cartilagine 50%**
- **Cornea 68%**
- **Osso 23%**
- **Aorta 20%**
- **Polmoni 10%**
- **Fegato 4%**

Collageno

- Ha un ruolo fondamentale nell'architettura e nell'integrità dei tessuti
- Media una vasta gamma di interazioni che si stabiliscono tra cellula e cellula e tra cellula e matrice
- Partecipa a diversi processi biologici

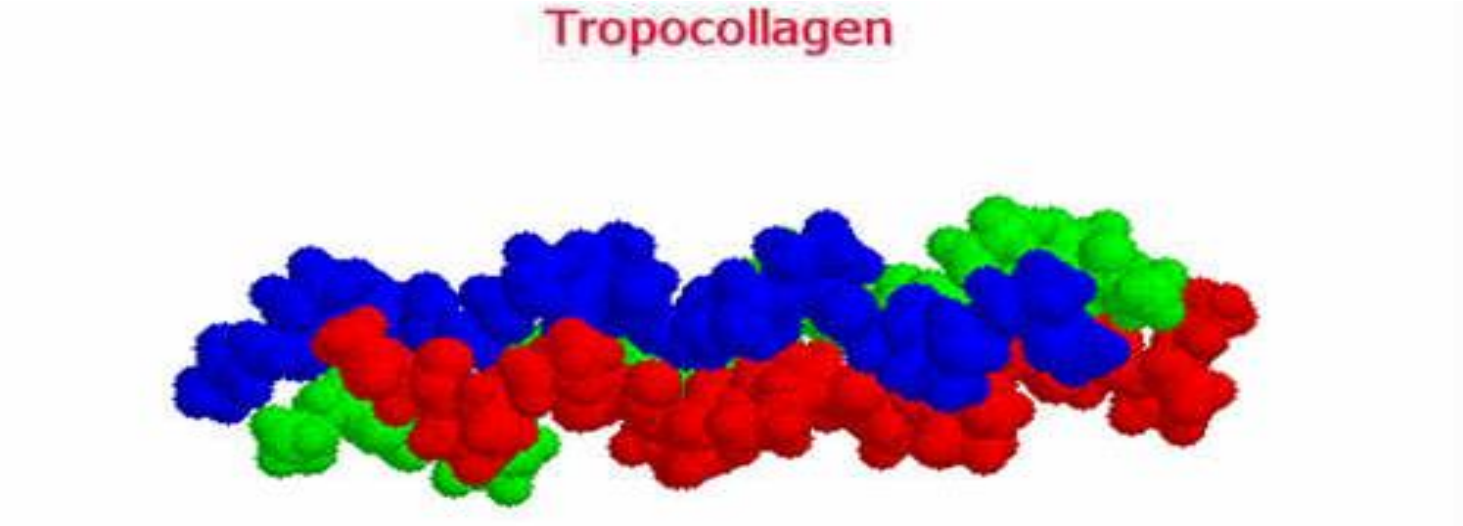
Collageno-processi biologici

- Adesione cellulare
- Migrazione cellulare

- Rimodellamento tissutale
- Crescita
- Differenziamento
- Morfogenesi
- Coagulazione del sangue
- Riparazione ferite

Collageno: struttura

Unità costitutiva è il tropocollageno (~ 300 kDa): formato dall'unione di **3 catene polipeptidiche** avvolte in una tripla elica destrorsa molto stretta (elica del collageno) di 300 x 1.5 nm.



Collageno: composizione e sequenza aminoacidica

31
-Gly-Pro-Met-Gly-Pro-Ser-Gly-Pro-Arg-
22
-Gly-Leu-Hyp-Gly-Pro-Hyp-Gly-Ala-Hyp-
31
-Gly-Pro-Gln-Gly-Phe-Gln-Gly-Pro-Hyp-
40
-Gly-Glu-Hyp-Gly-Glu-Hyp-Gly-Ala-Ser-
49
-Gly-Pro-Met-Gly-Pro-Arg-Gly-Pro-Hyp-
58
-Gly-Pro-Hyp-Gly-Lys-Asn-Gly-Asp-Asp-

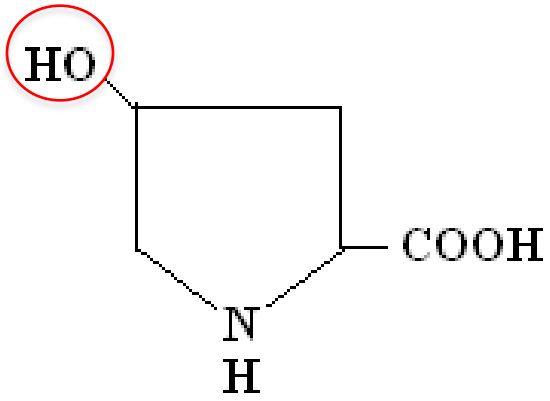
circa un terzo dei suoi residui sono Gly

il 15-30% è costituito da Pro e da 4-idrossiprolina.

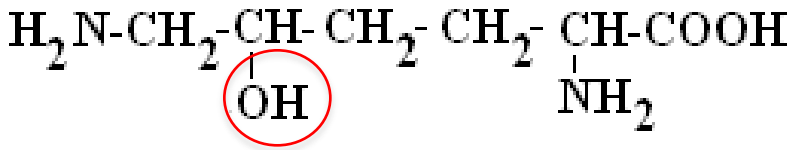
la sequenza Gly-Pro-Hyp ricorre frequentemente.

Composizione in aminoacidi

- Glicina (30%)
- Prolina (25%)
- Aminoacidi modificati: Idrossiprolina; Idrossilisina (25%)



4-OH Pro

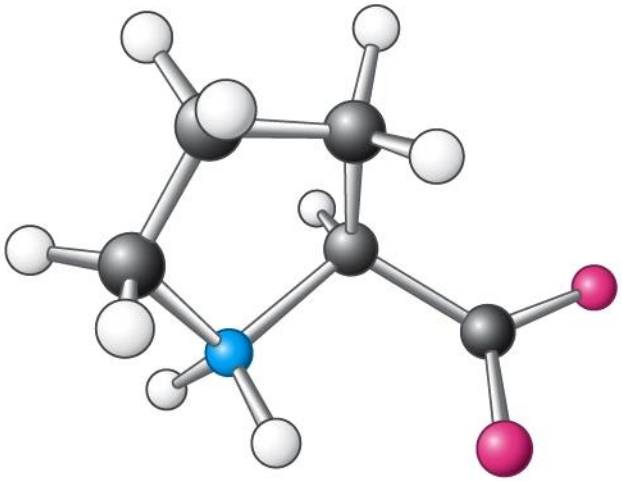


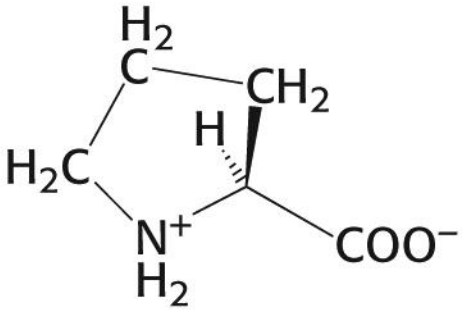
5-OH Lys

Prolina: struttura ciclica

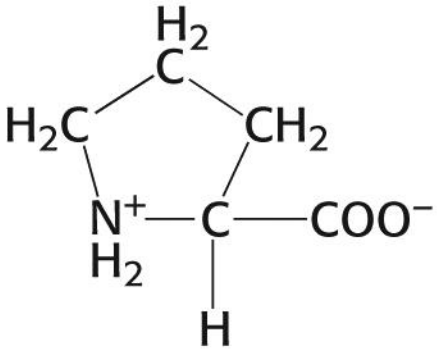
R alifatico non polare

Ring structure: Proline conformationally restricted, marked effect on protein architecture

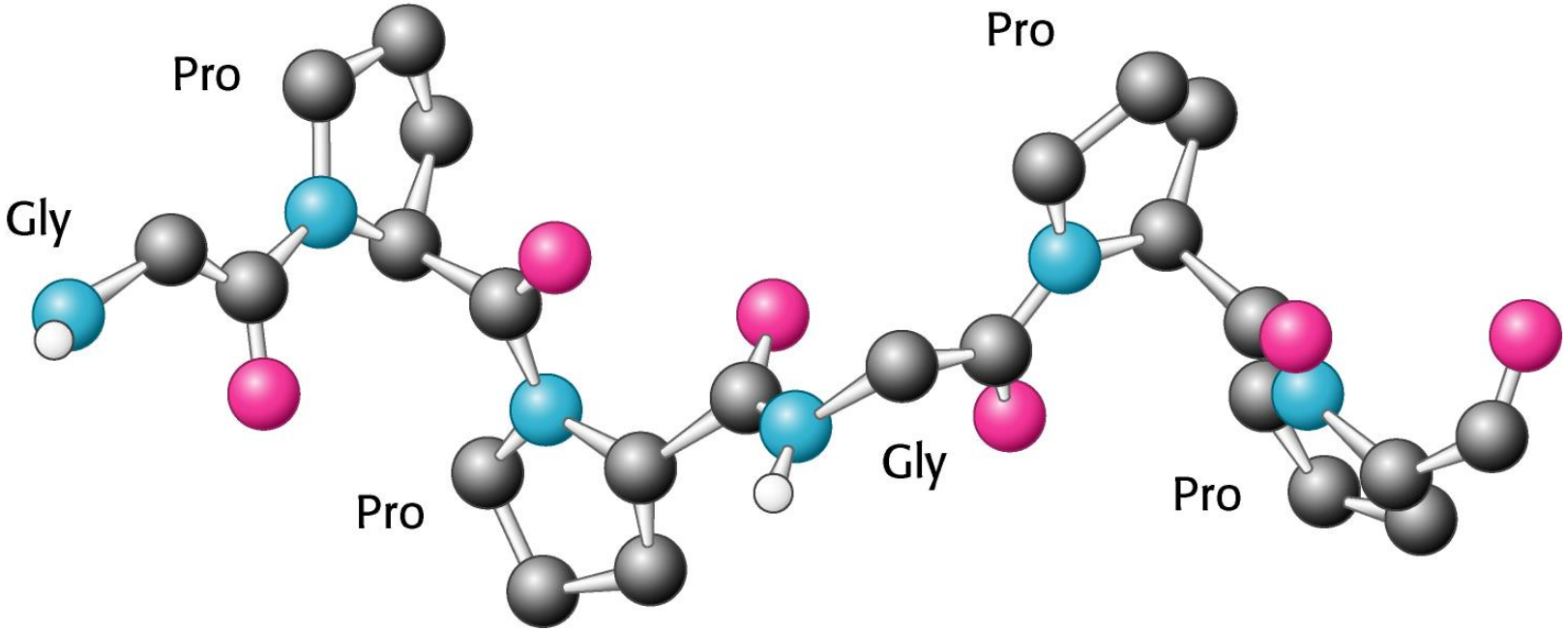




Proline
(Pro, P)

