Le peroxyde d'hydrogène : rôle dans l'assemblage de la matrice extracellulaire et les interactions cellule-matrice

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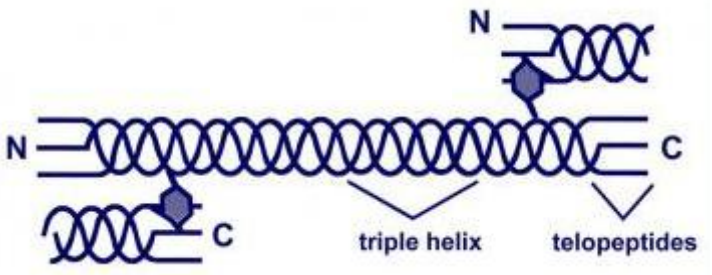
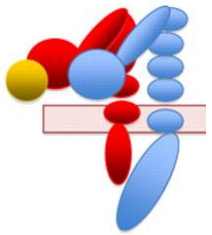
Hydrogen peroxide plays a role in extracellular matrix assembly and in cell-matrix interactions

- **Extracellular matrix** (ECM) **overview**
- ECM **supramolecular assemblies**
- **H_2O_2** and **cross-linking of collagens and elastin**
- **ROS** and **cell adhesion**

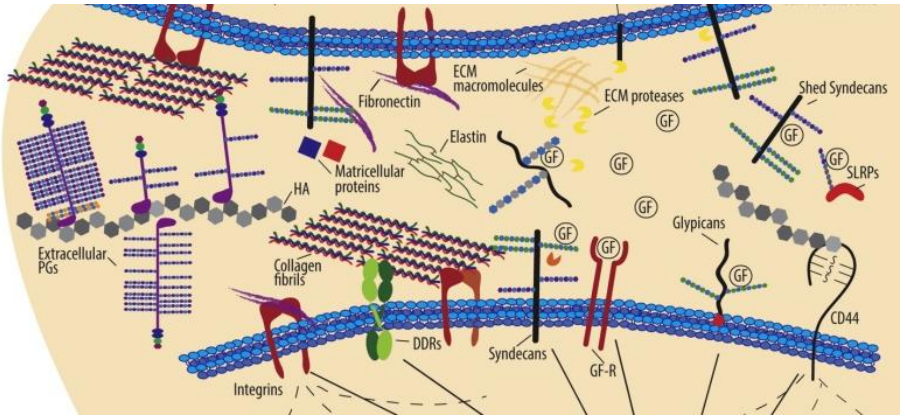




The human extracellular matrix

~ 1000 proteins

- The **core matrisome**
- **Matrisome-associated proteins**



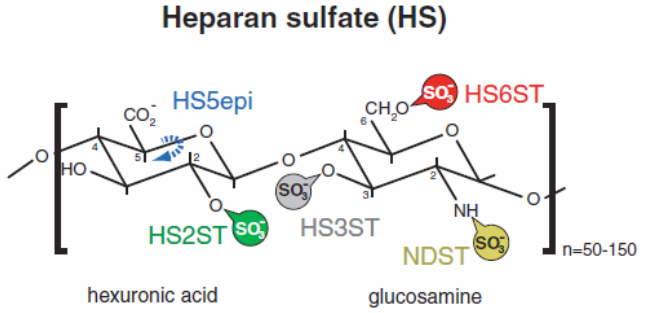
Proteoglycans: protein core + glycosaminoglycans

43 genes, more protein isoforms

6 glycosaminoglycans (GAGs)

e.g. heparin, heparan sulfate

hyaluronan

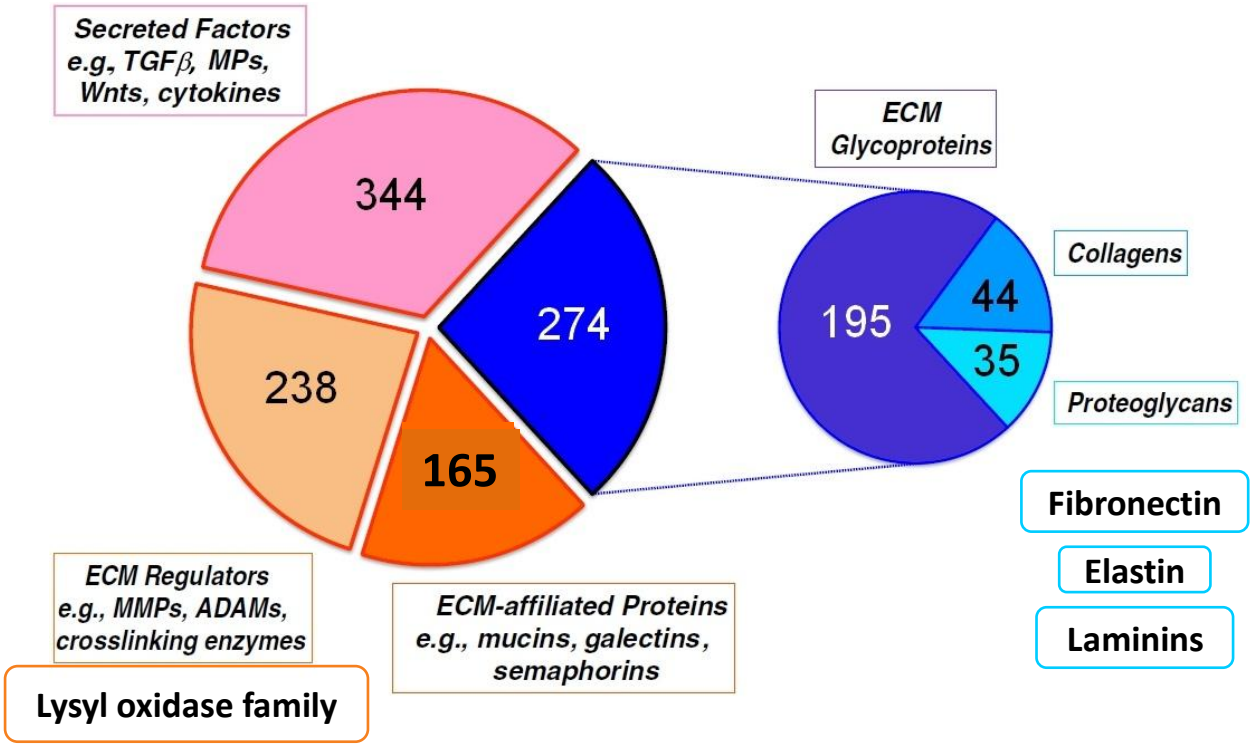


*(*Naba et al. Matrix Biol 2012 31:371-2, Peysselon et al. , 5. Structure and Function of Biomatrix. Control of Cell Behavior and Gene Expression. Ed A. Balazs. Matrix Biology Institute, Edgewater, New Jersey, USA 2012, pp 277-298, Iozzo and Schaefer 2015 Matrix Biol 42: 11-55)*

Molecular composition of the human ECM

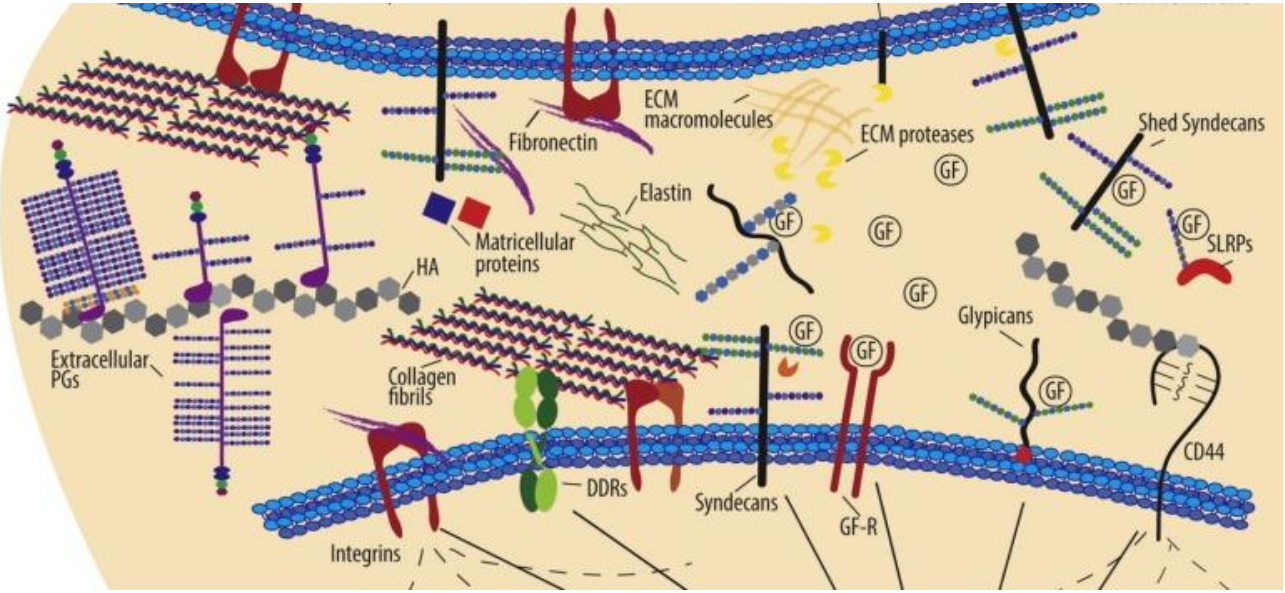
Matrisome- associated proteins (273)

Core matrisome 274 proteins



(Naba et al. Towards definition of an ECM parts list: an advance on GO categories Matrix Biol 2012 31: 371-372, http://matrisomeproject.mit.edu/)

An integrative view of the extracellular matrix (ECM)



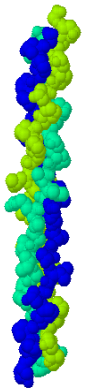
(Theocharis et al. 2016 Adv Drug Deliv Rev. 97:4-27)

A dynamic 3D network of **protein**-protein and protein-**glycosaminoglycan interactions**, which is **context-dependent**

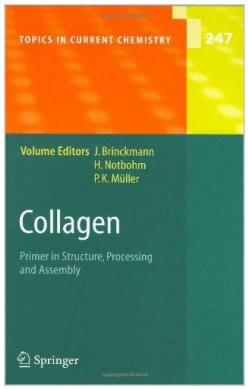
Structure - interaction - function relationships

The collagen superfamily: 28 types (I-XXVIII)

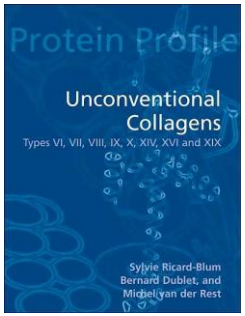
- Collagen **II** **1969**
- Collagen **XXVIII** **2006**
- α1 chain of collagen XXIX = α5 chain of collagen VI **2008**



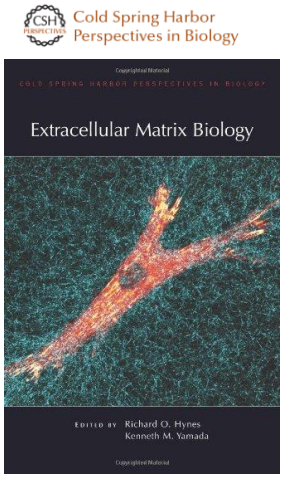
The most abundant proteins in mammals



(Ricard-Blum et al. 2005 Topics Curr Chem 247: 35-84)



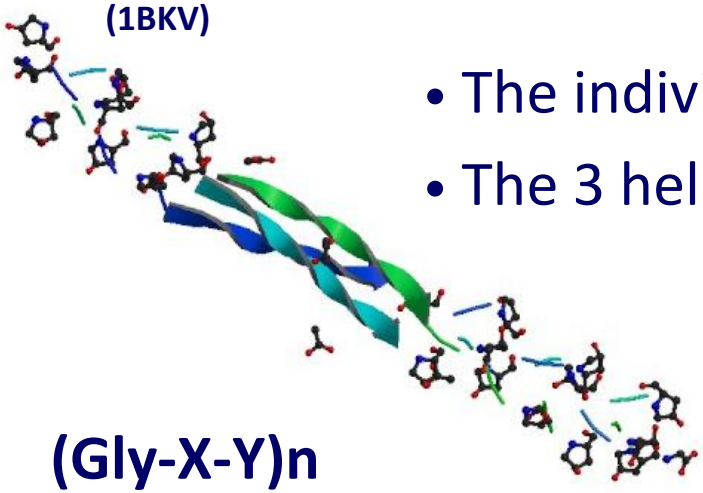
(Ricard-Blum et al. 2000 Protein Profile Series (Oxford University, Press)



(Ricard-Blum S. 2011 Cold Spring Harb Perspect Biol 3:a004978)

(Miller and Matukas Proc Natl Acad Sci USA 1969 64:1264-8, Veit et al. J Biol Chem 2006 281:3494-504, Fitzgerald et al. J Biol Chem 2008 283: 20170-80)

