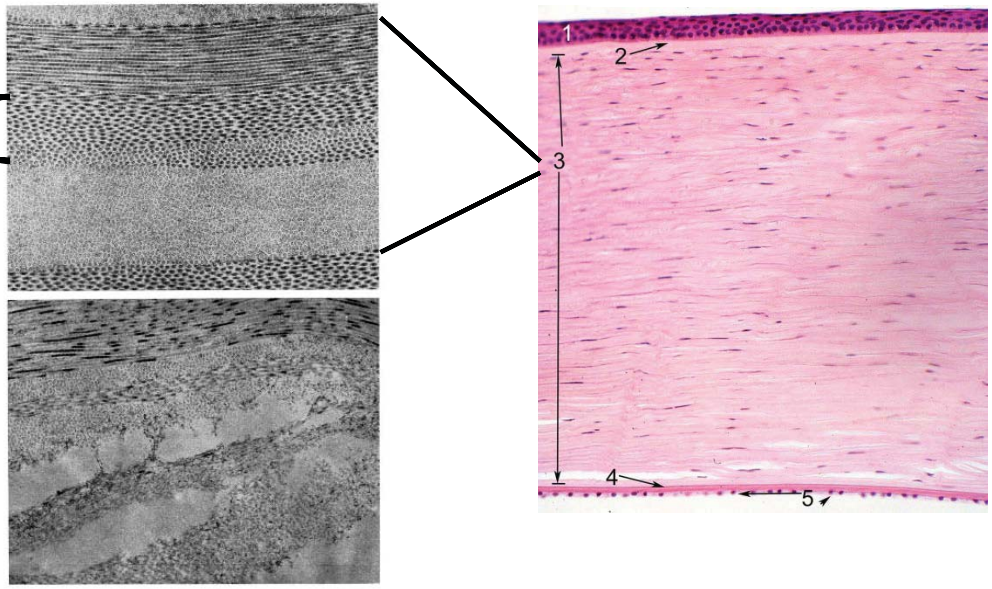
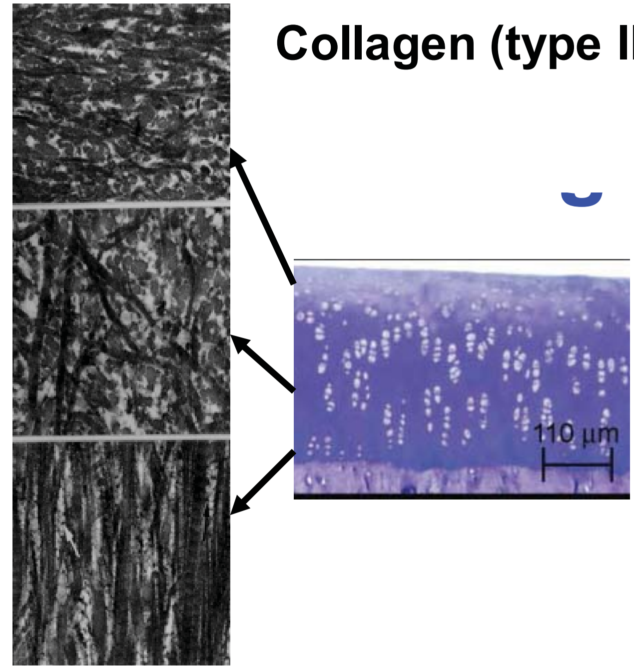
Cornea collagen architecture

Cornea

2 μm

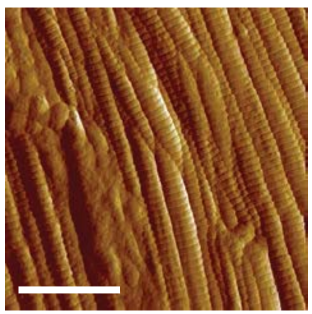


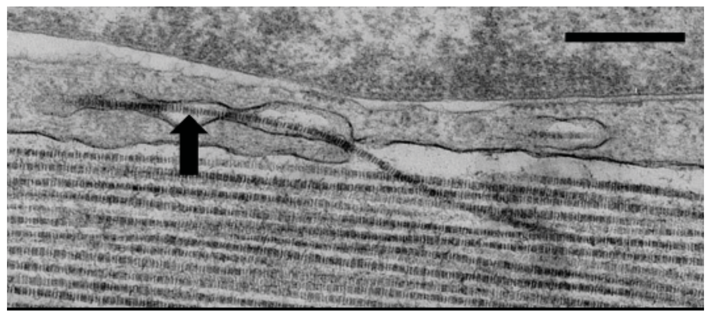
Collagen (type II)