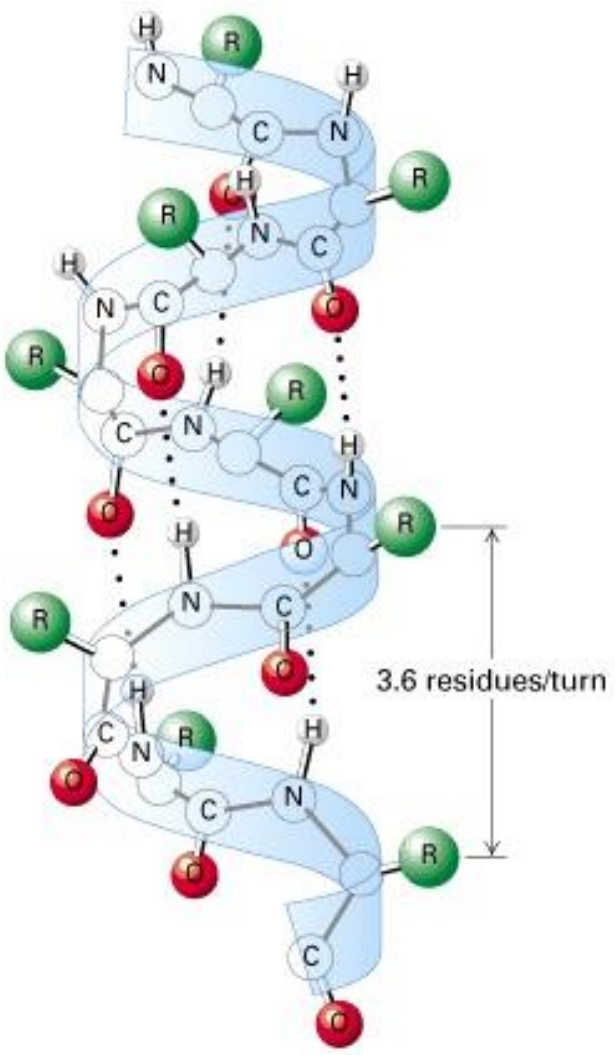


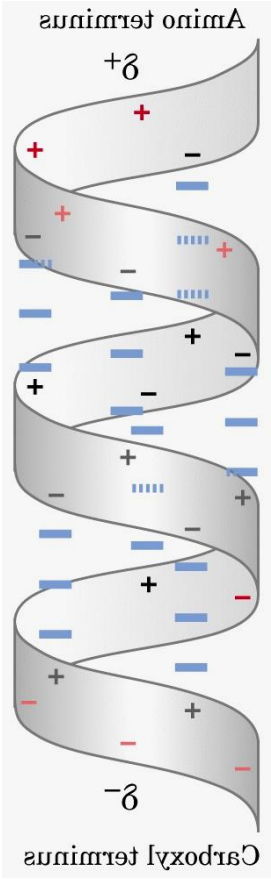
CONFORMAZIONE DI UNA SINGOLA CATENA DELLA TRIPLICE ELICA DEL COLLAGENO

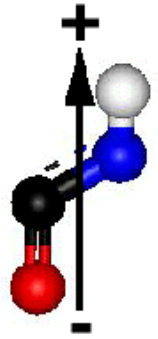


NB. Non è una struttura secondaria ad a elica

Struttura secondaria: l'α elica

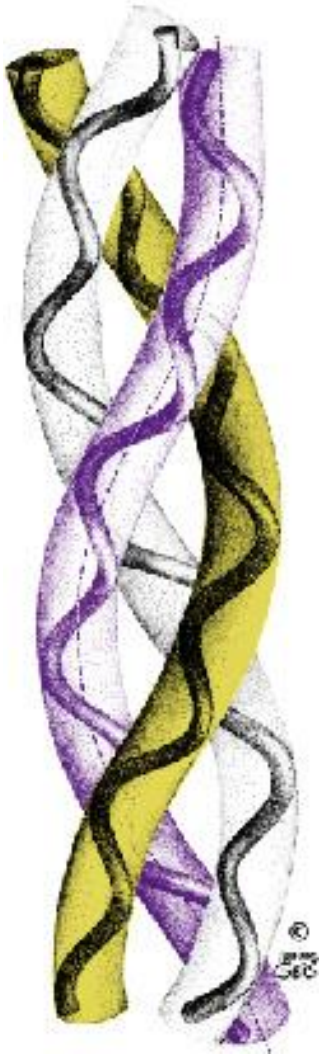


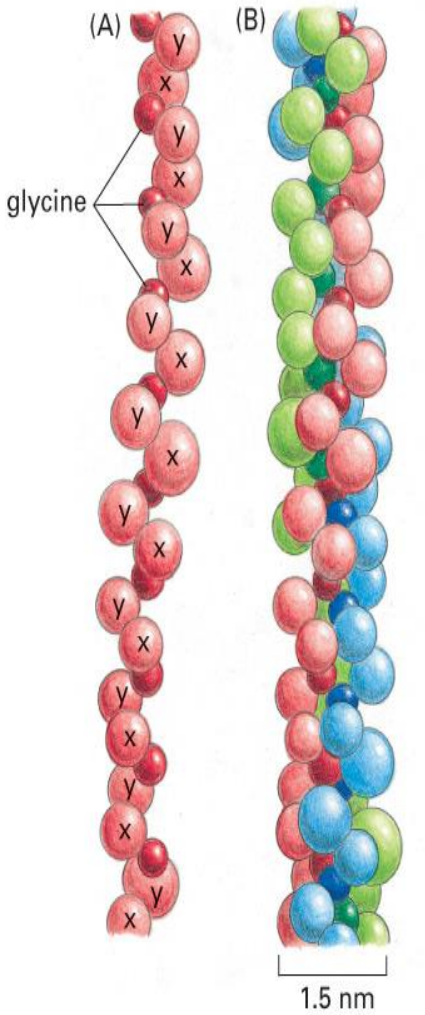




Il **dipolo elettrico** di un legame peptidico viene trasmesso lungo l'elica mediante i legami idrogeno intercatena, generando un dipolo complessivo dell'elica

Tripla elica del Collageno





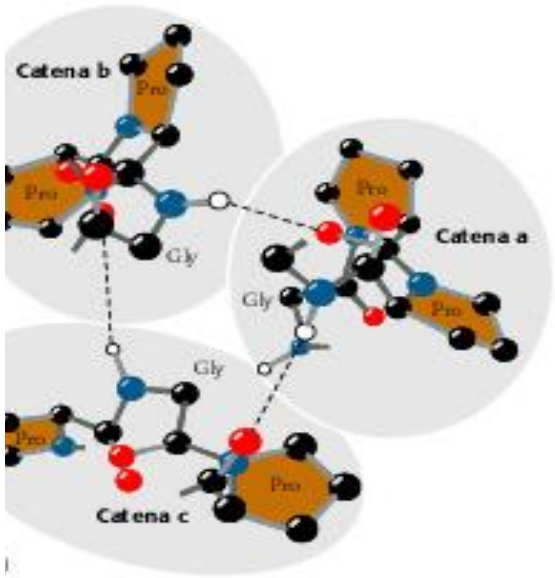
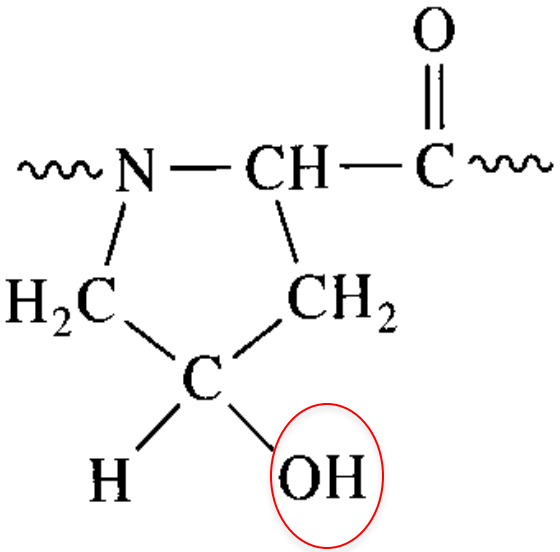
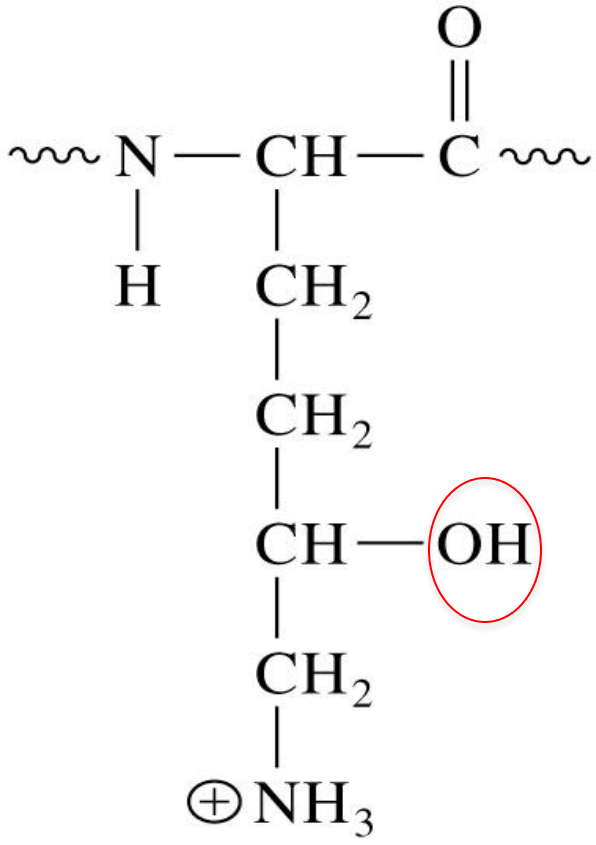


Figure 19–43. Molecular Biology of the Cell, 4th Edition.

Modificazioni postraduzionali nel collageno



idrossiprolina



idrossilisina

Idrossilazione di PRO e di LYS

PRO + O_2 + α-chetoglutarato → HyPRO + CO_2 + succinato

LYS + O_2 + α-chetoglutarato → HyPRO + CO_2 + succinato

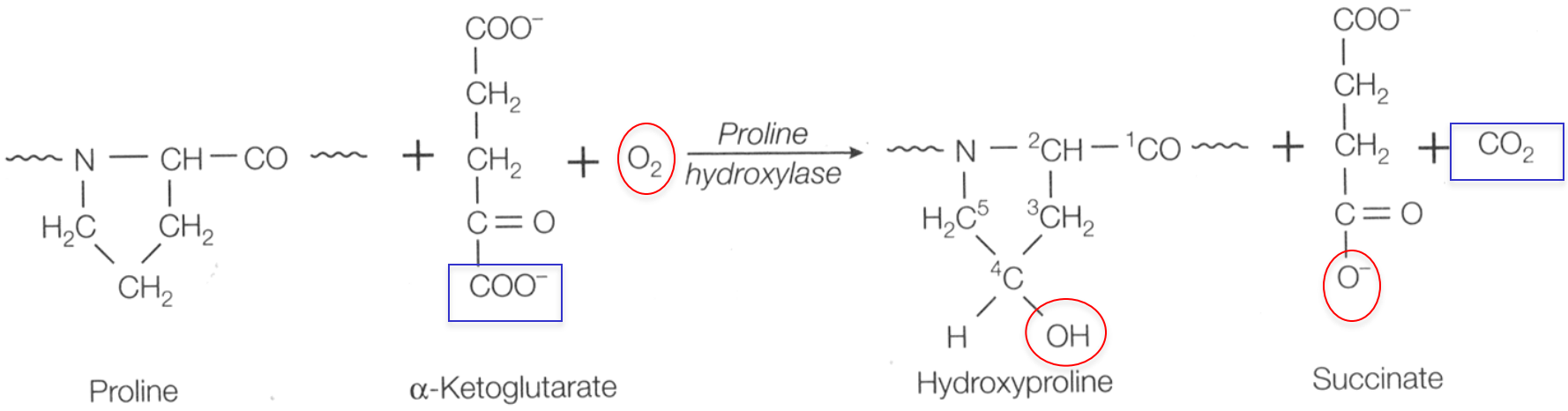
- Enzimi: prolil- idrossilasi, lisil-idrossilasi
- Fe^{++}
- acido ascorbico (vitamina C)
- O_2