A common structural motif: the triple helix

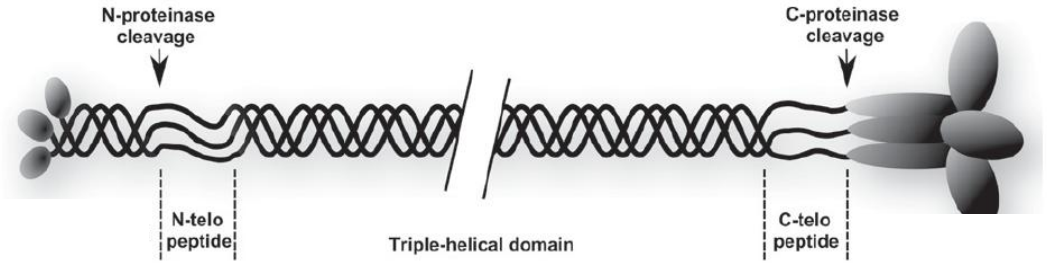


(Gly-X-Y)n

- The individual α chains fold into a polyproline II helix
- The 3 helices form a right-handed triple helix

(Kramer et al. J Mol Biol 2001 311:131-47, Shoulders & Raines Ann Rev Biochem 2009 78: 929-58)

One major triple-helix in fibrillar collagens



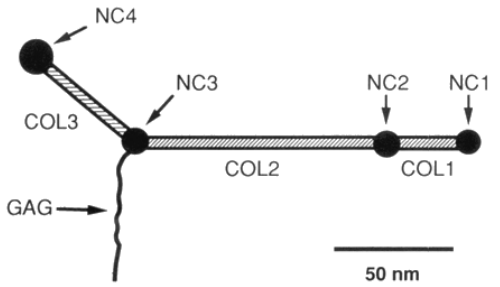
N-propeptide 50 kDa

(Boudko et al. Int J Biochem Cell Biol 2012 44:21-32)

C-propeptide 90 kDa

Several triple-helical domains interspersed by non-collagenous domains

FACITs (**F**ibril-**A**ssociated **C**ollagens with **I**nterrupted **T**riple Helices)

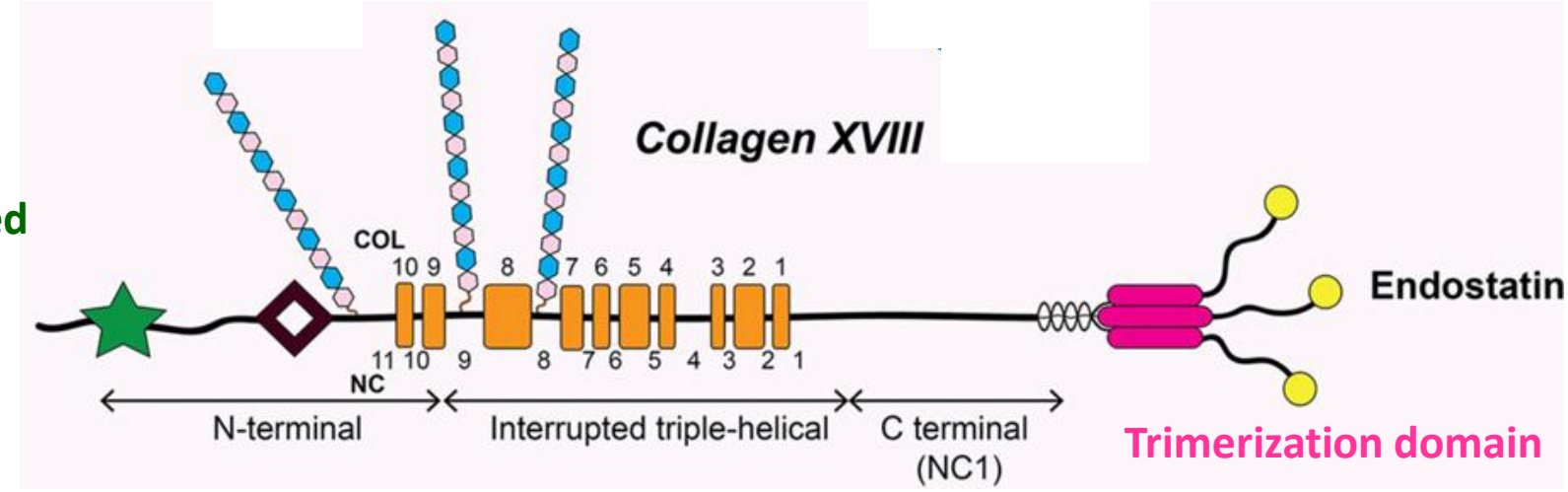


The structure of collagen IX

van der Rest et al. J Biol Chem 1985 260:220-5
Ricard-Blum et al. FEBS Lett 1982 146:343-7
Ricard-Blum et al. J Cell Biochem 1985 27:147-58

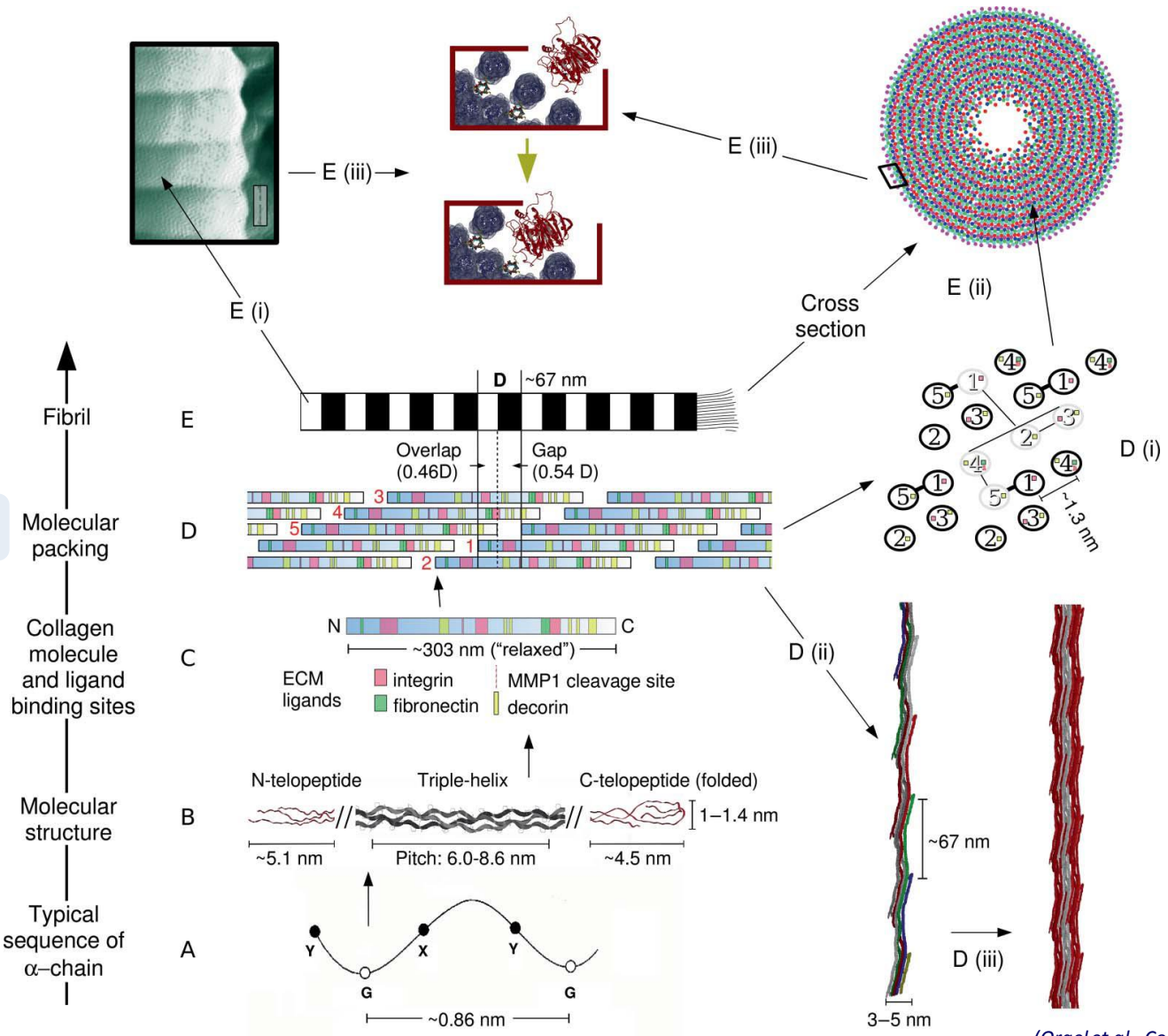
Multiplexins

Multiple triple-heli**x** domains and **in**terruptions



Schaefer and Iozzo Matrix Biol 2015 42: 11–55)

Organizational hierarchy of fibrillar collagen I



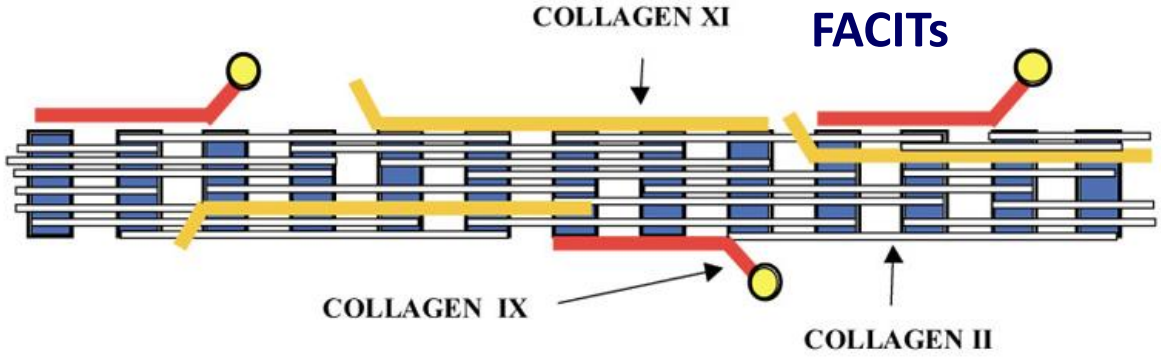
Cross-linking

(Orgel et al.. Connect Tissue Res. 2011 52:2-17)

Collagen fibrils are macromolecular alloys

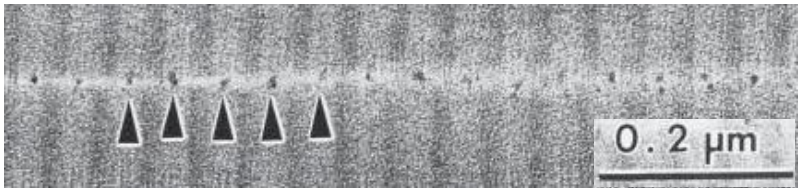
Several collagen types
(heterotypic fibrils)

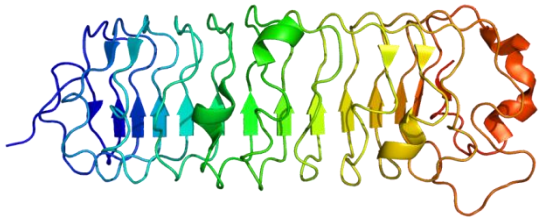
(Reginato, Olsen Arthritis Res 2002 4:337-345)



Proteoglycans bind via their protein core to collagen I fibrils

(Danielson et al. 1997 J Cell Biol 136:10729–743)

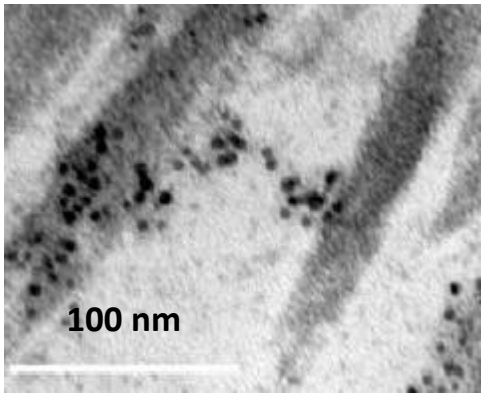




Decorin

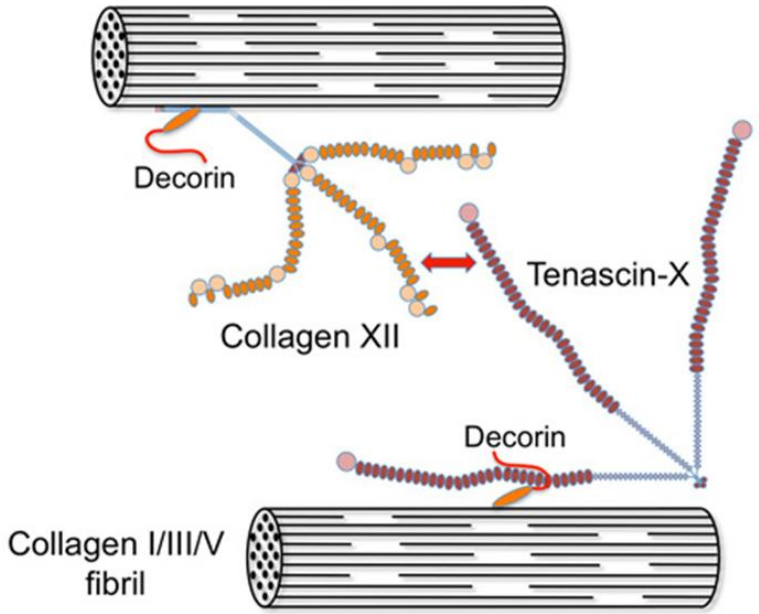
Cross-bridging of collagen fibrils

by **collagen XV**



(Amenta et al. J Histochem Cytochem 2005 53: 165-76)

by **collagen XII** and **tenascin-X**



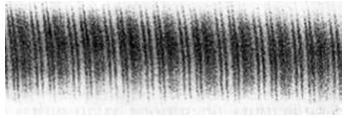
(Chiquet et al. Int J Biochem Cell Biol. 2014 53:51-4)