Tendon

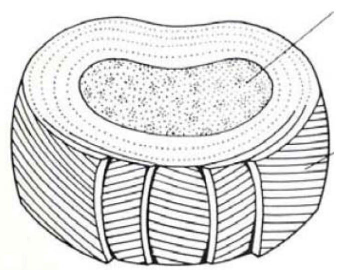
imaged by AFM

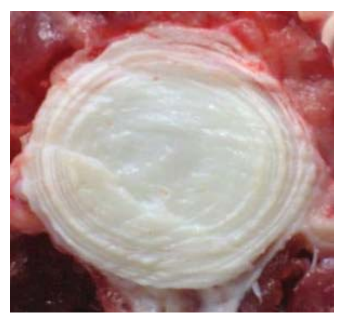




Tendon collagen fibrils (~28 nm) secreted and organized by tendon fibroblast

Collagen architecture of the disc

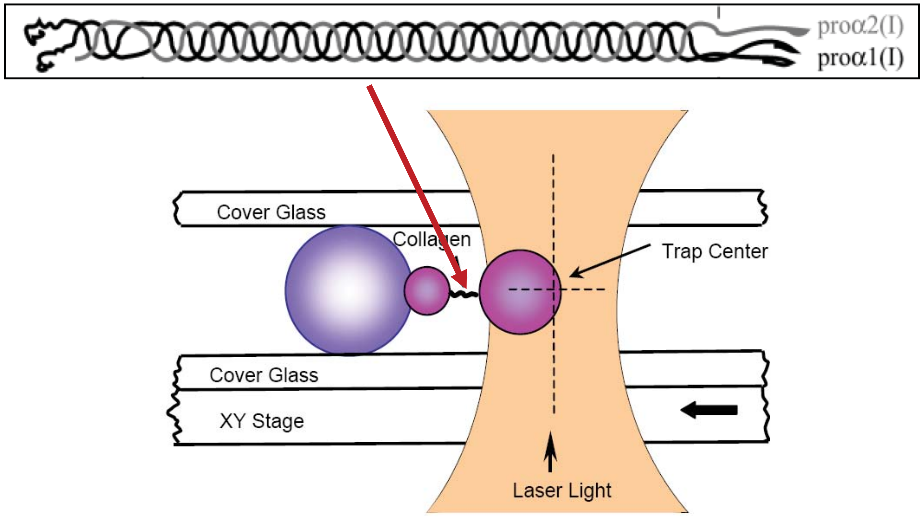




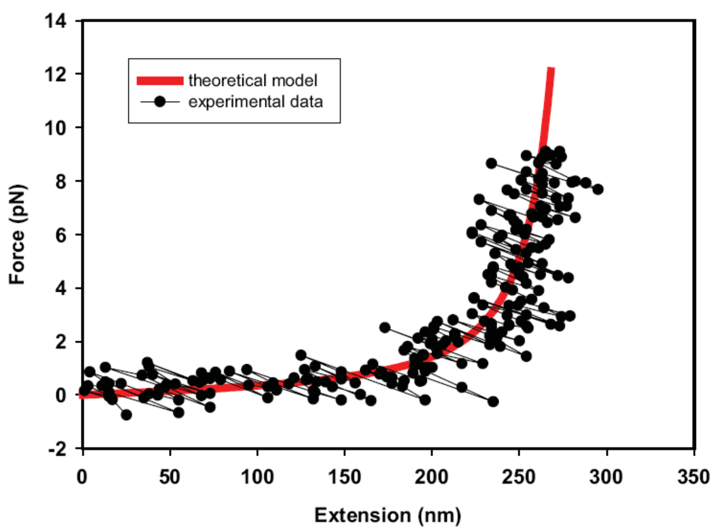
Human intervertebral

Disc

Pro-collagen molecule →



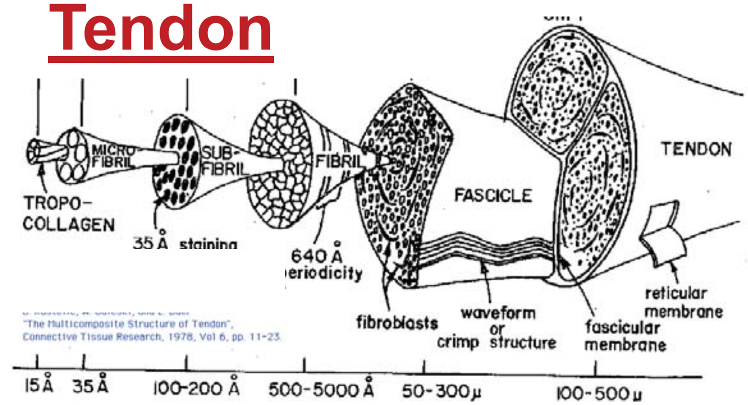
Force - extension



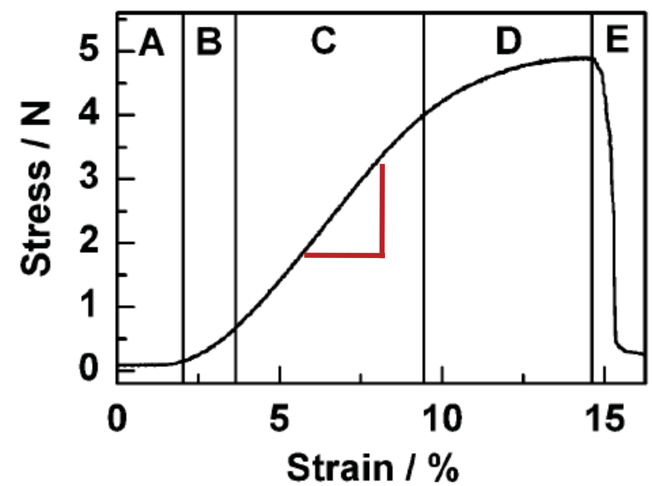
(Sun+, J Biomechanics, 2004)

Source: Sun, Yu-Long, et al. "Stretching Type II Collagen with Optical Tweezers." *Journal of Biomechanics* 37, no. 11 (2004): 1665-9.