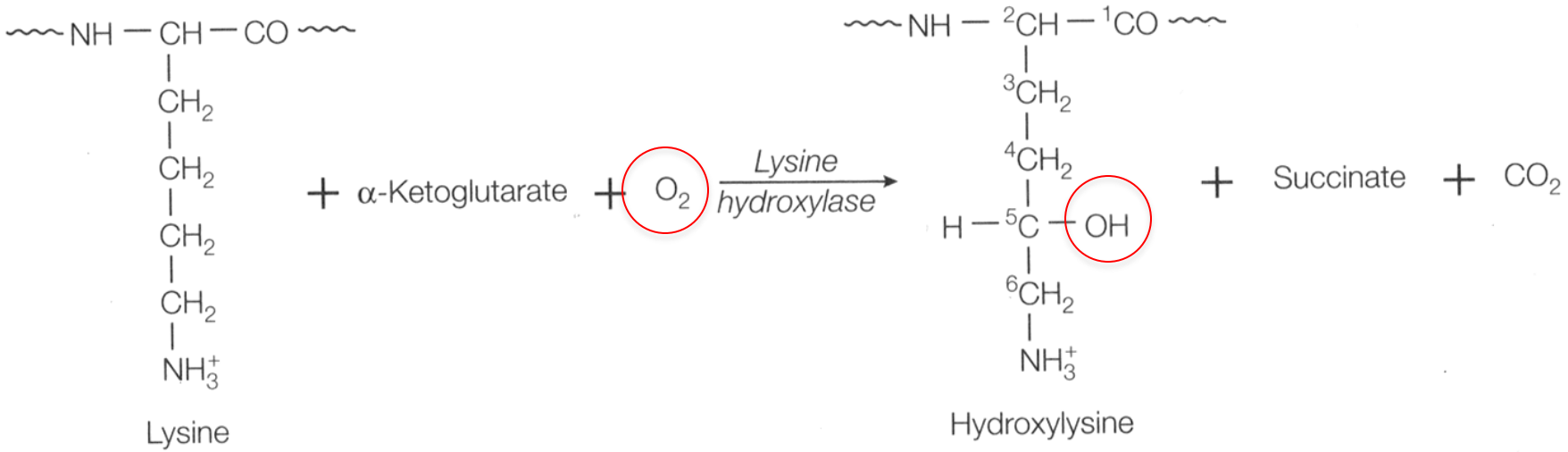
-Prolil- e lisil-idrossilasi sono diossigenasi:

trasferiscono entrambi gli atomi di O2 su 2 diverse molecole;

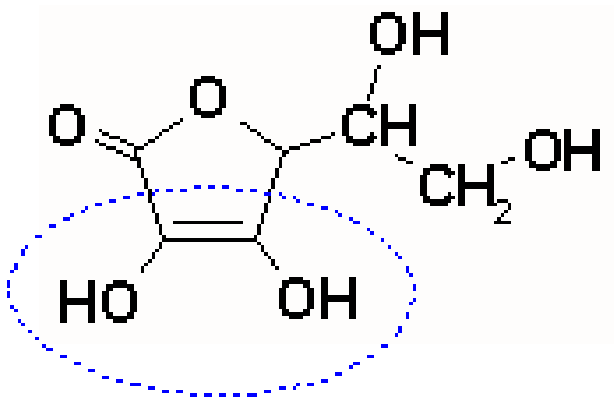
-sono sito-specifiche: una prolina puo' essere idrossilata solo se si trova in posizione –X-PRO-Gly

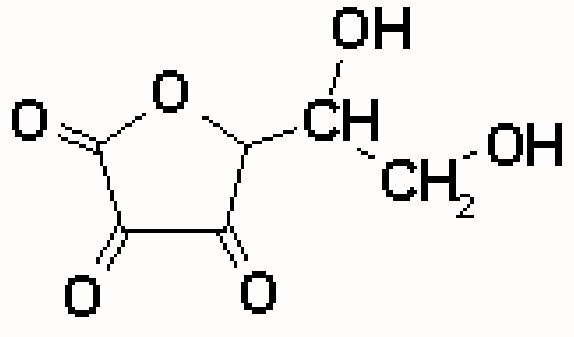
Idrossilazione di PRO e di LYS





ascorbic acid





dehydroascorbic acid

functions: selected hydroxylation reactions;
intestinal iron absorption

(1) electron donor in collagen hydroxylations and in the synthesis of carnitine and catecholamines;
(2) non-enzymatic uptake of iron in gastrointestinal tract;
(3) soluble anti-oxidant in blood and tissues

Glicosilazione del collageno

Aggiunta di carboidrati (glucosio e galattosio) che conferiscono rigidità alla molecola

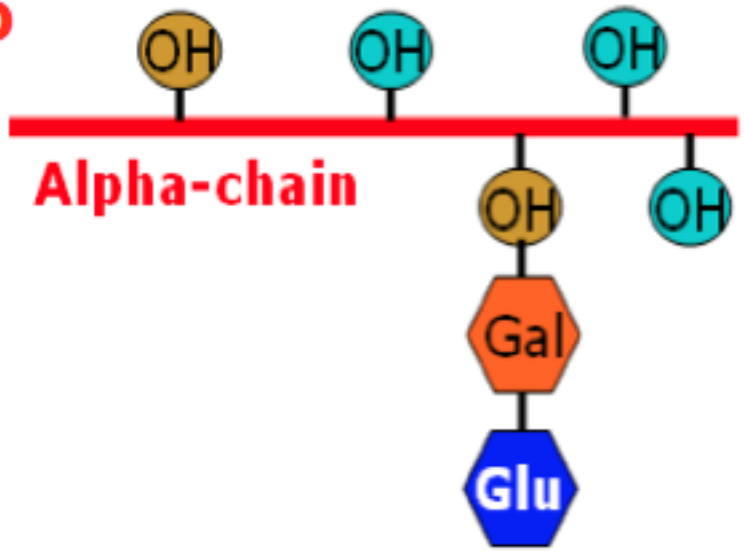
- 0.5% nella pelle
- 4% nella cartilagine
- >10% nella lamina basale

- ENZIMI: GLICOSILASI

Il Collageno e'una glicoproteina

- **Glicosilazione di alcuni residui di idrossilisina:**

  Glucosio e galattosio vengono aggiunti dagli enzimi



galattosil transferasi

glucosil transferasi

wv

Biosynthesis of collagen

- The polypeptide precursor of the collagen molecule is formed in fibroblasts (or in the related osteoblasts of bone and chondroblasts of cartilage), and is secreted into the extracellular matrix.

- After enzymatic modification, the mature collagen monomers aggregate and become cross-linked → collagen fibrils.

Biosintesi del collageno

- Tappe intracellulari
  - Trascrizione del RNA
  - Pro-catene α
  - **Idrossilazione** di Pro e Lys
  - **Glicosilazione**
  - **Assemblaggio** a formare la triplice elica

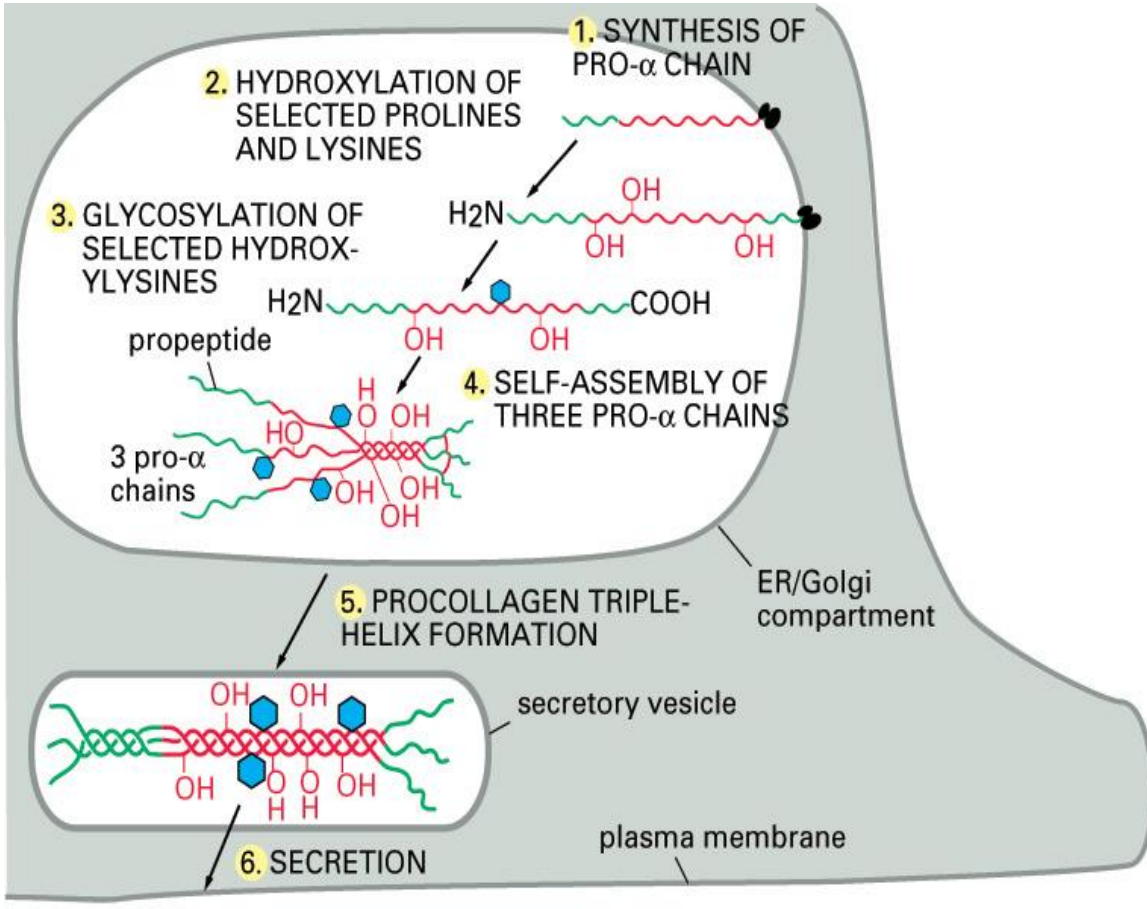


Figure 19–47 part 1 of 3. Molecular Biology of the Cell, 4th Edition.