- **Collagen XII** forms with its partners **flexible bridges** between neighboring fibrils that might **absorb shear stresses upon loading**
- It regulates **organization** and **mechanical properties** of collagen fibrils

Diversity of supramolecular assemblies

Fibrillar collagens

I, II, III, V, XI, XXIV, XXVII



Unconventional collagens

Networks

Beaded filaments

Anchoring fibrils

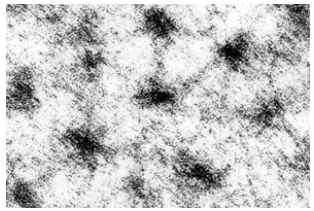
Fibril-Associated Collagen with Interrupted triple Helices (FACITs)

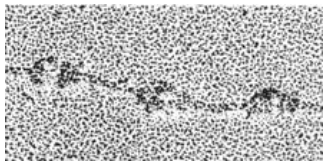
IV, VIII, X

VI

VII

IX, XII, XIV, XVI, XIX, XX, XXI, XXII



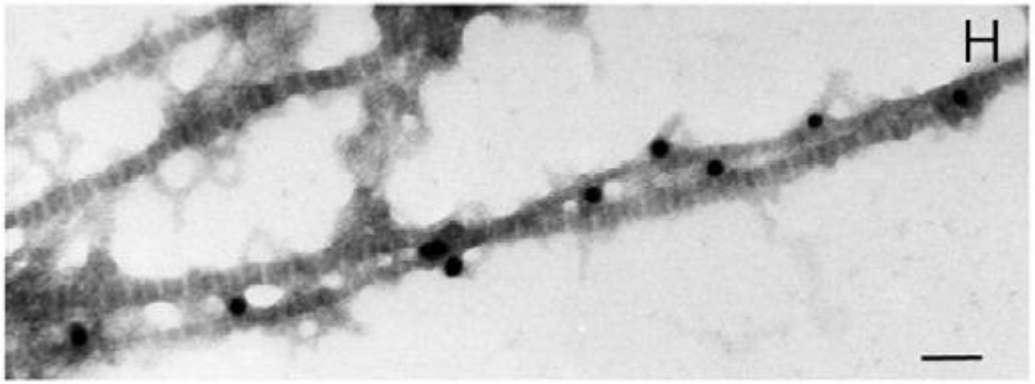


Membrane collagens

XIII, XVII, XXIII, XXV

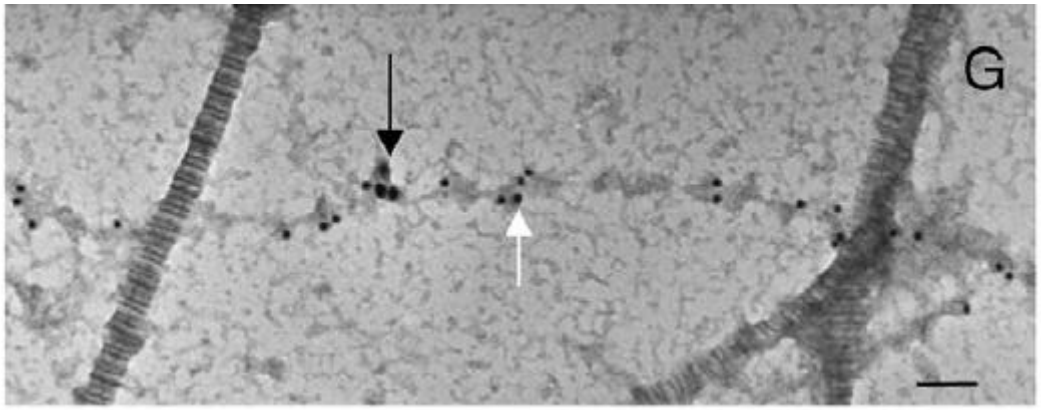
Plasticity of supramolecular assemblies: a matter of context for collagen XVI

Collagen fibrils in **cartilage**



Bar: 0.18 µm

Beaded microfibrils in **skin**



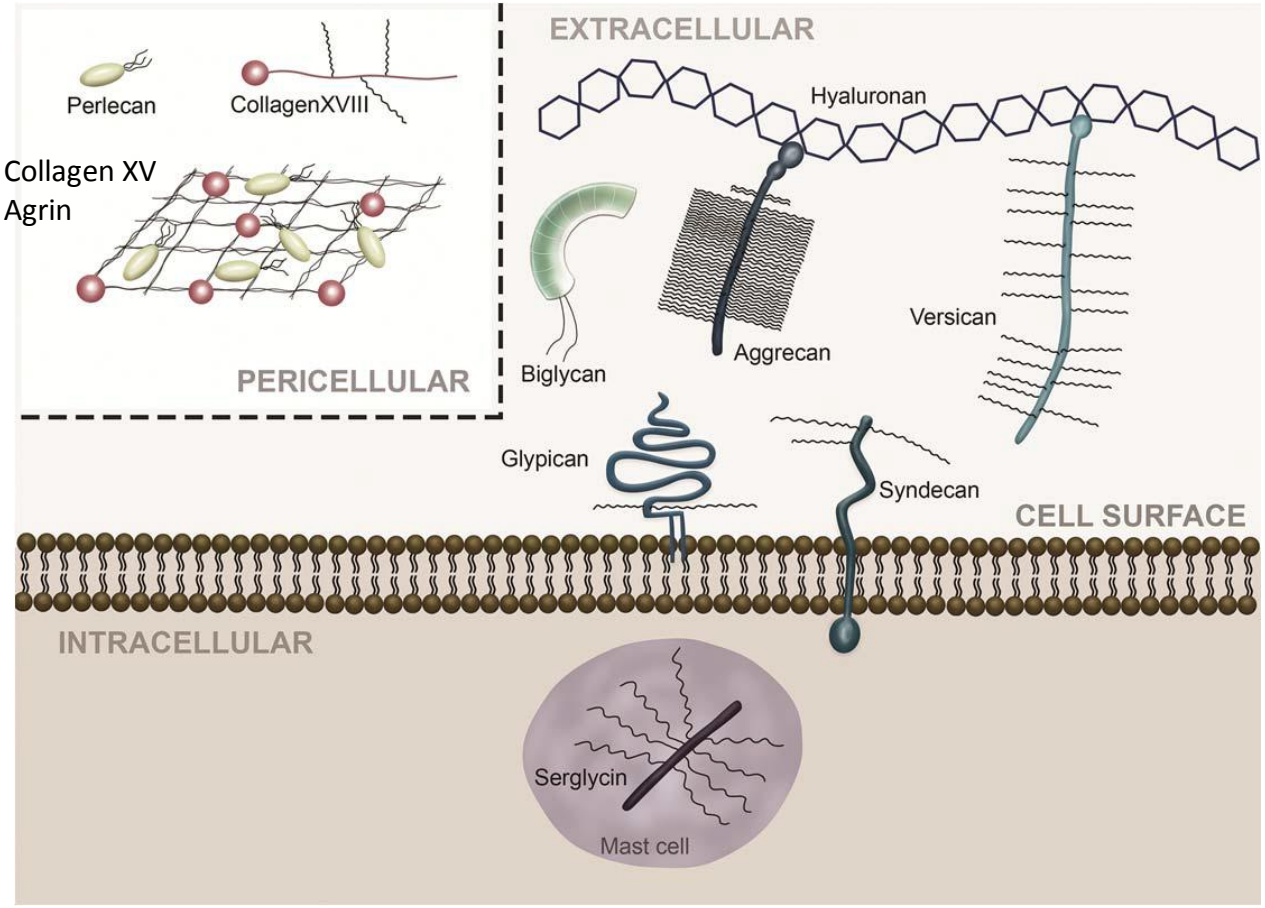
Bar: 0.12 µm

Collagen XVI →

Fibrillin-1 *(white arrow)*

Grassel & Bauer Matrix Biol 2013 32: 64–73)

Proteoglycans (PGs)



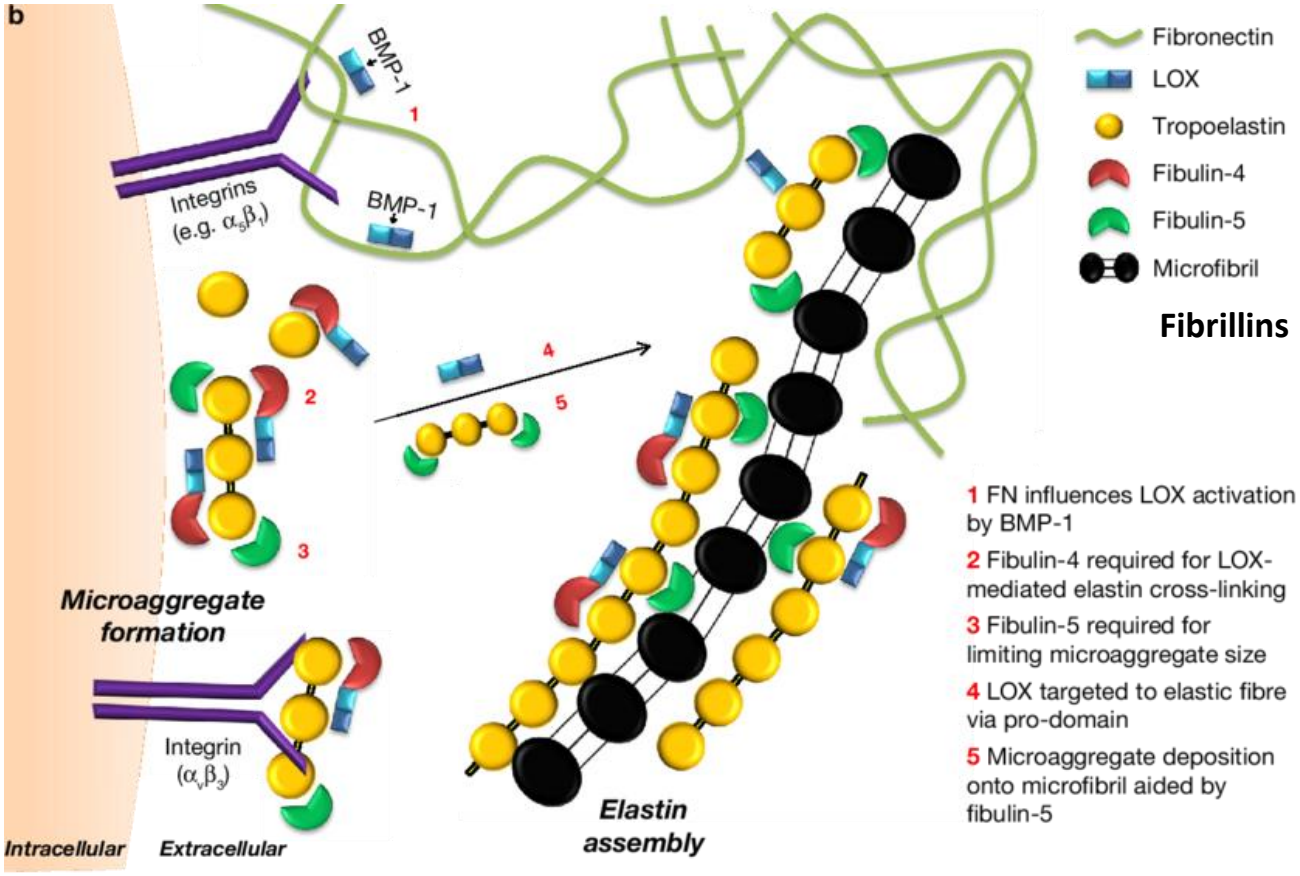
- **Hyaluronan-binding PGs**
- **Small Leucine-Rich Proteins**
- **SPOCK* family**

- **NG2**
- **Phosphacan**
- **Betaglycan**

***SPARC/Osteonectin, CWCV, and Kazal-like**

(*Nastase et al. Antioxid Redox Signal. 2017 27:855-873*)