Tendon



Source: Kastelic, J., A. Galeski, et al. "The Multicomposite Structure of Tendon." *Connective Tissue Research* 6, no. 1 (1978): 11-23.



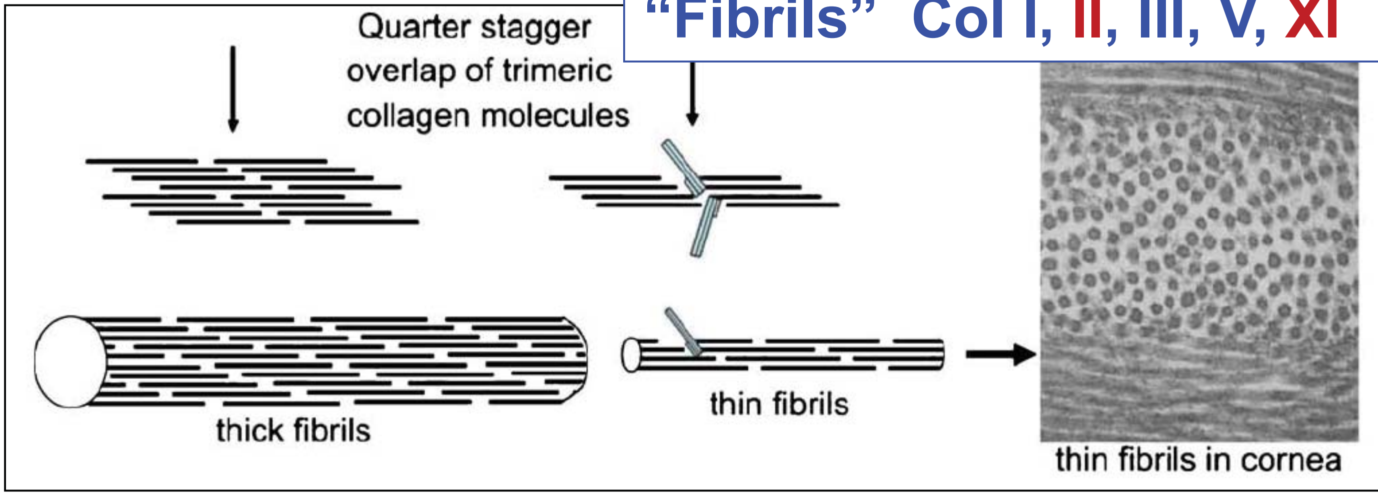
Source: Gutsmann, Thomas. "Force Spectroscopy of Collagen Fibers to Investigate their Mechanical Properties and Structural Organization." *Biophysical Journal* 86, no. 5 (2004): 3186-93.

Stress vs strain curve of a rat tail tendon: (A-B) Toe - heel region, **(C) linear region**, (D) plateau, (E) rupture of the tendon.