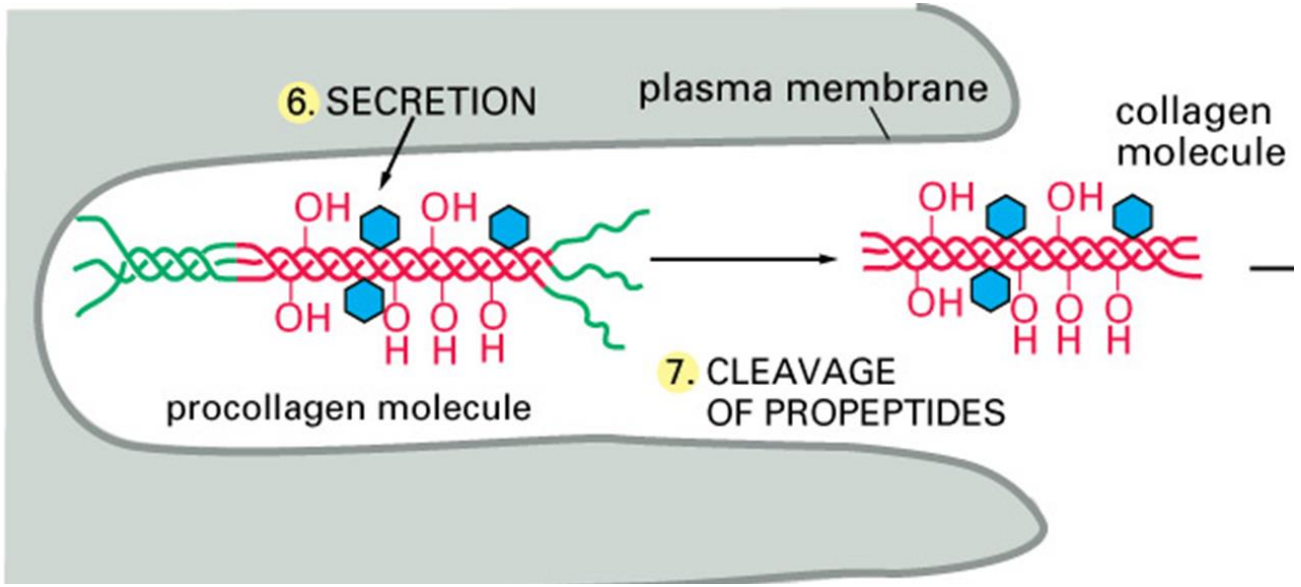
Biosintesi del collageno

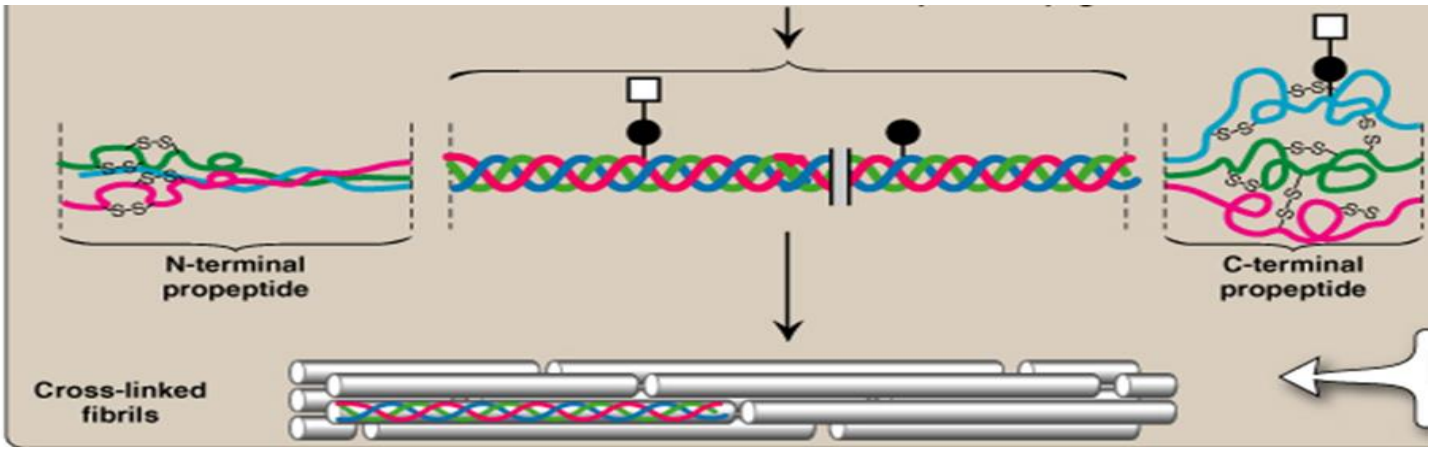
tappe extracellulari

Tappe extracellulari

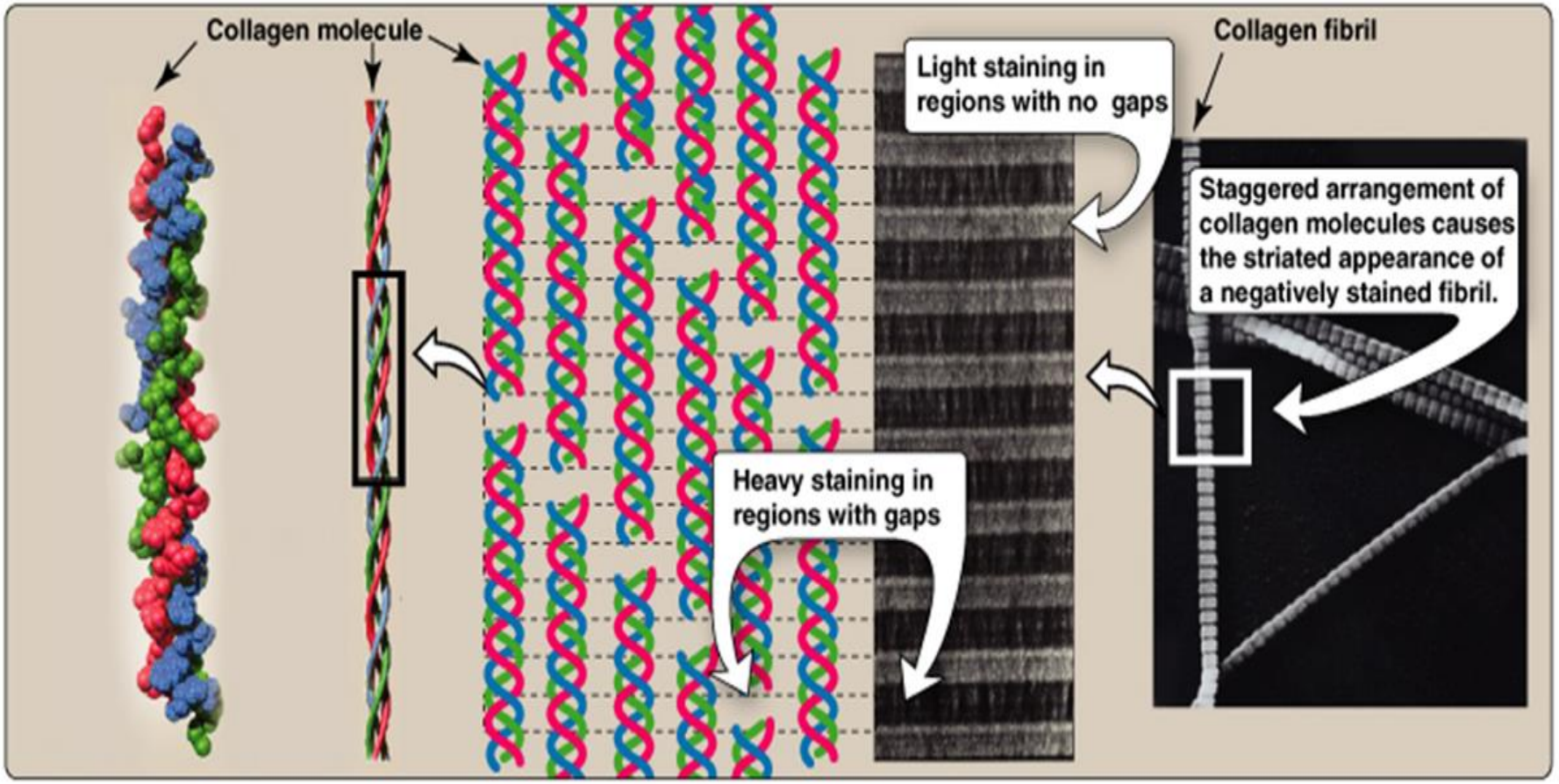
- **Proteolisi** dei C- e N-terminali del pro-collagene