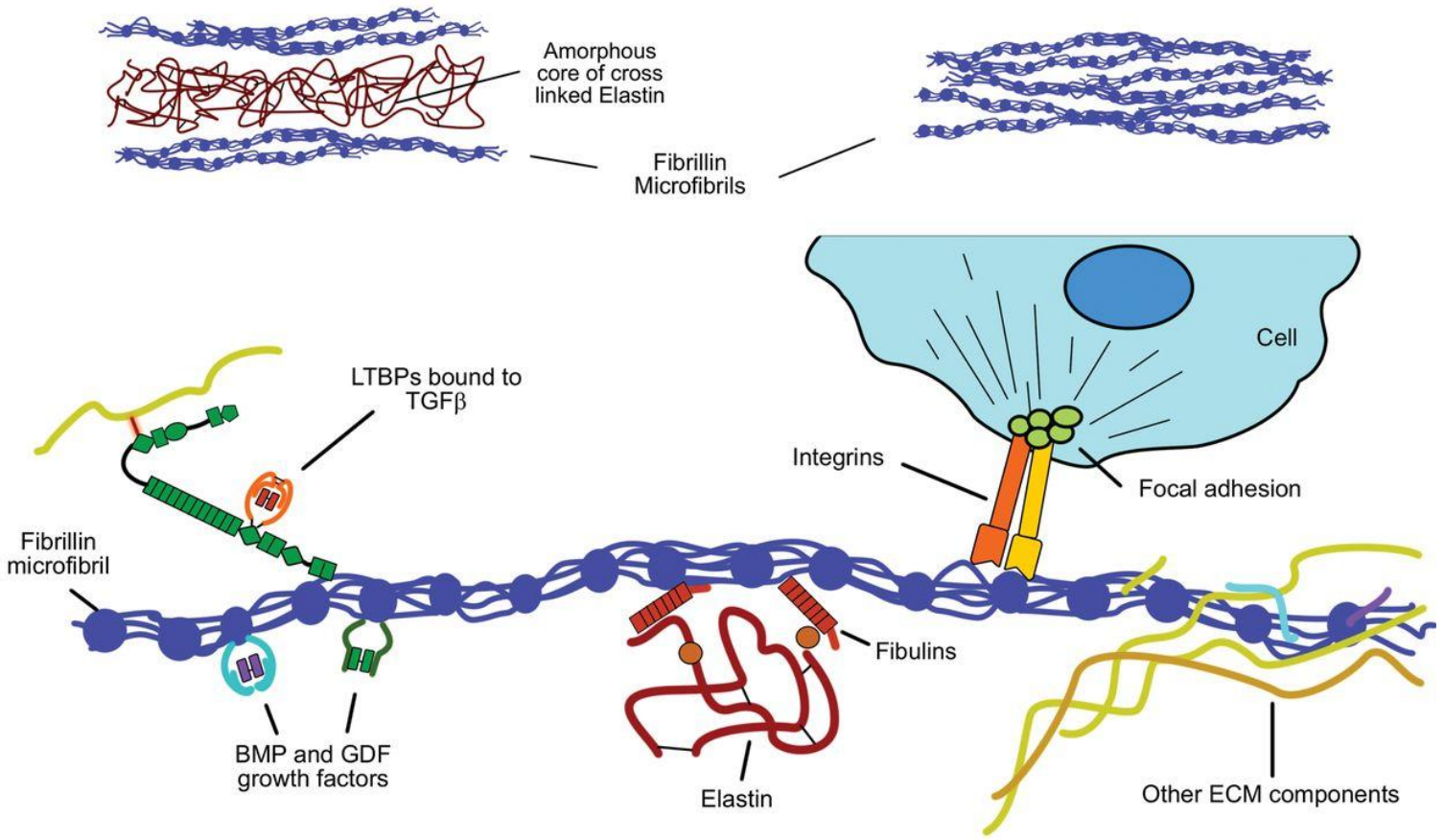
Elastin supramolecular assembly



BMP-1	Bone Morphogenetic Protein-1
FN	Fibronectin
LOX	**Lysyl oxidase**

(Baldwin et al. Expert Rev Mol Med. 2013 15:e8)

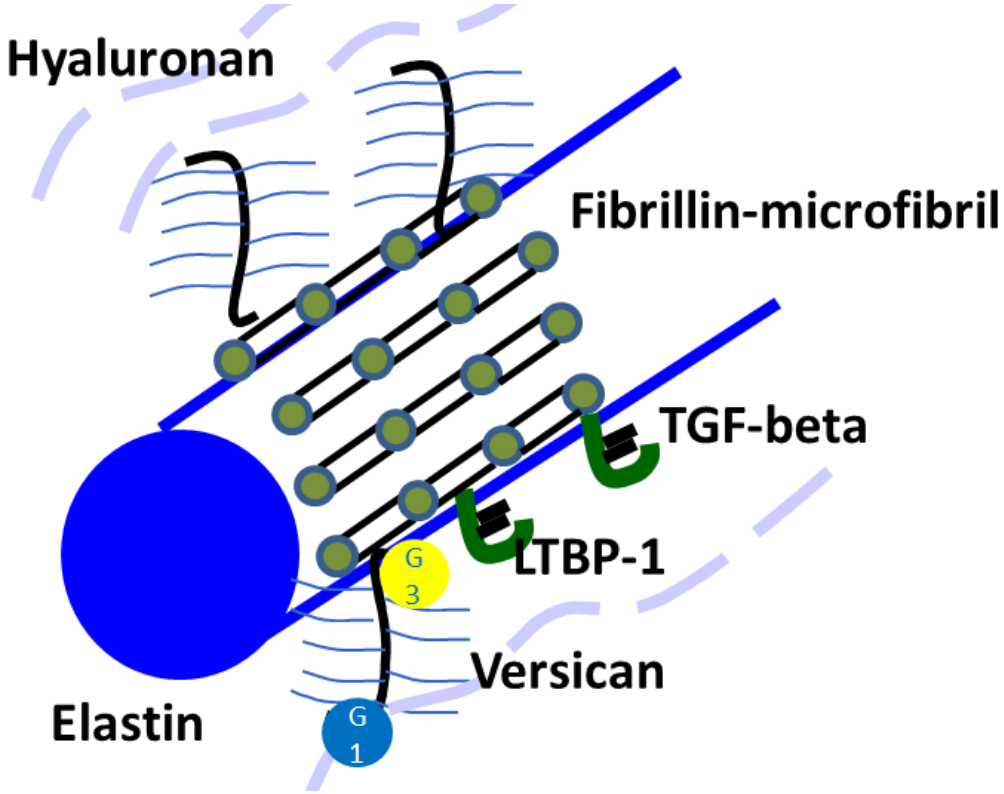
Microfibril and elastic fiber assemblies



BMP	Bone Morphogenetic Protein
GDF	Growth and Differentiation Factor
LTBP	Latent Transforming Growth Factor β-binding protein
TGFβ	Transforming Growth Factor β

(*Robertson et al. Biochem. J 2011 433: 263–276)*

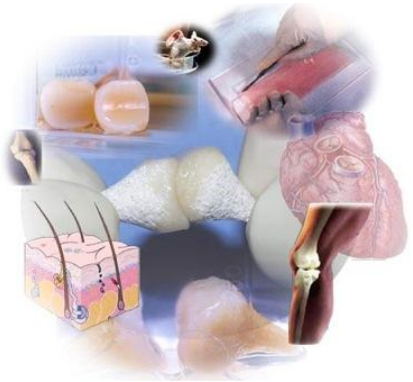
Versican links elastic fibers to the surrounding ECM

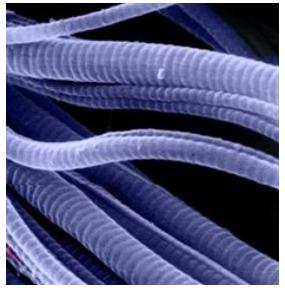


(Nemoto et al. 2012 From Theory to Biological Applications, Dr. Juan De Vicente (Ed.), InTech, DOI: 10.5772/50146)

Roles of the extracellular matrix

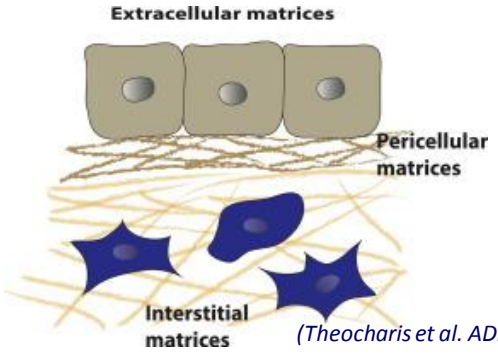
- **Structural scaffold** (tissus shape)





- **Mechanical properties of tissues**
  Mediated by cross-linking

- **Interactions with cells**
  Regulation of cell behavior



(Theocharis et al. ADDR 2016 97: 4-27)

- **Highly dynamic structure**
  - Reservoir of growth factors
  - Release of **bioactive fragments** upon remodeling (e.g. endostatin)

Bioactive fragments: matricryptins/matrikines

Substrates : collagens, elastin, proteoglycans, fibronectin, laminins, matricellular proteins, enzymes

Cysteine and serine proteinases | Metalloproteinases

Matricryptins

Receptors

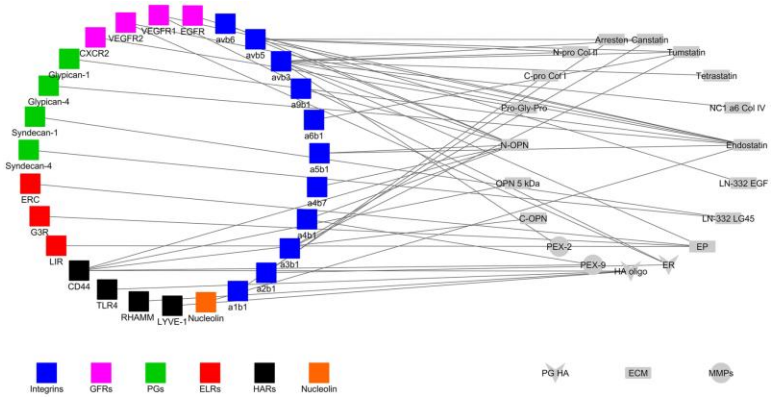
Signaling pathways

- Enzymatic activities
  Protease inhibition
  Protein synthesis inhibition
- Apoptosis
  Cell growth
  Cell migration
  Cell proliferation
- Angiogenesis
  Cancer, metastasis
  Diabetes, obesity
  Infection
  Inflammation
  Wound healing

The extracellular matrix interaction database

MatrixDB

http://matrixdb.univ-lyon1.fr/
Launay et al. 2015 Nucleic Acids Res 43:D321-7)

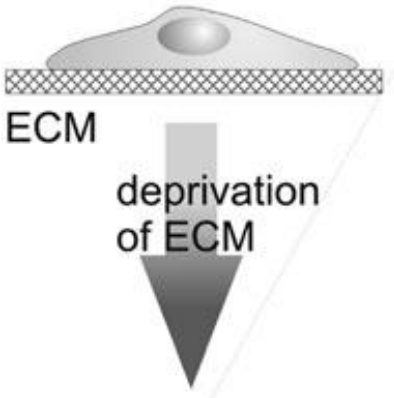


Interaction network of matricryptins and their receptors on endothelial and cancer cells

(Ricard-Blum and Vallet Biochimie 2016 122: 300-313, Ricard-Blum, Vallet. Front Pharmacol. 2016 7:11)

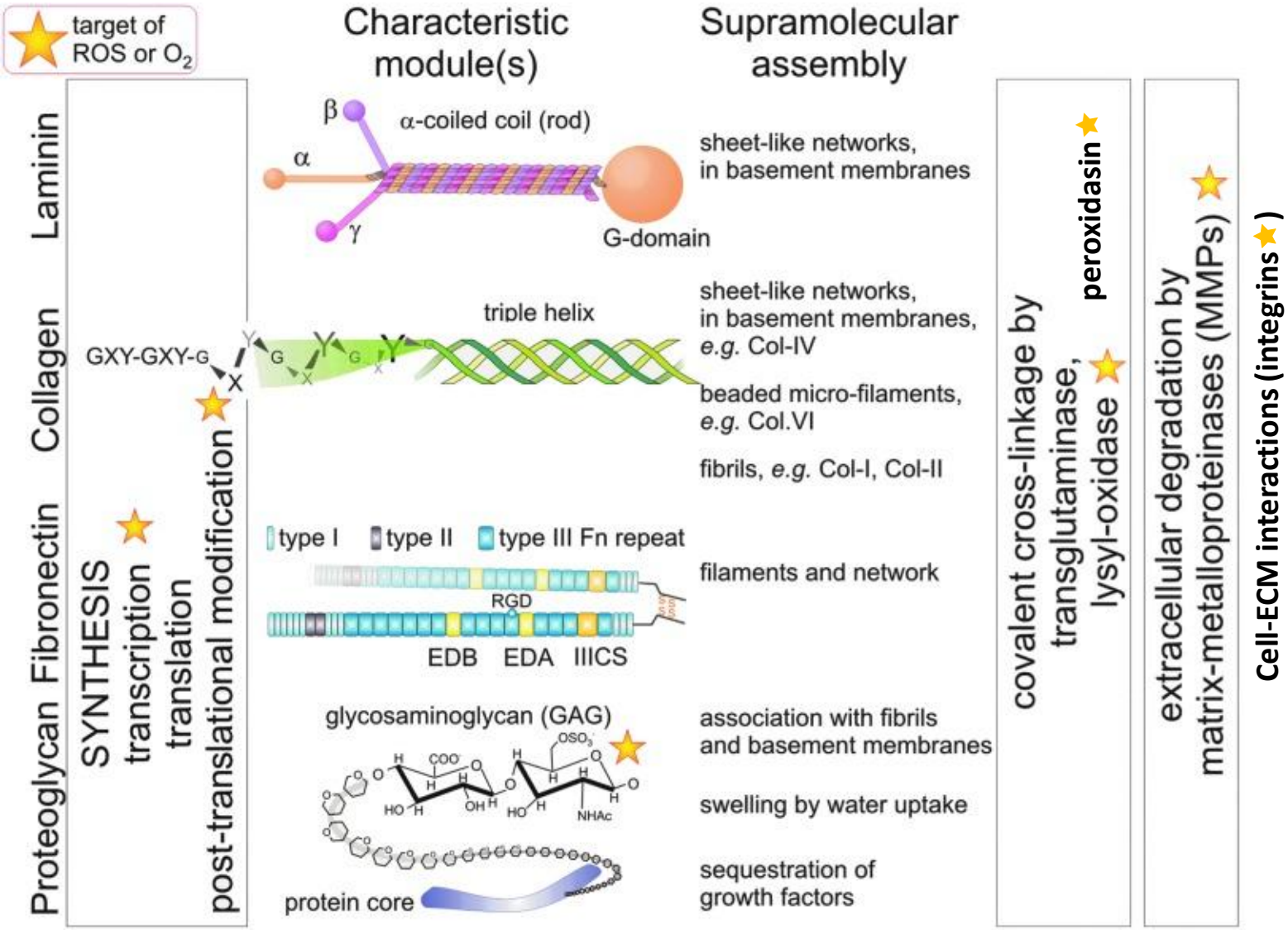
ECM and Reactive Oxygen Species (ROS) interplay

- ROS affect the production, assembly and turnover of the ECM
- The ECM regulates the production of ROS by cells
- Deprivation of matrix contacts triggers ROS production in many adherent cells



(Laragione et al. 2003 PNAS 100: 14737-41 , Metcalfe et al. 2011 Open Biol 1: 110010 , de Rezende et al. 2012 Free Rad Biol Med 53:521-31, Eble & de Rezende 2014 Antioxid Redox Signal 20: 1977-93)

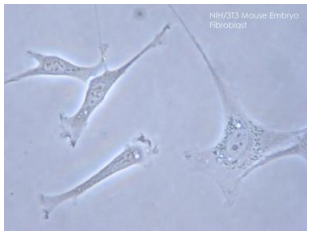
Components of the ECM targeted by ROS



(Eble & de Rezende 2014 Antioxid Redox Signal 20: 1977-93)

Extracellular matrix and H_2O_2

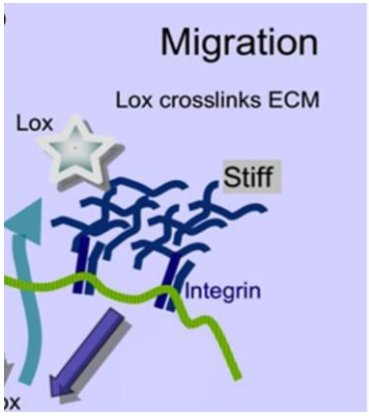
- H_2O_2 is a second **messenger in several growth factor-induced signaling cascades**



- **Cysteine residues** may operate as **redox switches using H_2O_2 as thiol oxidant**, which control **cell-matrix adhesion**

3IJE

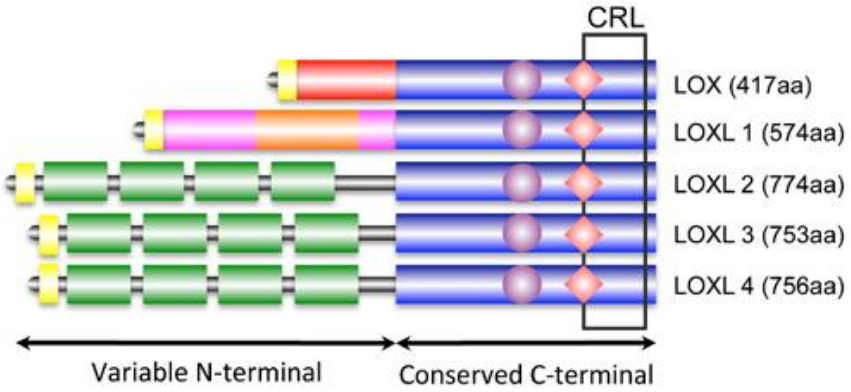
- Role of H_2O_2 in **ECM cross-linking**



(Kim et al. Development 2014 141:3233-42)

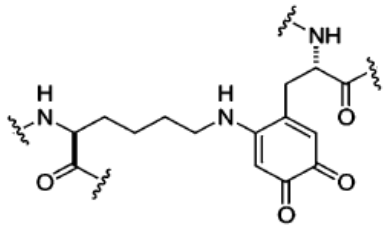
H_2O_2 and ECM cross-linking: the lysyl oxidase family

Lysyl oxidase (LOX) family



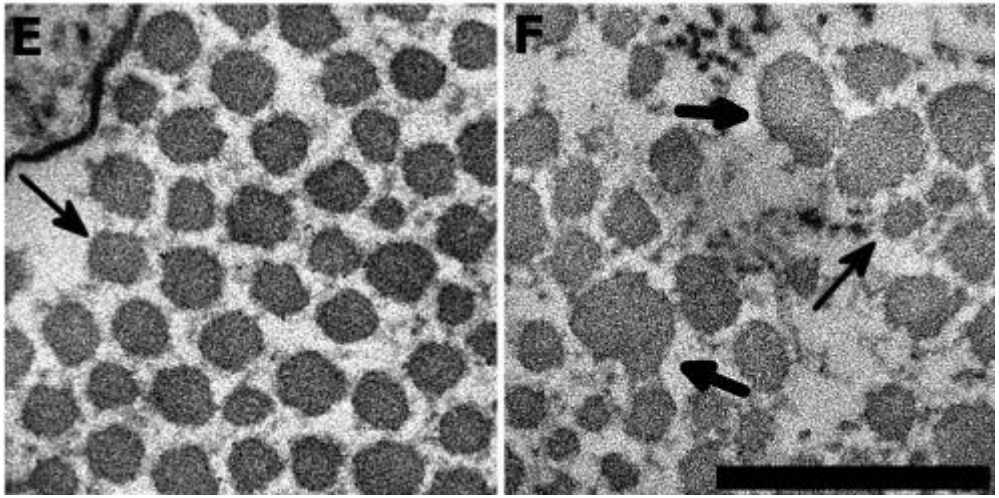
Copper binding domain
Lysyl-tyrosyl quinone (LTQ)
Scavenger receptor cystein rich domain (SRCR)
Proline rich domain
Catalytic domain
LOX Pro sequence
LOXL1 Pro sequence
Signal Peptide (~20aa)

CRL Cytokine-like receptor



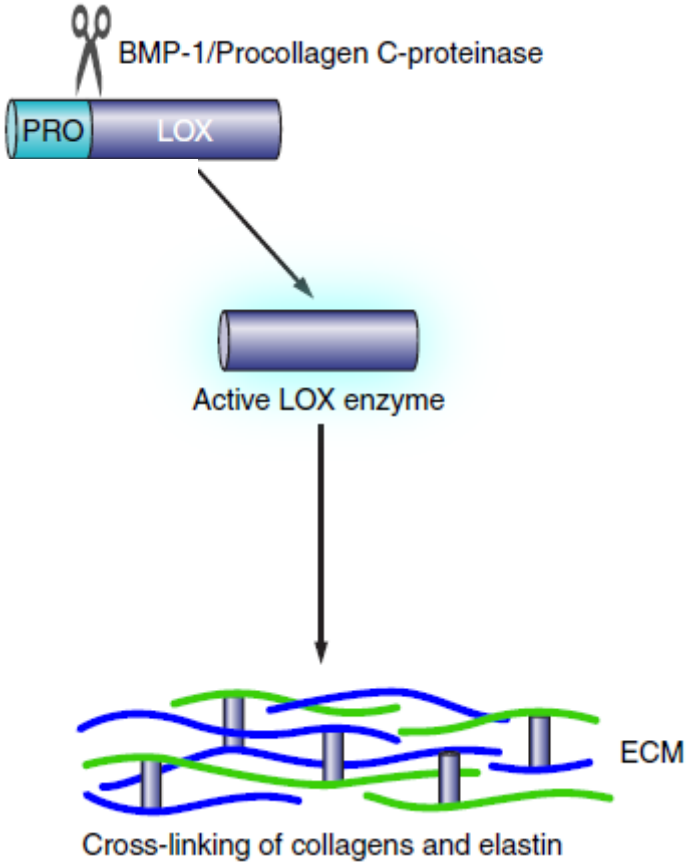
(Cox and Erler 2013 J Carcinogene Mutagene S13)

Lysyl oxidase activity is required for ordered collagen fibrillogenesis



(Herchenhan et al. J Biol Chem. 2015 290: 16440-50)

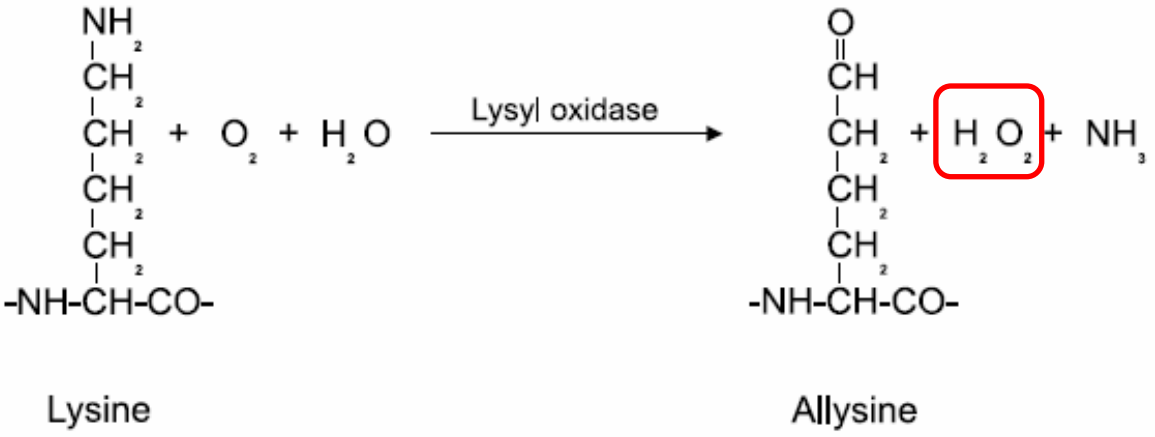
300 nm

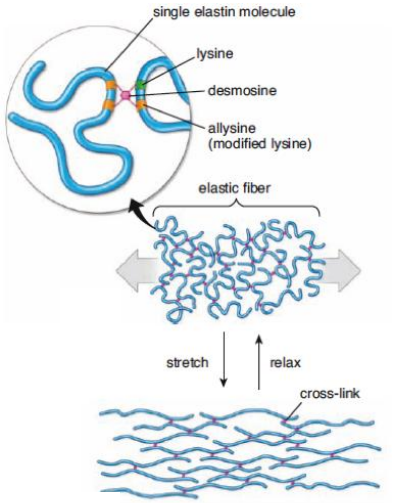


LOX is essential for correct collagen fibril shape formation in tendon

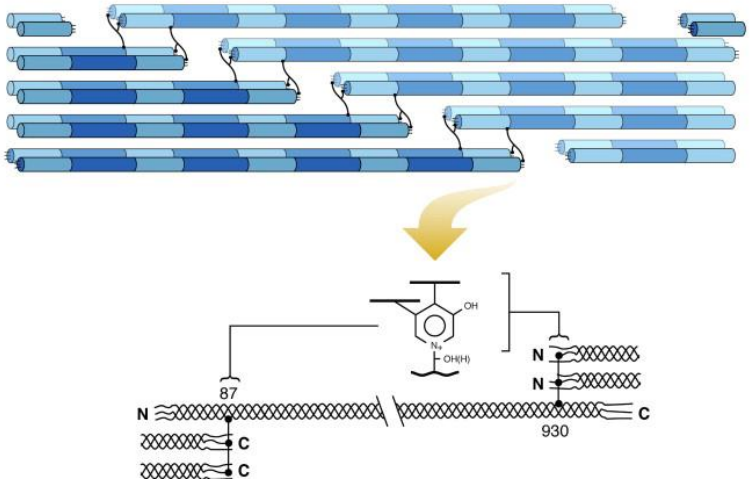
(Cox & Erler 2013 Am J Physiol Gastrointest Liver Physiol 305: G659–66)

Lysyl oxidase generates H_2O_2



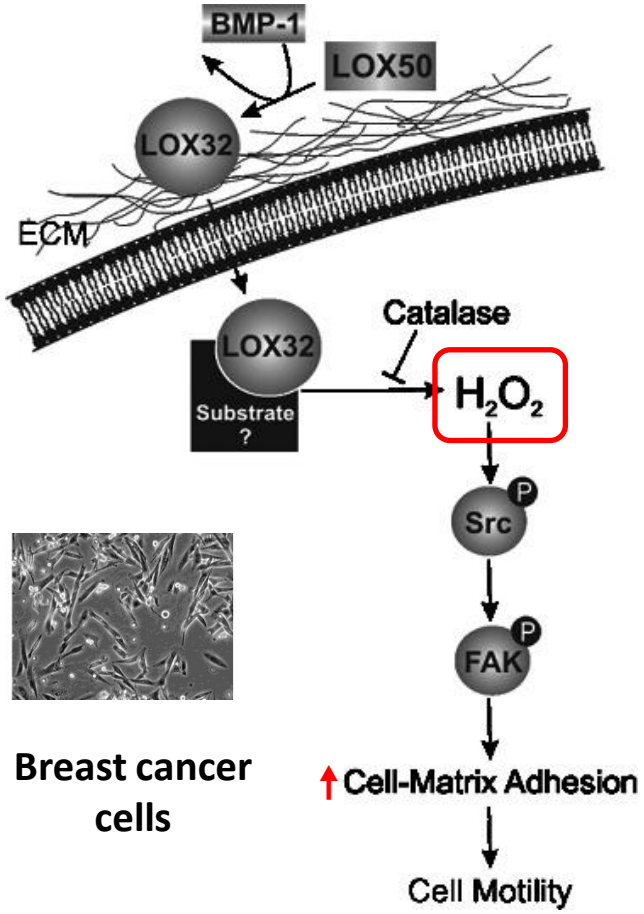


Cross-linking contributes to the **ECM stiffness**, which modulates cell proliferation and differentiation