- **Assemblaggio** delle molecole fibrille

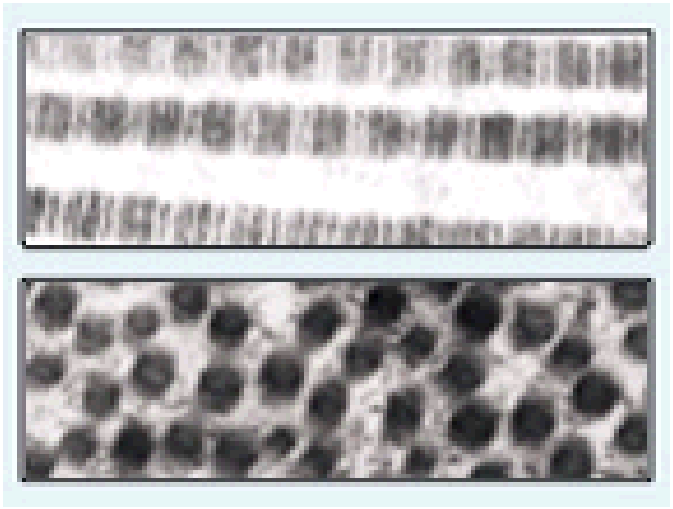


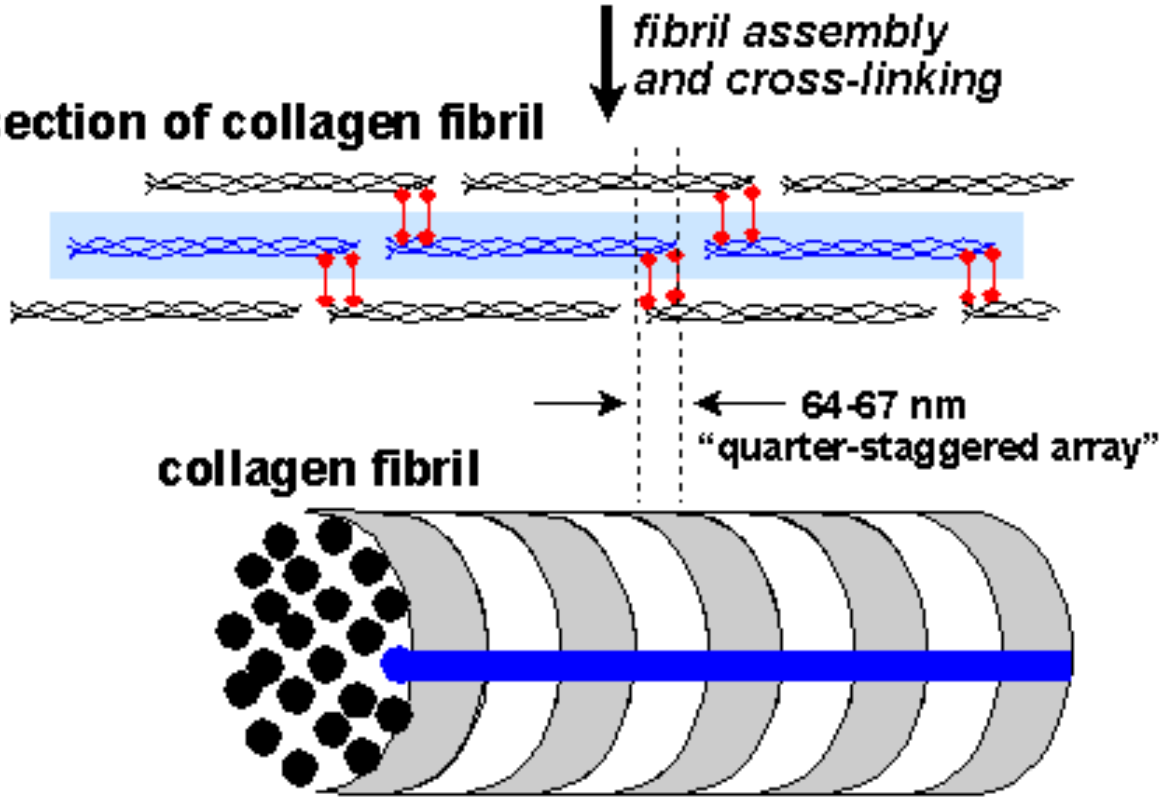


Collageno – assemblaggio in fibre

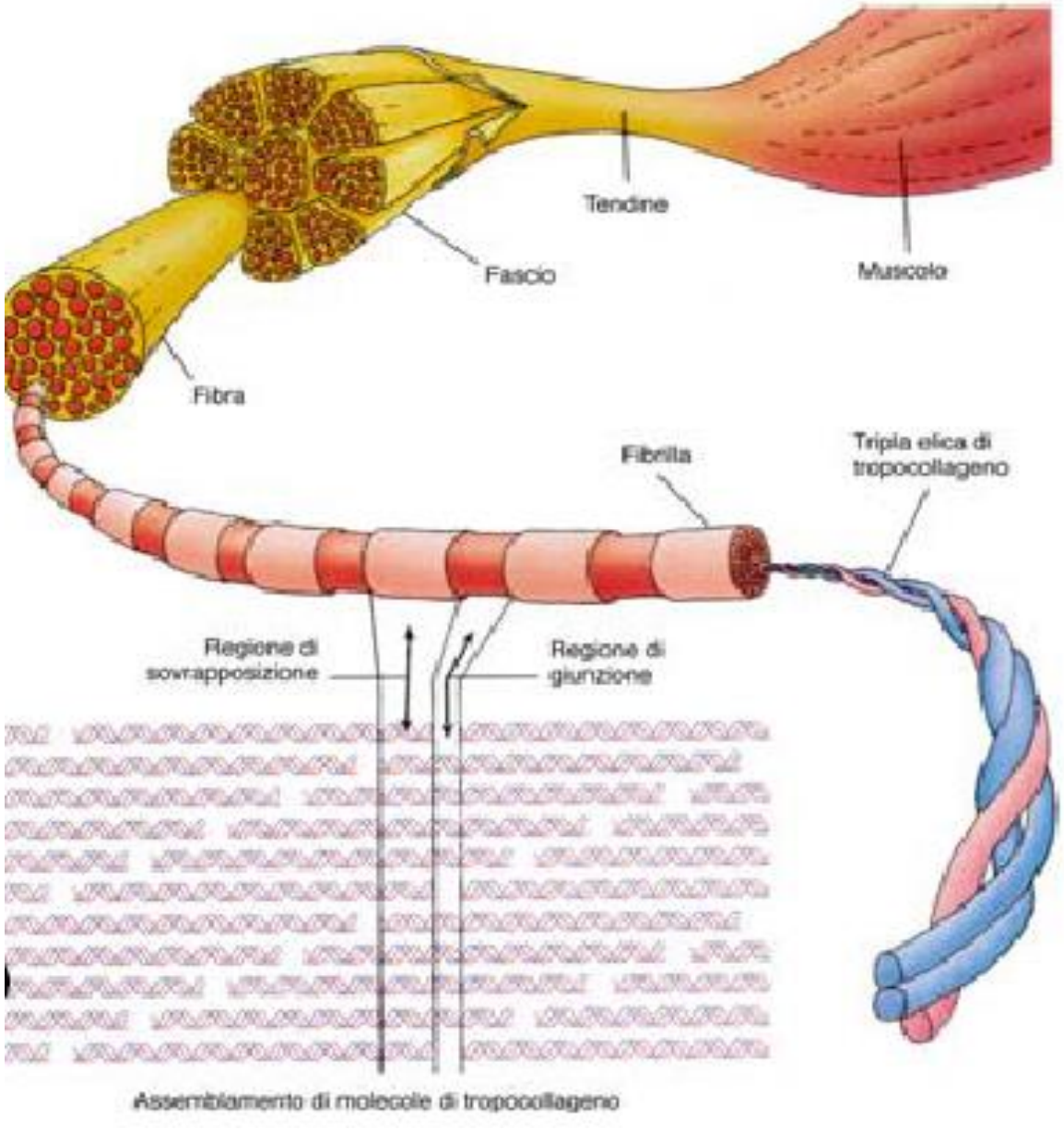


Biosintesi del collageno
assemblaggio in fibre





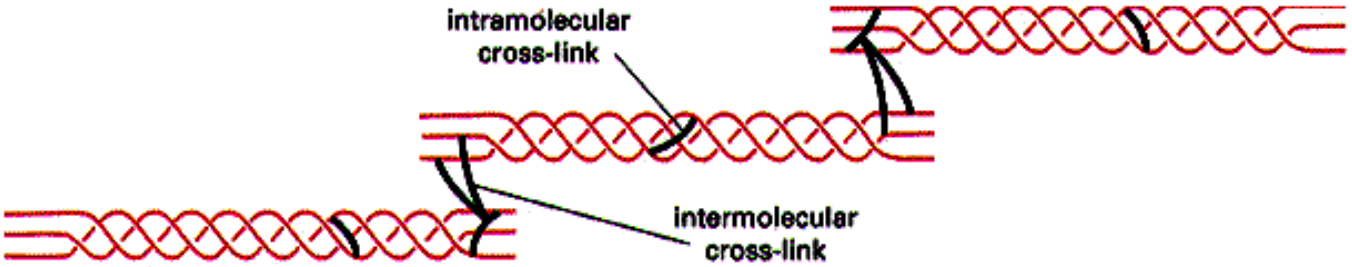
Collageno


Tendine
Fascio
Muscolo
Fibra
Fibrilla
Tripla elica di tropocollageno
Regione di sovrapposizione
Regione di giunzione
Assemblamento di molecole di tropocollageno

Legami intra - e inter-molecolari covalenti Lys-Lys

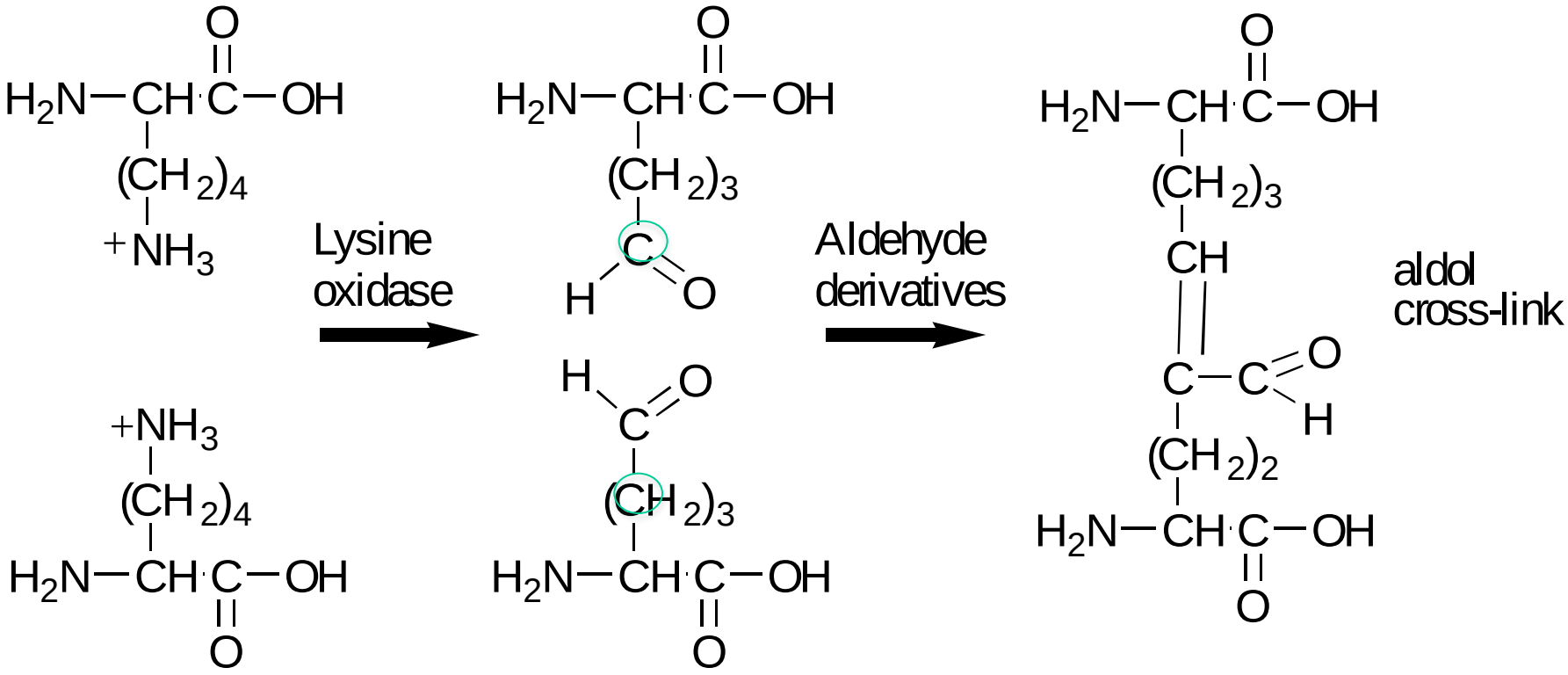
Enzima: lisil amino ossidasi

- Lys + Lys → aldeide + aldeide → cross-link aldolico
- Lys + Lys → Lys + aldeide → cross-link lysinorleucine (Base di Schiff)



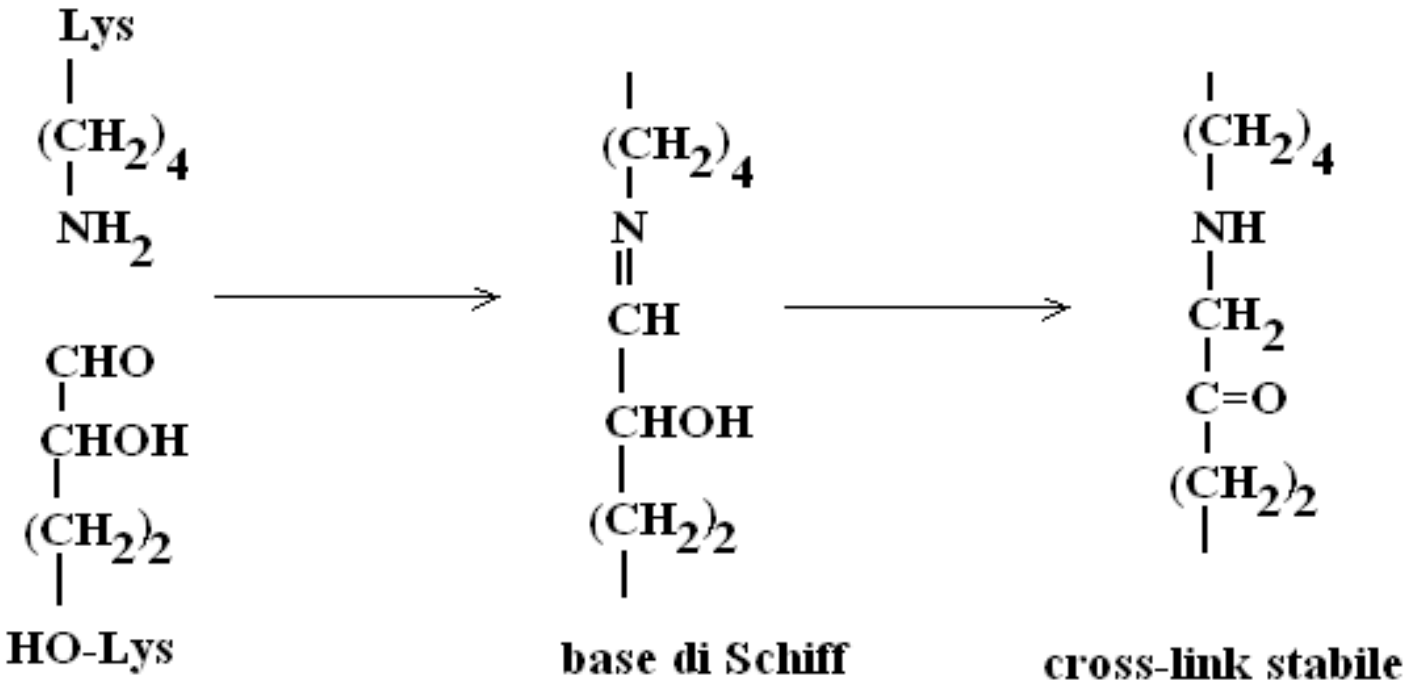
- I legami crociati conferiscono al collageno **resistenza meccanica**, **resistenza alla trazione** ed elevata stabilità chimica.
- Quantità e tipo di legami crociati variano con il tipo e l'età del tessuto