(Robins 2008 Biochem Soc Trans. 2007 35:849-52, Eyre et al. Methods 2008 45:65-74, Eyre et al. J Biol Chem 2010 285:16675-82, Omar 2012 https://www.studyblue.com/notes/note/n/histology-connective-tissue/deck/1998230)

H_2O_2 generated by LOX controls cancer cell behavior

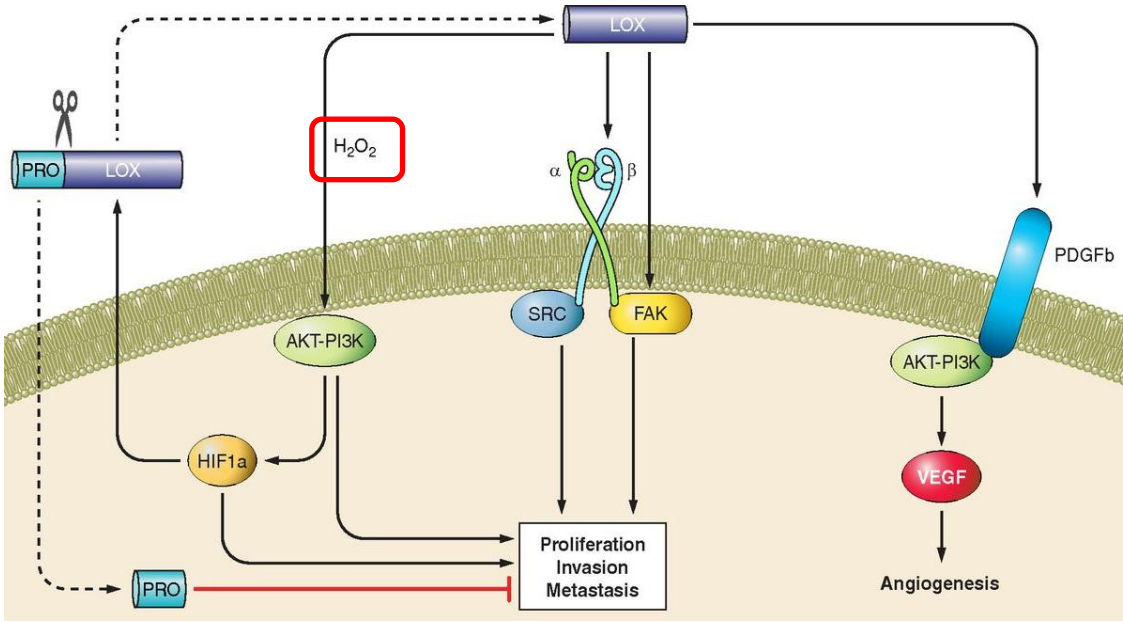


Breast cancer cells

Migration and **adhesion**

(Payne et al. 2005 Cancer Res 65: 11429-36)

Colorectal cancer cells



HIF-1 Hypoxia-Inducible Factor-1

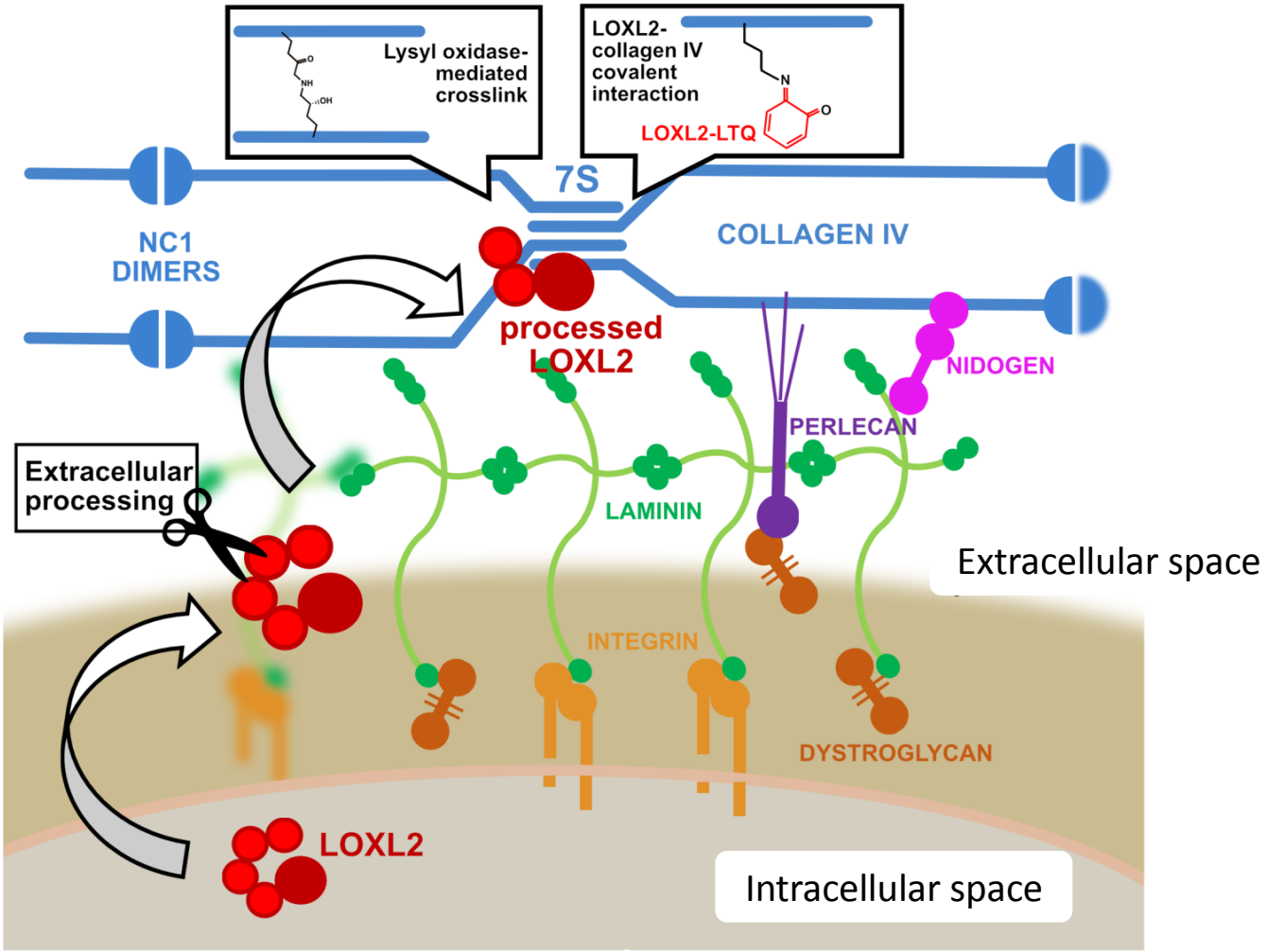
Proliferation

(Guadall et al. Front Biosci 2011 3: 955-67, Pez et al. Cancer Res 71: 1647–1657, 2011, Cox & Erler 2013 Am J Physiol Gastrointest Liver Physiol 305: G659–66)

Lysyl oxidase like-2 (LOXL2) cross-links collagen IV and generates H_2O_2

Cross-linking of NC1 domains by peroxidasin

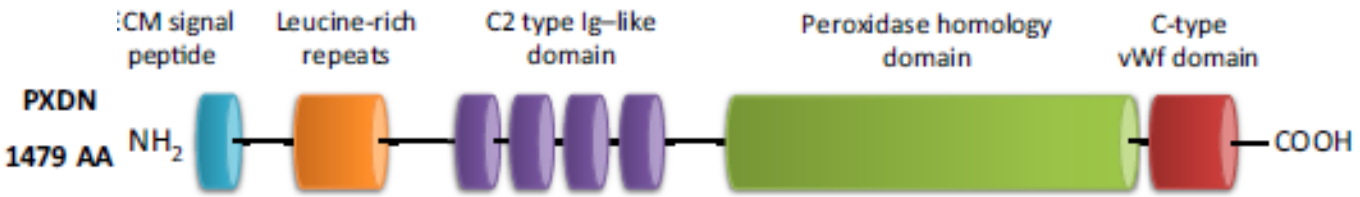
Serine protease



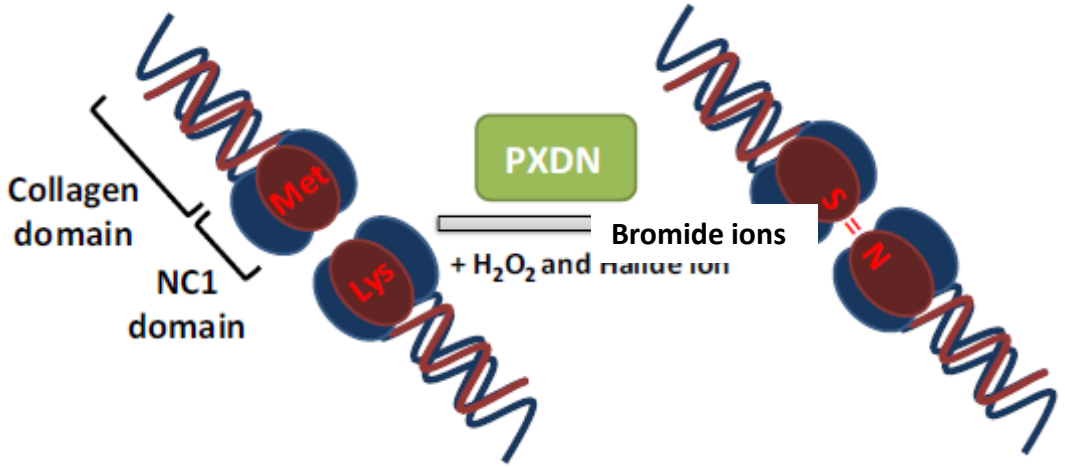
(Lopez-Jimenez et al. J Biol Chem. 2017 Sep 1. pii: jbc.M117.798603. doi: 10.1074/jbc.M117.798603. [Epub ahead of print]

Peroxidasin, a cross-linking enzyme, uses H_2O_2

- Peroxidasin is found in basement membranes



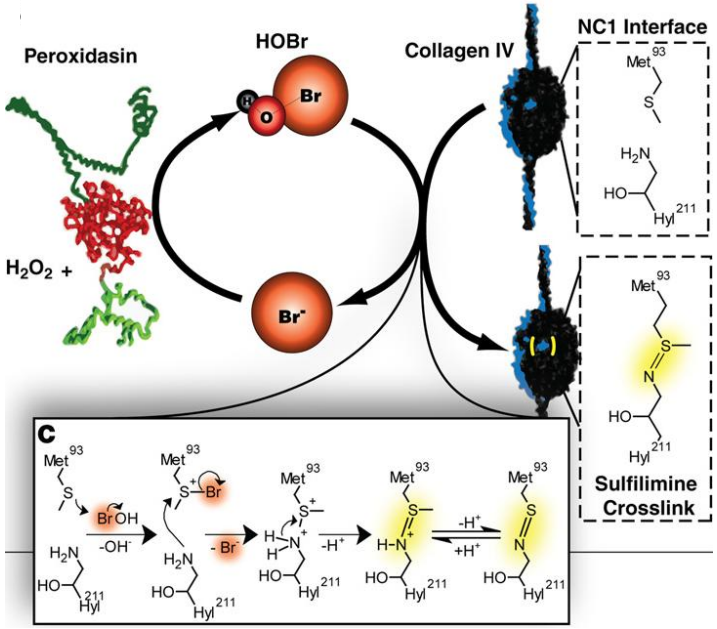
- It catalyzes the formation of a **sulfilimine (S=N)** bond in collagen IV, which requires its **catalytic** and **immunoglobulin domains**



(Bhave et al. 2012 Nat Chem Biol 8: 784-790, Weiss Nat Chem Biol 2012 740-741, Peterfi & Geiszt 2014 Trends Biochem Sci 39: 305-7)

Peroxidasin uses H_2O_2 as a substrate

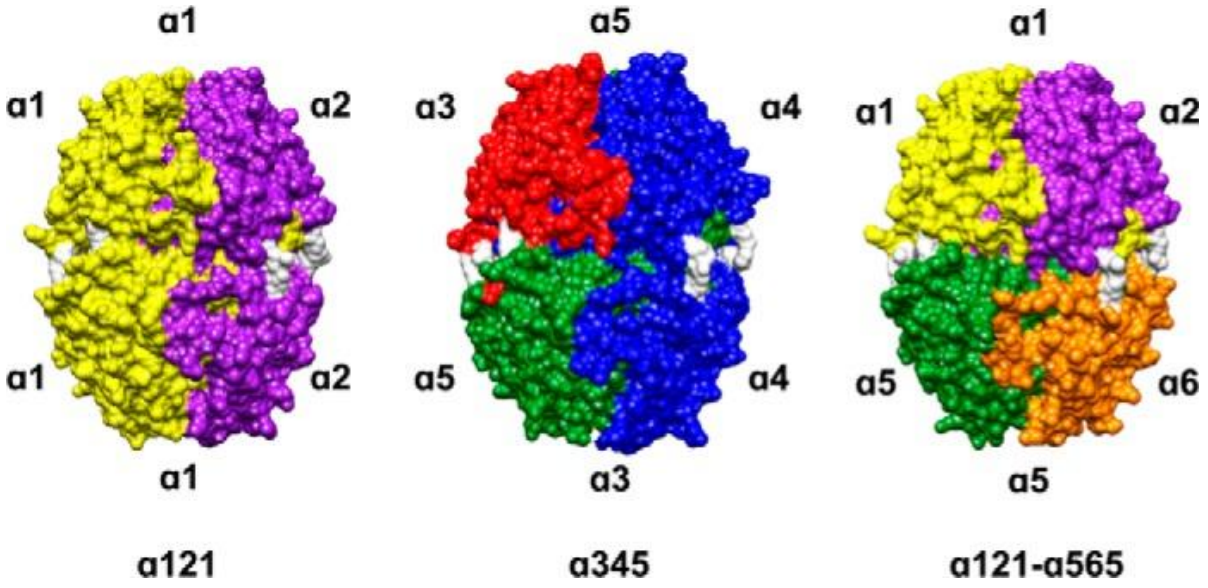
- The sulfilimine is formed by **peroxidasin**, **H_2O_2**, and **bromide ions** (Br^-)
- Peroxidasin generates **hypohalous acid** (HOBr) as intermediate
- **HOBr oxidizes Met-93** to create a **bromosulfonium intermediate**



➔ **Anabolic role** for this **usually destructive oxidant**

(Bhave et al. 2012 Nat Chem Biol 8: 784-790, Weiss Nat Chem Biol 2012 740-741, Peterfi & Geiszt 2014 Trends Biochem Sci 39: 305-7)

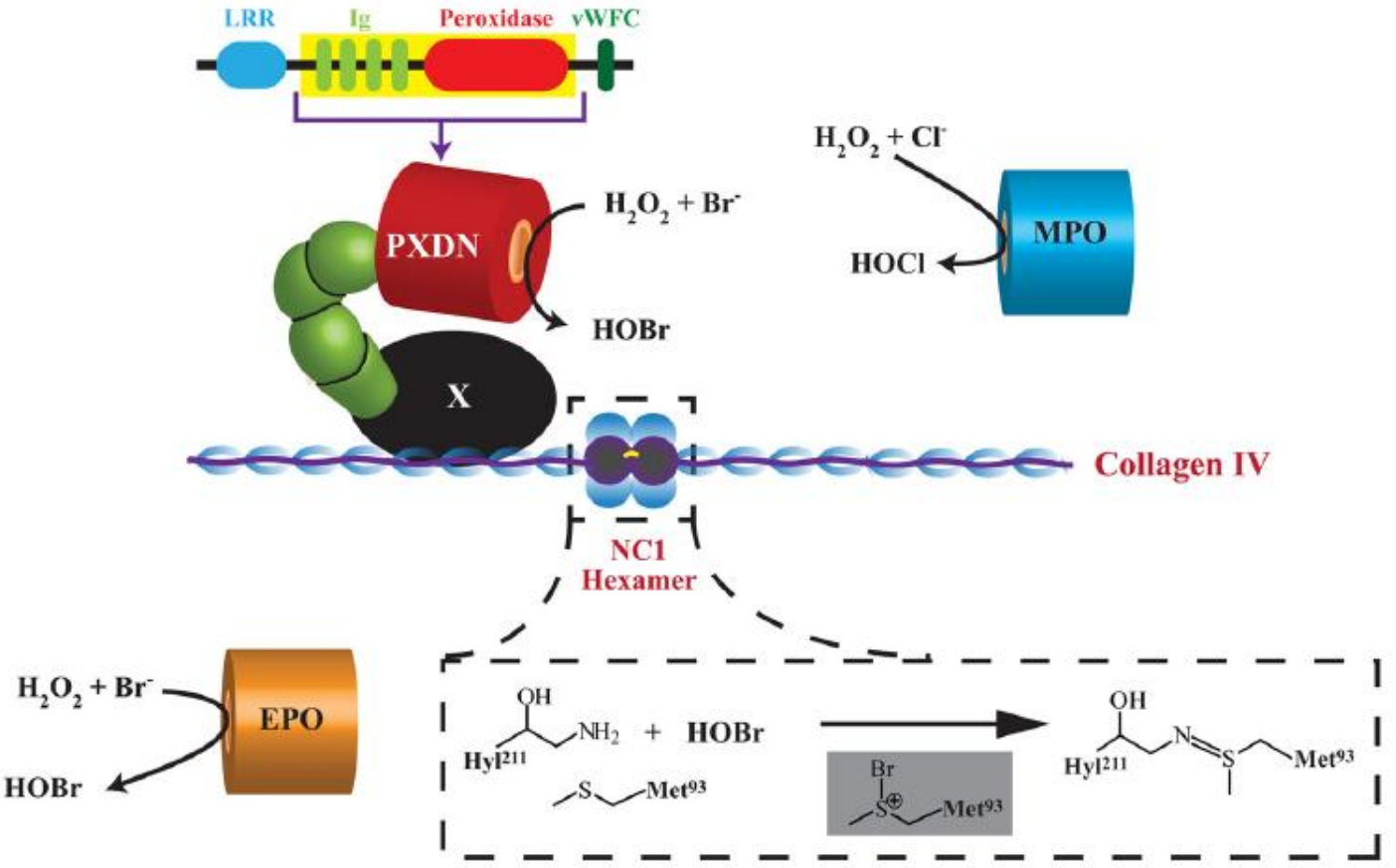
Peroxidasin cross-links the 3 collagen IV networks



- **NC1 hexamers** of collagen IV networks are stabilized by sulfilimine bonds
- **They cross-link α1 to α5** and **α2 to α6 NC1 domains**, orientating interacting α121 and α565 trimers

(*Robertson et al. Biol Chem. 2014 289:25601-10)*

Model for sulfilimine bond formation in collagen IV

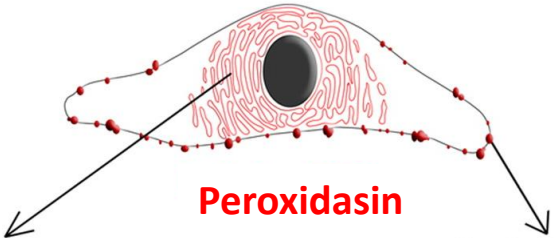


- Only peroxidasin efficiently cross-links collagen IV
- Its N-terminal Ig domain of peroxidasin interacts with an unknown intermediary matrix protein (X) to form a ternary complex with collagen IV
- HOBr generated is placed in position allowing the formation of sulfilimine bonds

(Ero-Tolliver et al. J Biol Chem 2015 290: 21741–8)

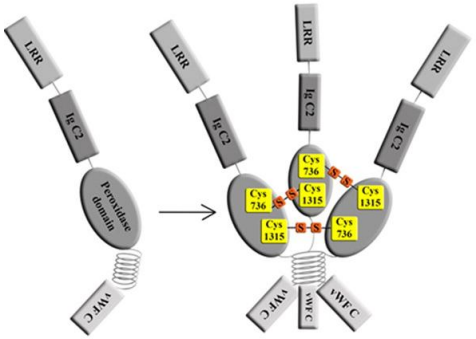
Peroxidasin oligomerizes and adheres to the cells

Endothelial and epithelial cells, fibroblasts



Peroxidasin

Endoplasmic reticulum



Trimerization through conserved cysteine residues

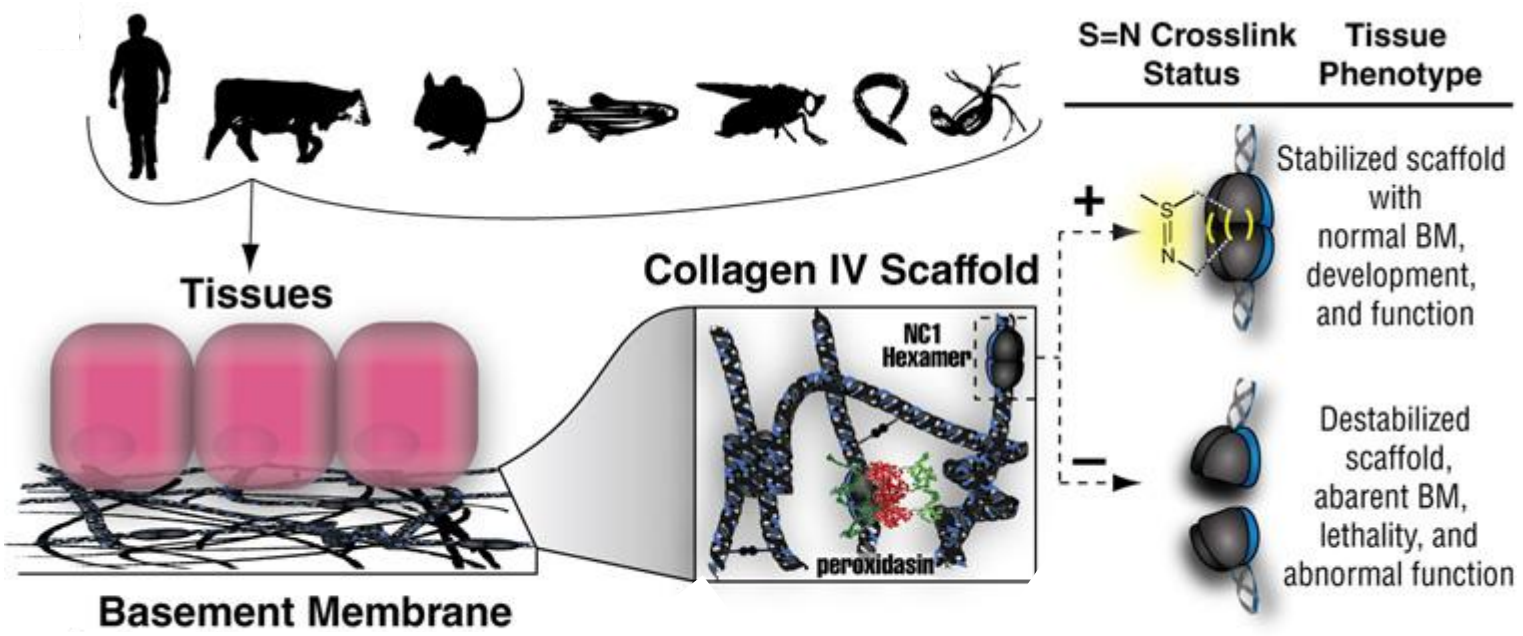
Cell surface hot spots

Cross-linking of collagen IV

- Optimal cross-linking of collagen IV is catalyzed by trimers
- **Oligomers adhere on the cell surface** in **hot spots** (locations of collagen IV cross-linking)

(Lazar et al. 2015 Free Radical Biology Medicine 86: 273-82)