I legami crociati



La lisil ossidasi richiede Cu++ e O2

I legami crociati



Le basi di Schiff tra residui di Lys e HO-Allisina sono + stabili perché riarrangiano come mostrato sopra → importanza dell'idrossilazione (vit. C-dipendente)

Tipi di collageno (principali)

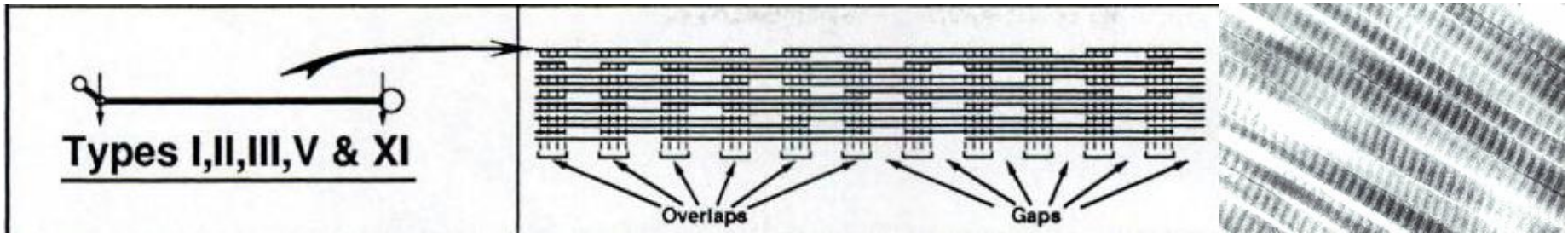
Tipo	Geni	Proteina	Distribuzione
I	Col 1A1; Col1A2	$2\alpha_1 + 1\alpha_2$	Pelle, tendini, osso, tessuto cicatriziale
II	Col 2A1	$3\alpha_1$	Cartilagine articolare, corpo vitreo
III	Col3A1	$3\alpha_1$	Tessuto di granulazione, pelle, muscolo (insieme al tipo I), feto
IV	Col4A1; Col4A2 **Non fibrillare**	$2\alpha_1\ 1\alpha_2$	Membrane basali; cristallino
V	Col 5A1; Col5A2 **Non fibrillare**	$2\alpha_1\ 1\alpha_2$	**Tessuto interstiziale** (insieme al tipo I)

TYPE	TISSUE DISTRIBUTION
	Fibril-forming
I	Skin, bone, tendon, blood vessels, cornea
II	Cartilage, intervertebral disk, vitreous body
III	Blood vessels, fetal skin
	Network-forming
IV	Basement membrane
VII	Beneath stratified squamous epithelia
	Fibril-associated
IX	Cartilage
XII	Tendon, ligaments, some other tissues

Classification of collagen

1. Fibril-forming collagens

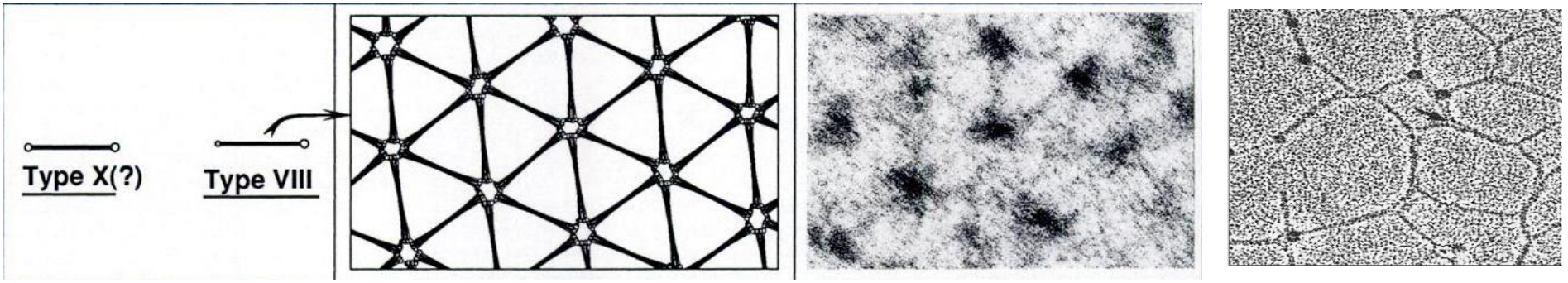
- No interruptions in triple helix
- Regular arrangement results in characteristic "D" period of 67 nm
- Diameter : 50-500 nm
- Example : Types I, II, III, V, XI



Classification of collagen

2. Network-forming collagens

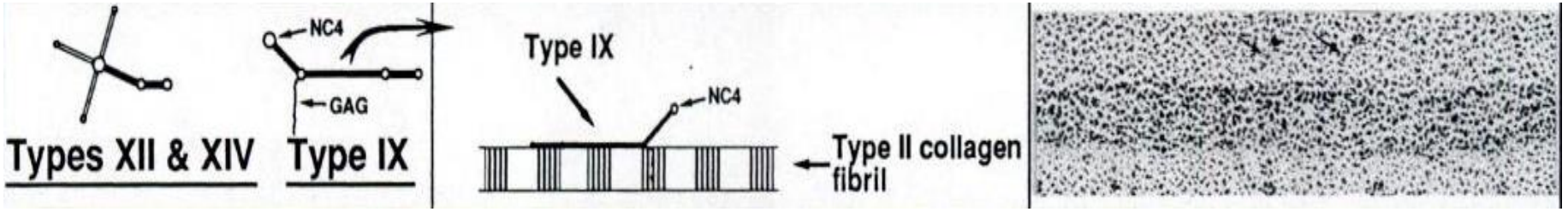
- Forms network in basement (Collagen IV) and Descemet's membrane (Collagen VIII)
- Molecular filtration
- Example : Types IV, VIII, X



Classification of collagen

3. Fibril-associated collagens with interrupted triple helices (FACITs)

- Short collagens with interruptions
- Linked to collagen II and carries a *GAG* chain
- Found at the surface of fibril-forming collagens
- Example : Types IX, XII, XIV

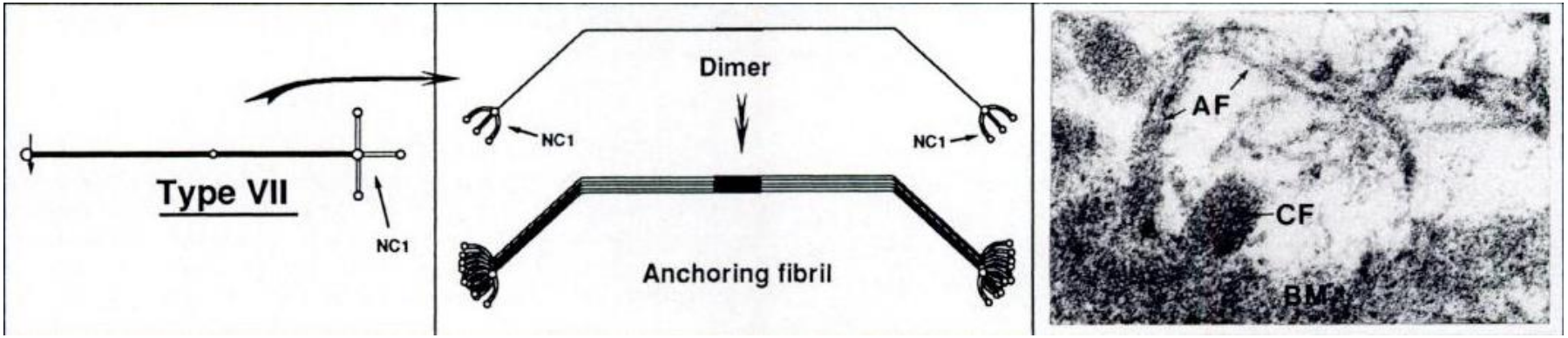


Importanti per la integrita' e plasticita' del tessuto?

Classification of collagen

4. Anchoring collagens

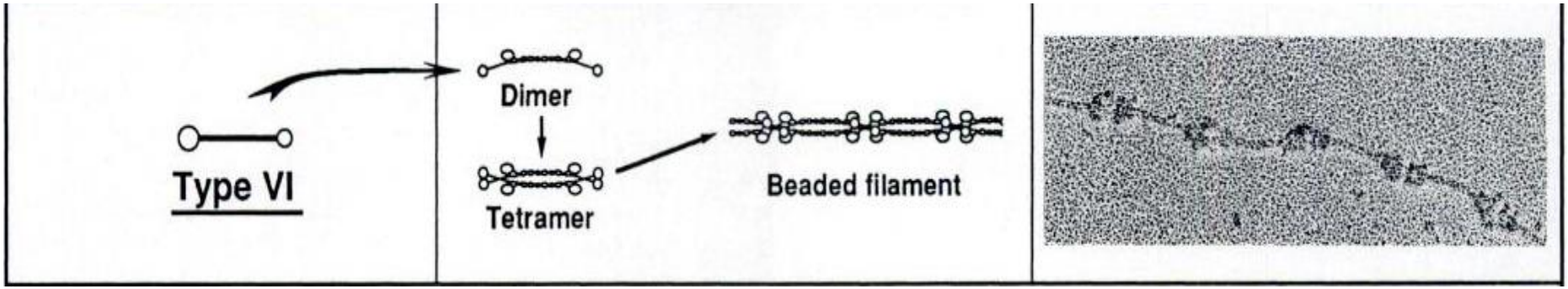
- Provides functional integrity by connecting epithelium to stroma
- Example : Type VII



Classification of collagen

5. Beaded-filament-forming collagens

- Form structural links with cells
- Example : Type VI
- Collagen VI crosslink into tetramers that assemble into long molecular chains (microfibrils) and have beaded repeat of 105 nm



Turnover del collageno

- Il turnover del collageno nei tessuti dell'adulto è molto lento.

- L'emivita della proteina è di circa 60-70 giorni.

- Il turnover del collageno è modulato "in vivo" da ormoni e dallo stato nutrizionale.

Degradation of collagen

- Normal collagens are highly stable molecules, having half-lives as long as 2-3 months.

- However, connective tissue is dynamic and is constantly being remodeled, often in response to growth or injury of the tissue.