Relationship between collagen IV sulfilimine formation and tissue phenotype

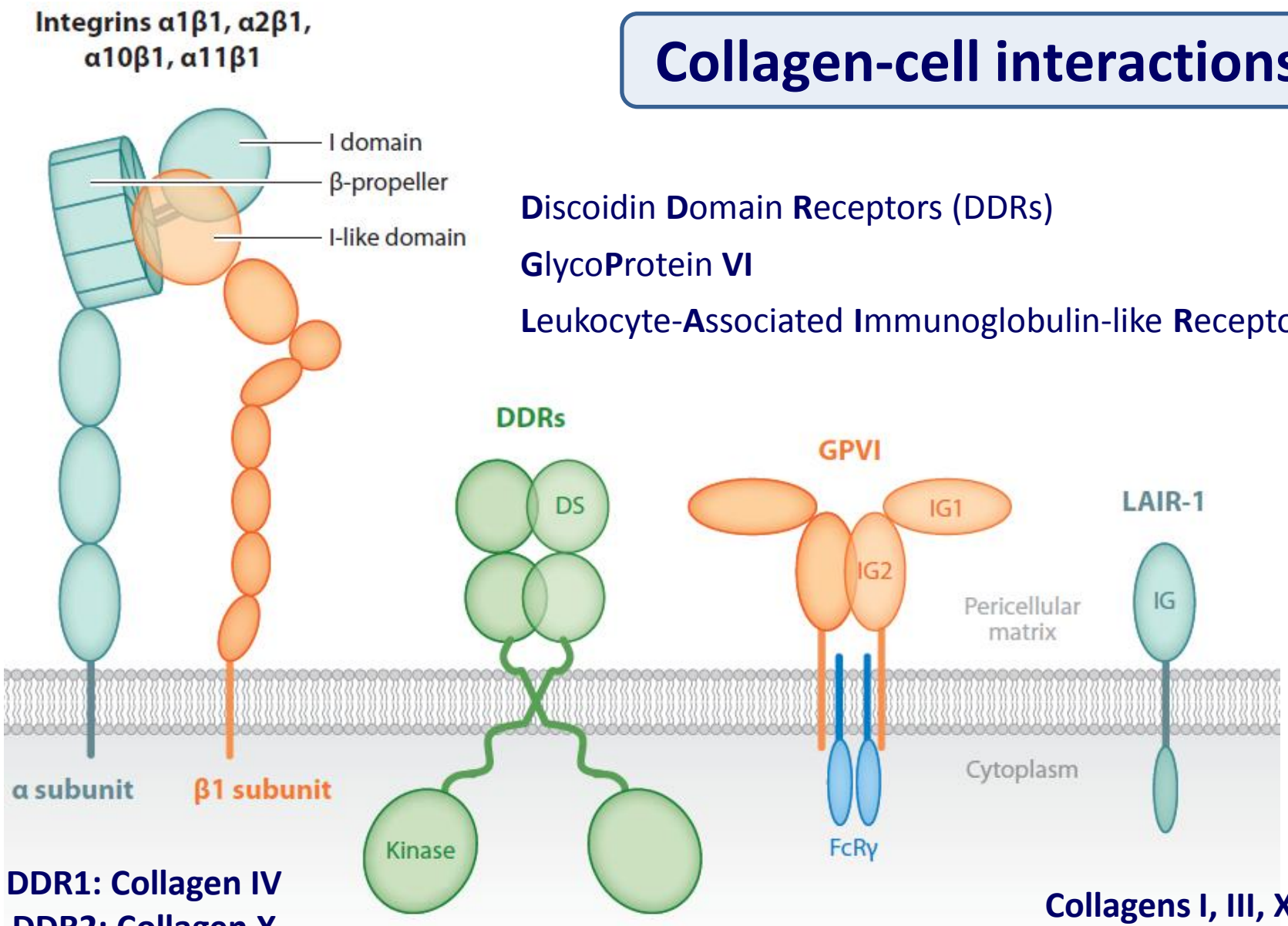


(collagen IV, peroxidasin, laminins, nidogen, perlecan, dystroglycan, GAGs)

Sulfilimine cross-links contribute to kidney tubular basement membrane stiffness

(Mc Call et al. Cell 2014 157:1380-92, Bhave et al. Am J Physiol Renal Physiol. 2017 313:F596-F602)

Collagen-cell interactions



Discoidin **D**omain **R**eceptors (DDRs)

Glyco**P**rotein **VI**

Leukocyte-**A**ssociated **I**mmunoglobulin-like **R**eceptor-1

DDR1: Collagen IV
DDR2: Collagen X

Collagen XV binds to DDR1

Fibrillar collagens I-III
GVMGFO motif

Collagens I, III, XVII

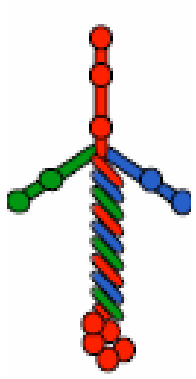
(Leitinger Annu Rev Cell Dev Biol 2011 27:265–90)

ECM modulates H_2O_2 degradation and redox signaling in endothelial cells

- Decreased H_2O_2 consumption rates
- Decreased glutathione peroxidase activity
- Increased PTEN* oxidation

  in cells cultured on **laminin-111** compared to gelatin

***Phosphatase Tensin homolog**

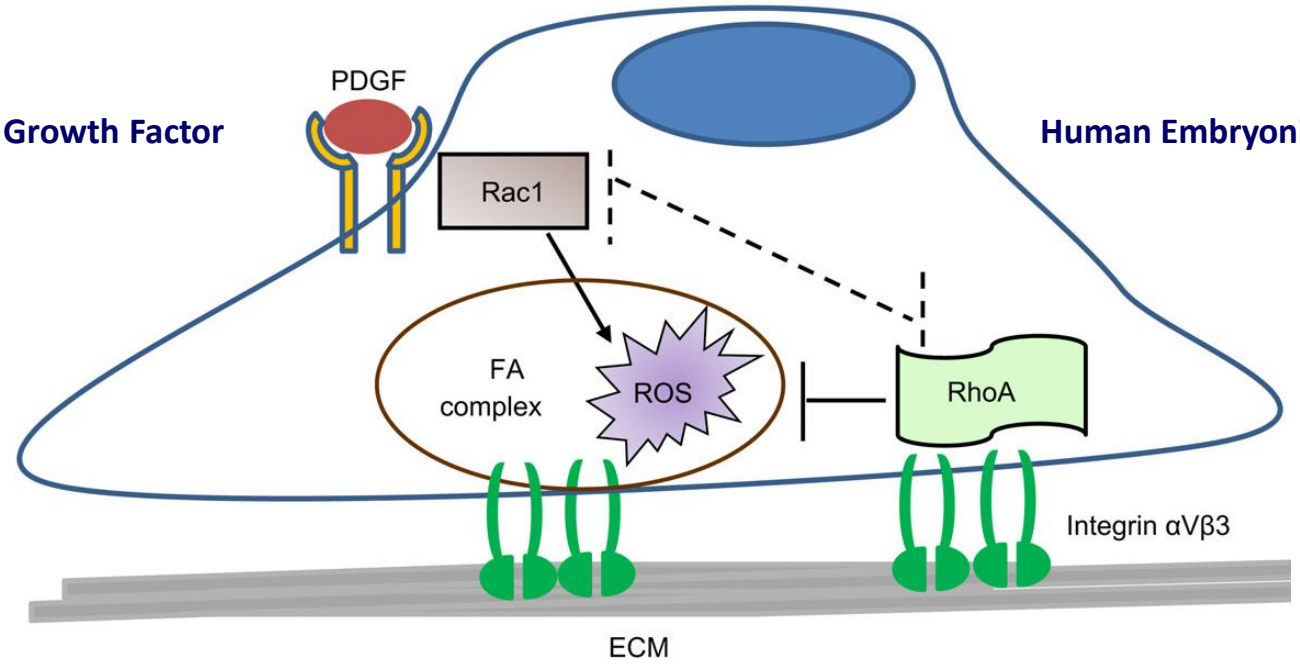


(Eble & de Rezende 2014 Antioxid Redox Signal 20: 1977-93, Bagulho et al. redox Biol 2013 6: 454-460)

Antagonistic roles of PDGF and integrin αvβ3 in regulating ROS production at focal adhesions

Platelet Derived Growth Factor

Human Embryonic Kidney cells



- **PDGF induces ROS production** at focal adhesions (FA) via Rac1
- **ECM proteins** (e.g. fibronectin) can interact with **integrin αvβ3** to **inhibit local ROS production** *via* RhoA

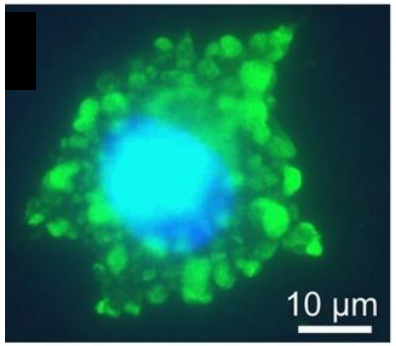
(Lin et al. Biomaterials 2013 34: 454-460)

ROS production is influenced by cell adhesion

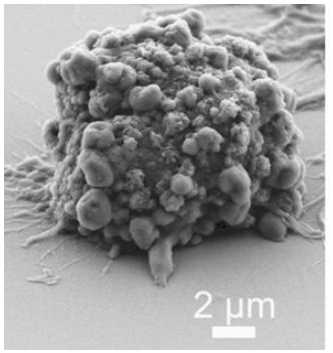
- During adhesion, the **generation of ROS is not homogenously distributed** throughout the cell and is **temporally transient**

- **High [ROS] oxidize proteins in membrane protrusions** at sites of new cell-matrix interactions: **redox hotspots**

- They depend on the activity of the **NADPH oxidase 4**



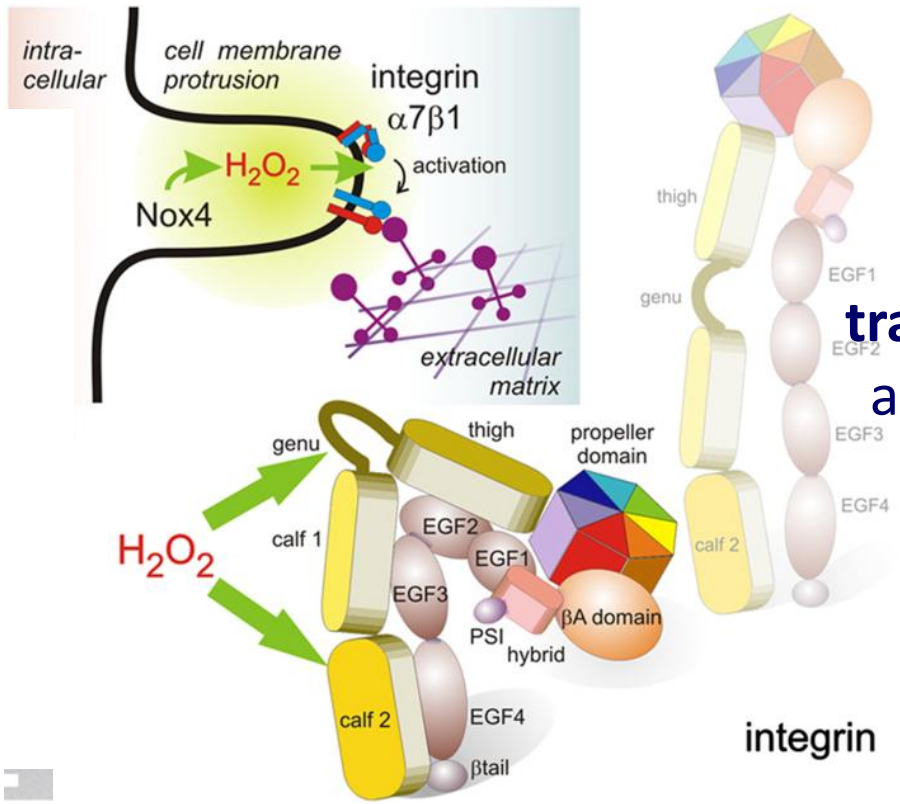
Vascular smooth muscle cells onto laminin-111



Protrusion diameter: 2.9 ± 0.8 µm Area: 5.6 ± 3.2 µm^2

(Laragione et al. 2003 PNAS 100: 14737-41 , Metcalfe et al. 2011 Open Biol 1: 110010 , de Rezende et al. 2012 Free Rad Biol Med 53:521-31, Eble & de Rezende 2014 Antioxid Redox Signal 20: 1977-93)

Cell adhesion is redox-regulated at the integrin level

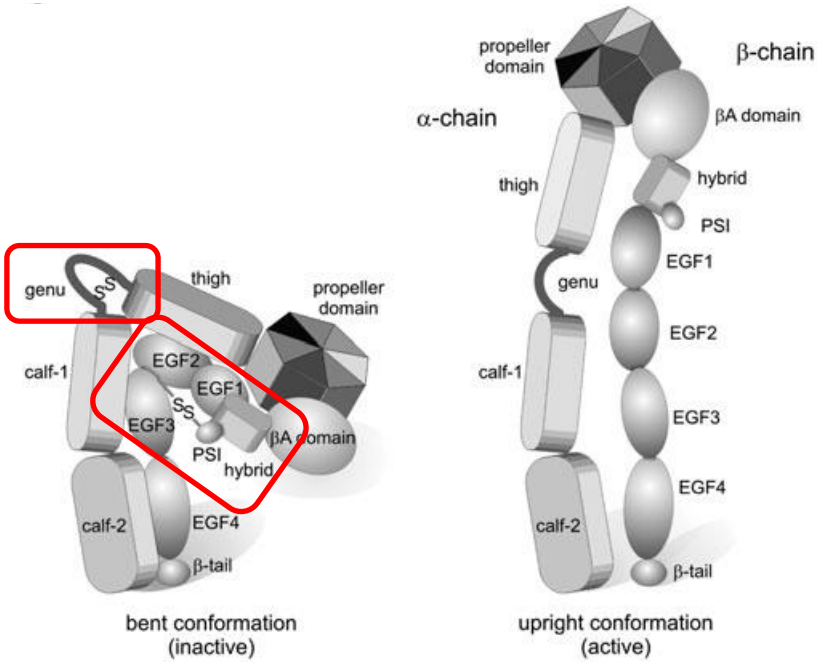


Disulfide bridges may control the **transition** between the **bent/inactive** and **upright/active** conformation of the integrin ectodomain

- The **ligand-binding headpiece** is **unaffected** by redox-regulation
- The thiol-based intramolecular **cross-links** occur in the **integrin legs**

(de Rezende et al. 2012 Free Rad Biol Med 53:521-31, Eble & de Rezende 2014 Antioxid Redox Signal 20: 1977-93)

Thiol-based redox-regulation of α7β1 integrin



The **redox-regulated cleavage** of the **S-S bridge** in **the genu** region of the α-subunit and the **long-range S-S bridge**(between the PSI and the EGF-domains of the β-subunit are **involved in the conformational transition of the integrin ectodomain**

(de Rezende et al. 2012 Free Rad Biol Med 53:521-31, Eble & de Rezende 2014 Antioxid Redox Signal 20: 1977-93)

Acknowledgements

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