Degradazione del collageno
ruolo delle metallo proteasi della matrice (MMPs)

- Le Collagenasi degradano il collageno fibrillare
- Le Gelatinasi degradano i collageni non fibrillari e i collageni denaturati

- Collagenasi batteriche (batterio *Clostridium histolyticum)*
- Proteasi lisosomiali: degradazione intracellulare (rimuovono i peptidi anormali)

L'attività collagenolitica è monitorabile con l'escrezione urinaria di HyPro, che non è metabolizzabile dall'organismo

PATOLOGIE PRINCIPALI DEL COLLAGENE

CONGENITE

- Mutazioni nei geni del collagene- sostituzioni aa

  Struttura molecolare anomala: Osteogenesis imperfecta

- Mutazioni nei geni che codificano per enzimi coinvolti nella maturazione post-traduzionale del collagene

  Sindromi di Ehlers-Danlos

ACQUISITE

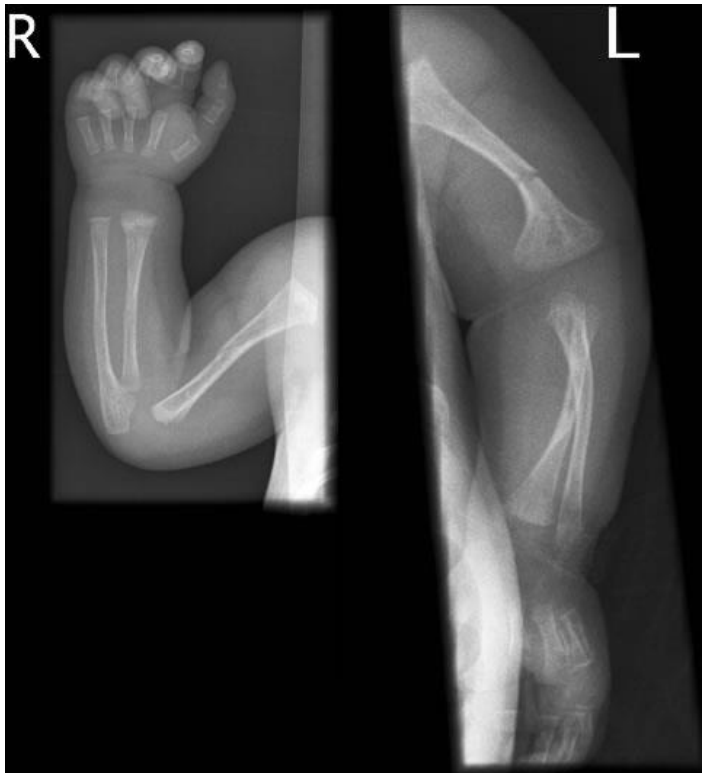
- Scarso apporto di vitamina C (acido ascorbico)

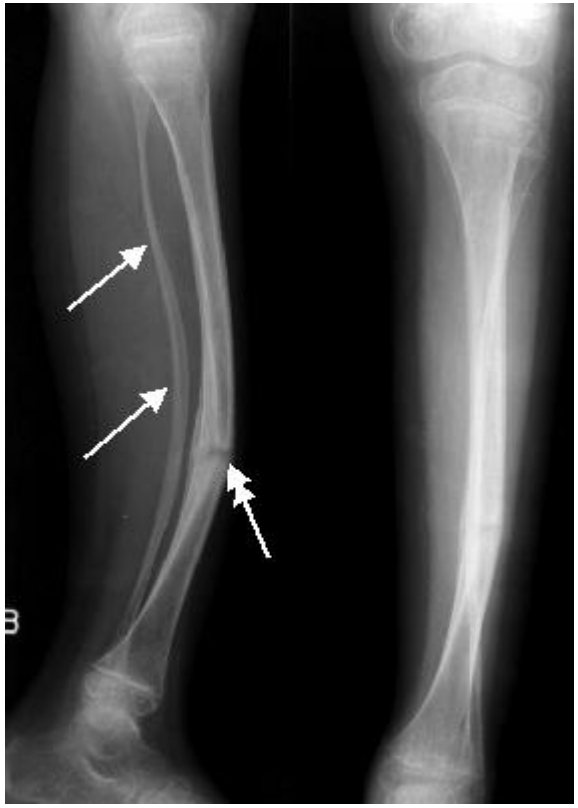
  Scorbuto

Osteogenesi Imperfetta

- **Almeno 4 tipi con diversa patogenesi e gravità dei sintomi, tutte hanno in comune una** alterata sintesi di collagene tipo I (mutazione nei geni Col 1 A1 e Col 1 A2)
- **Incidenza:** <1:10.000
- **Tipo I è il meno grave (mutazioni multiple): fratture osse in età pediatrica**
- **Tipo II (mutazione di una singola glicina) è il piu' grave: gravi deformazioni ossee, insuff. resp., morte pre- o immed. post-natale**

Osteogenesi imperfetta

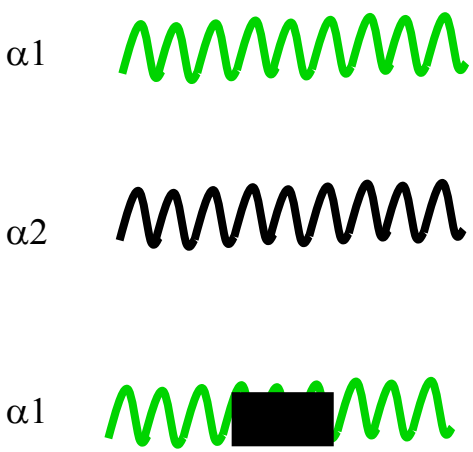


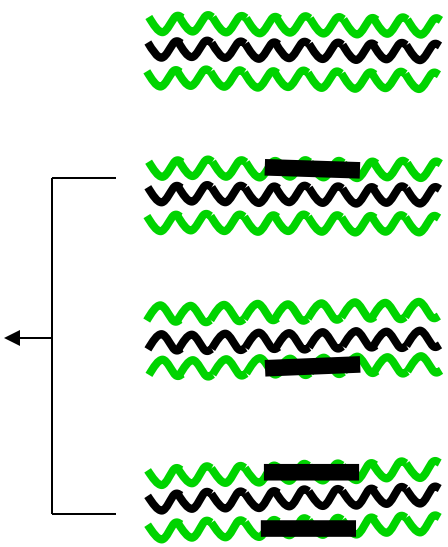


Deformità ossee, deficit accrescimento, fratture multiple spontanee, sordità, dentinogenesi difettosa.

Perche' la Osteogenesi imperfetta e' una patologia dominante

MUTAZIONE IN UN ALLELE DEL GENE PER LA CATENA α1





Nonostante un solo allele del gene COL1A1 e' mutato ben il 75% delle triple eliche sono anomale.

La mutazione riguarda il 50% del prodotto genico, ma influenza il 75% della proteina. L'EFFETTO E' DOMINANTE anche in caso di eterozigosi

Una mutazione dominate negativa è una mutazione che porta alla formazione di un prodotto mutato il quale "interferisce" anche con la funzione di quello normale.

Sindrome di Ehlers Danlos

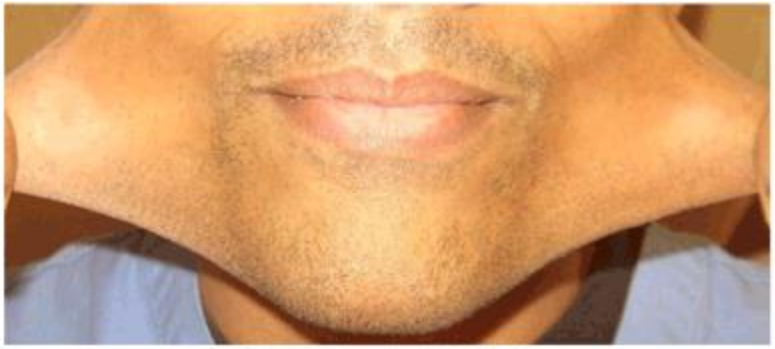


Figure 1—Hyperelastic facial skin.

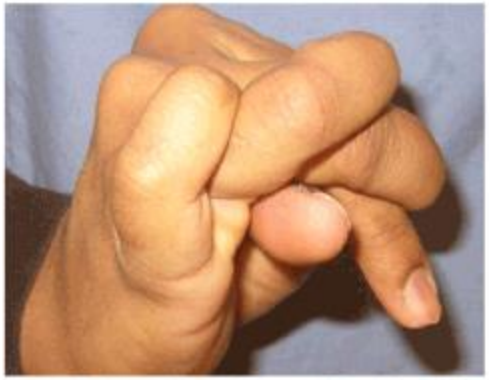
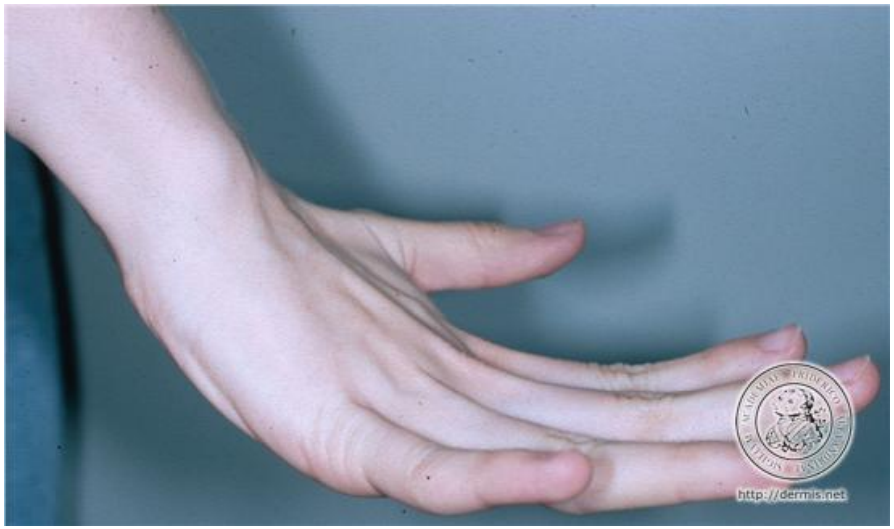
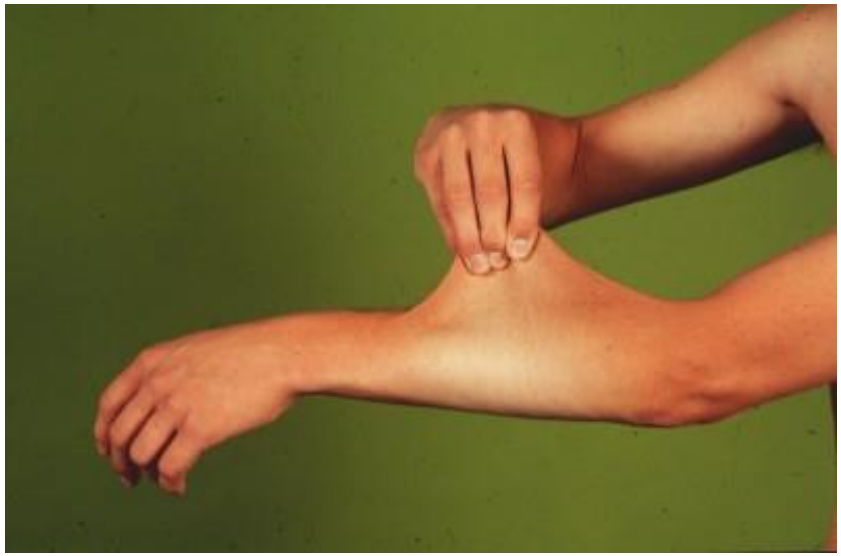


Figure 2—Extreme laxity and hypermobility of finger joints.

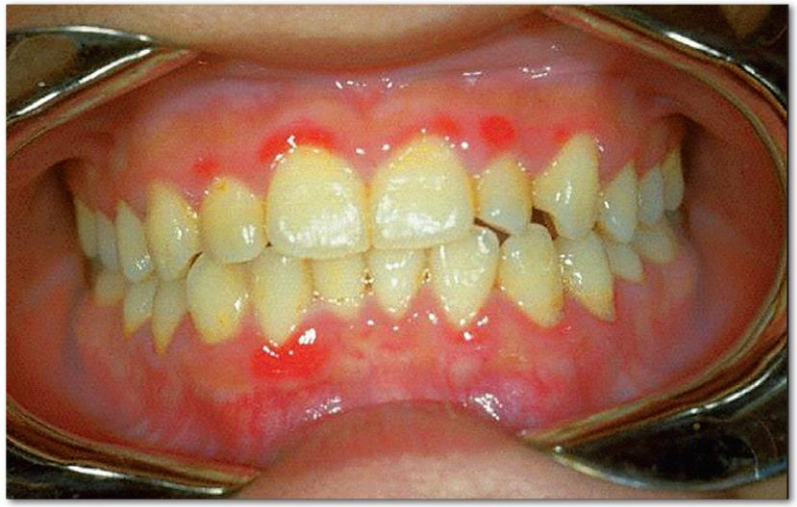


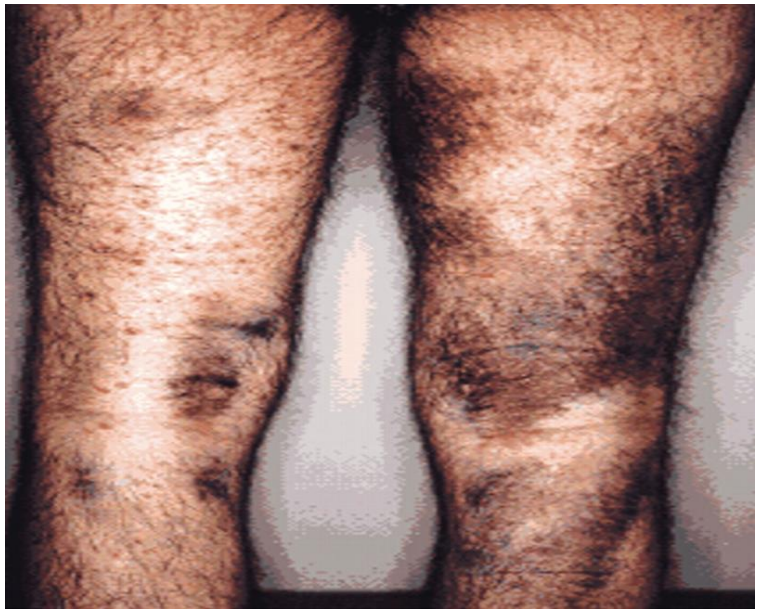


Iperlassità di legamenti; ipermobilità articolare; aneurismi aortici; emorragie interne (intestino, utero)

Lo scorbuto

- diminuita stabilità della tripla elica per riduzione dei cross-links covalenti tra fibrille (tutti i tipi di collageno)
- Fragilità capillare, ematomi ed emorragie, lenta cicatrizzazione ferite, deficit accrescimento nei bambini.





Sindrome di Marfan

Mutazione della **fibrillina** (FBP1, cr. 15), glicoproteina che forma microfilamenti cui aderisce l'elastina nel tessuto connettivo (particolarmente nella parete vasale e nel cristallino).

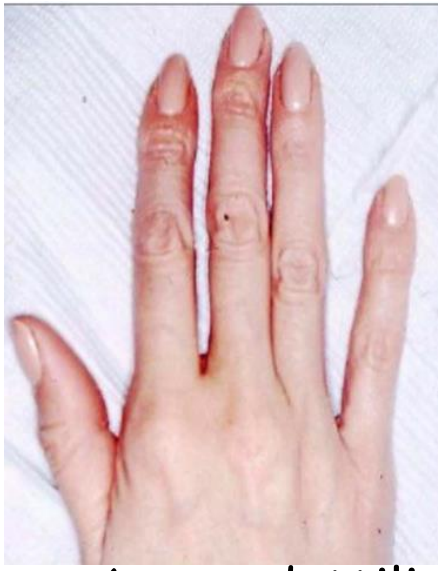
Incidenza: 1:5.000 (la + frequente tra le malattie del "collagene")

Trasmissione: Aut. Dom

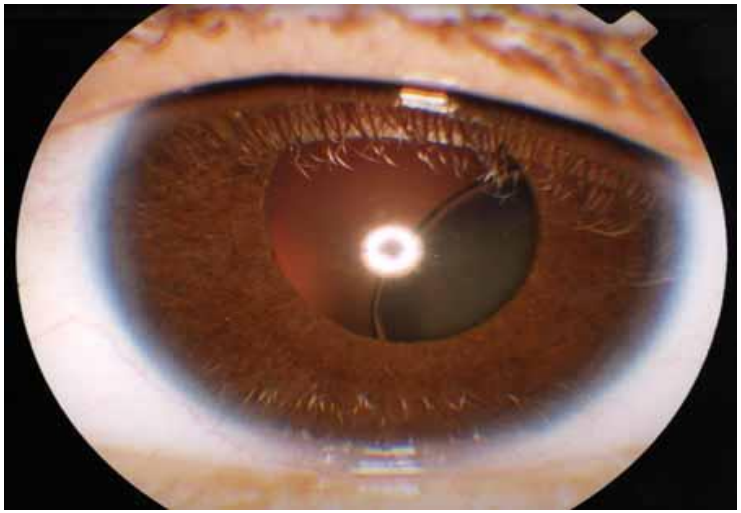
Lussazione cristallino, dilatazione-aneurismi aorta e difetti valvolari cardiaci (prolasso mitrale).
Arti e dita lunghi e sottili (aracnodattilia).
Ipermobilità articolare, scoliosi.

Sindrome di Marfan

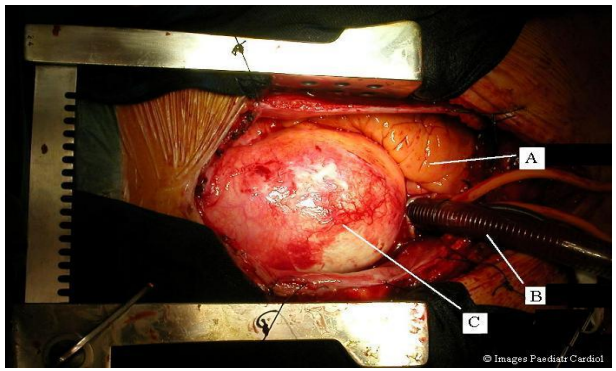
Iperlassità legamenti



Aracnodattilia



Lussazione cristallino



Aneurisma aorta ascendente

Elastin

- The main component of elastic fibers is elastin

- A highly hydrophobic protein, which, like collagen, is unusually rich in **proline** and **glycine**
- But, unlike collagen, is not glycosylated
- Contains some **hydroxyproline** but no hydroxylysine

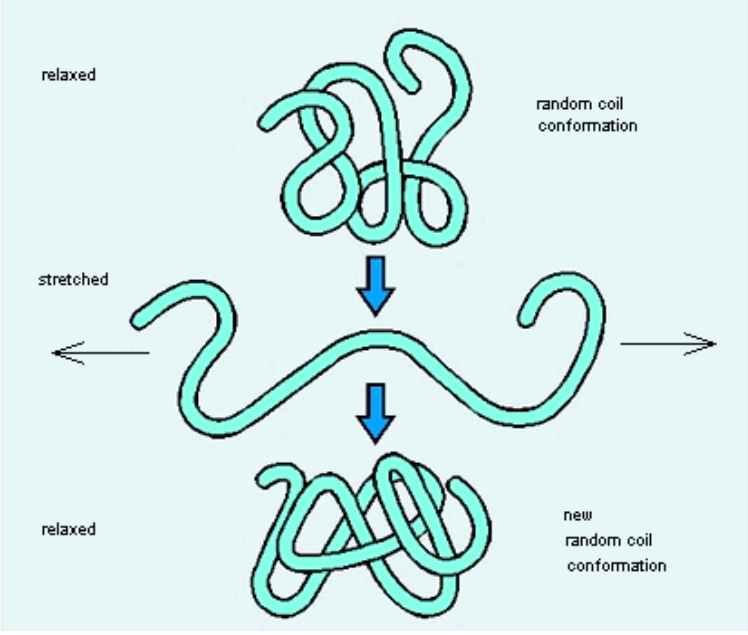
- Elastin is common in many connective tissues, along with collagen, especially if the tissue undergoes physical stress. It surrounds arteries, is in the lung and in ligaments.

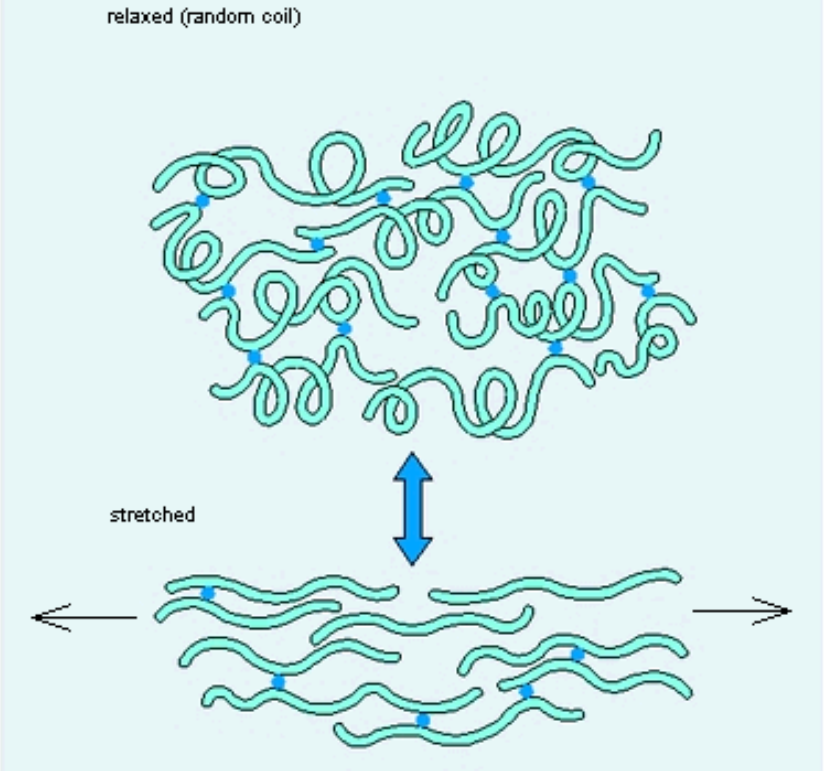
Elastin structure

- The elastin protein is composed largely of two types of short segments that alternate along the polypeptide chain:
  - hydrophobic segments, which are responsible for the elastic properties of the molecule;
  - alanine- and lysine-rich a-helical segments, which form cross-links between adjacent molecules

Elastin

- As a result, its has uncommon secondary structure (more *random structure* than found in other proteins): beta spiral. It does not have a stable tertiary structure.
- Elastin is very resilient. It can be stretched to lengths many times greater than in its relaxed state. It can also be compressed.





Role of α_1-antitrypsin in elastin degradation

- Blood and other body fluids contain a protein, α_1-antitrypsin (α_1-AT) or (α_1-antiproteinase) $\rightarrow$ inhibits a number of proteolytic enzymes (*proteases* or *proteinases*) $\rightarrow$ hydrolyze and destroy proteins.

- 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.

Role of α_1-AT in the lungs

- In the normal lung, the alveoli are chronically exposed to low levels of *neutrophil elastase,* which on the contrary is 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).*
- Because lung tissue cannot regenerate, emphysema results from the destruction of the connective tissue of alveolar walls.

Emphysema resulting from α_1-AT deficiency

- inherited defects in α_1-AT $\rightarrow$ ~ 2-5% of patients having emphysema.
- A number of different mutations in the α_1-AT gene $\rightarrow$ deficiency of this protein, but one single purine base mutation (GAG $\rightarrow$ AAG, $\rightarrow$ substitution of lysine for glutamic acid at position 342 of the protein) is clinically the most widespread.

Enfisema e deficienza di α_1-antitripsina

- An individual must inherit 2 abnormal α_1- AT alleles to develop 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, but if he his a smoker…….

- Smokers with α_1-AT deficiency (heterozygous), have ↑↑ rate of lung destruction → poorer survival rate than nonsmokers with the deficiency.

- A specific α_1-AT methionine is required for the binding of the inhibitor to its target proteases.

- Smoking → oxidation of methionine residue → the inhibitor is unable to bind elastase.