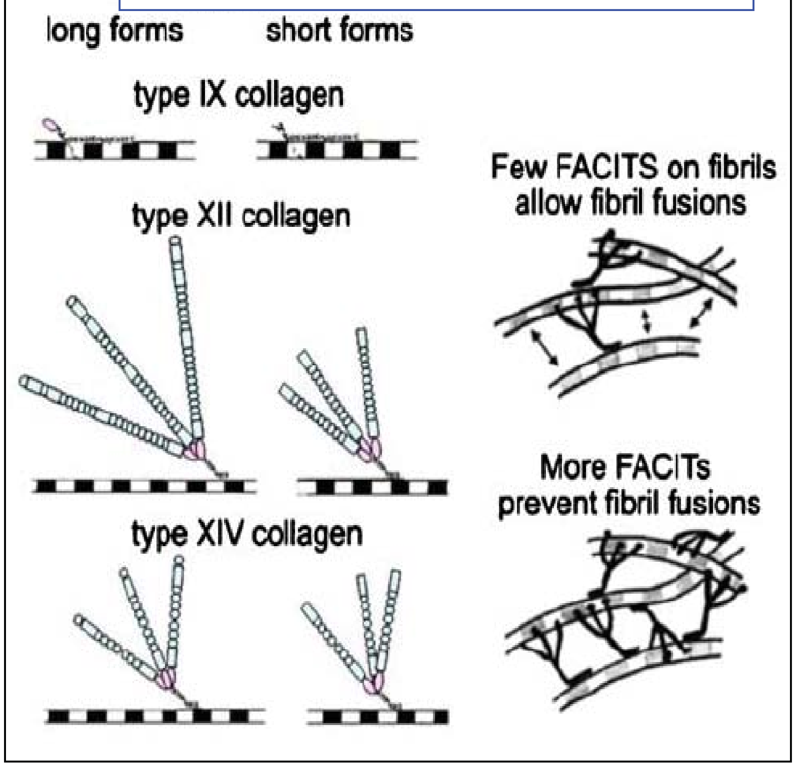
(Gutsmann+, Biophys J, 2004)

28 Types of Collagens forms fibrils, networks, other aggregates…

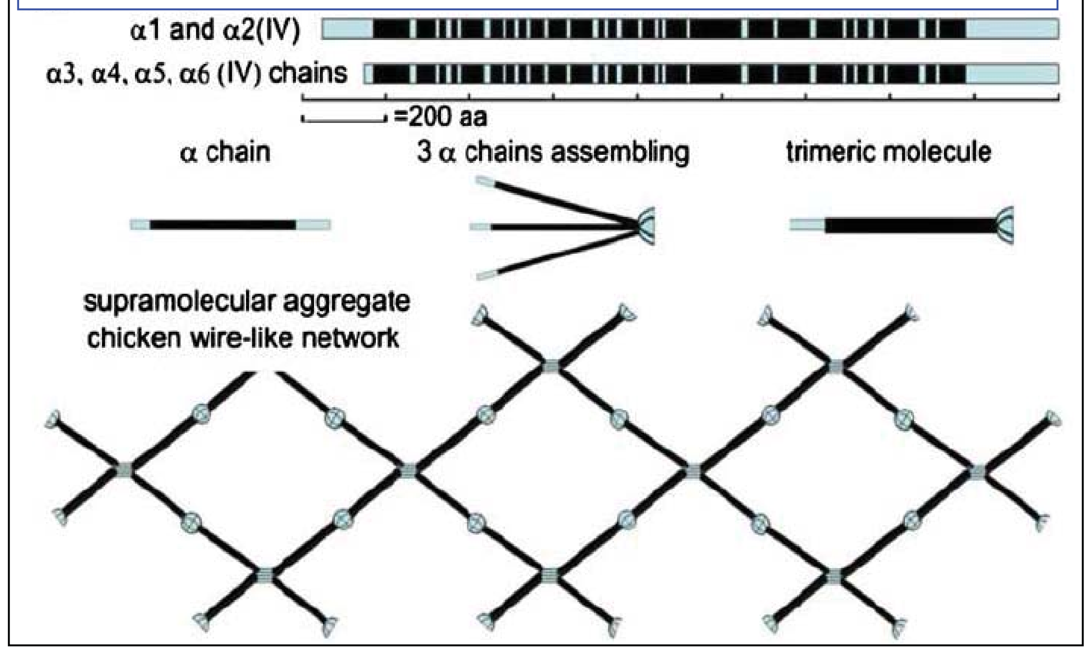
"Fibrils" Col I, II, III, V, XI



"FACIT" Col IX, XII, …



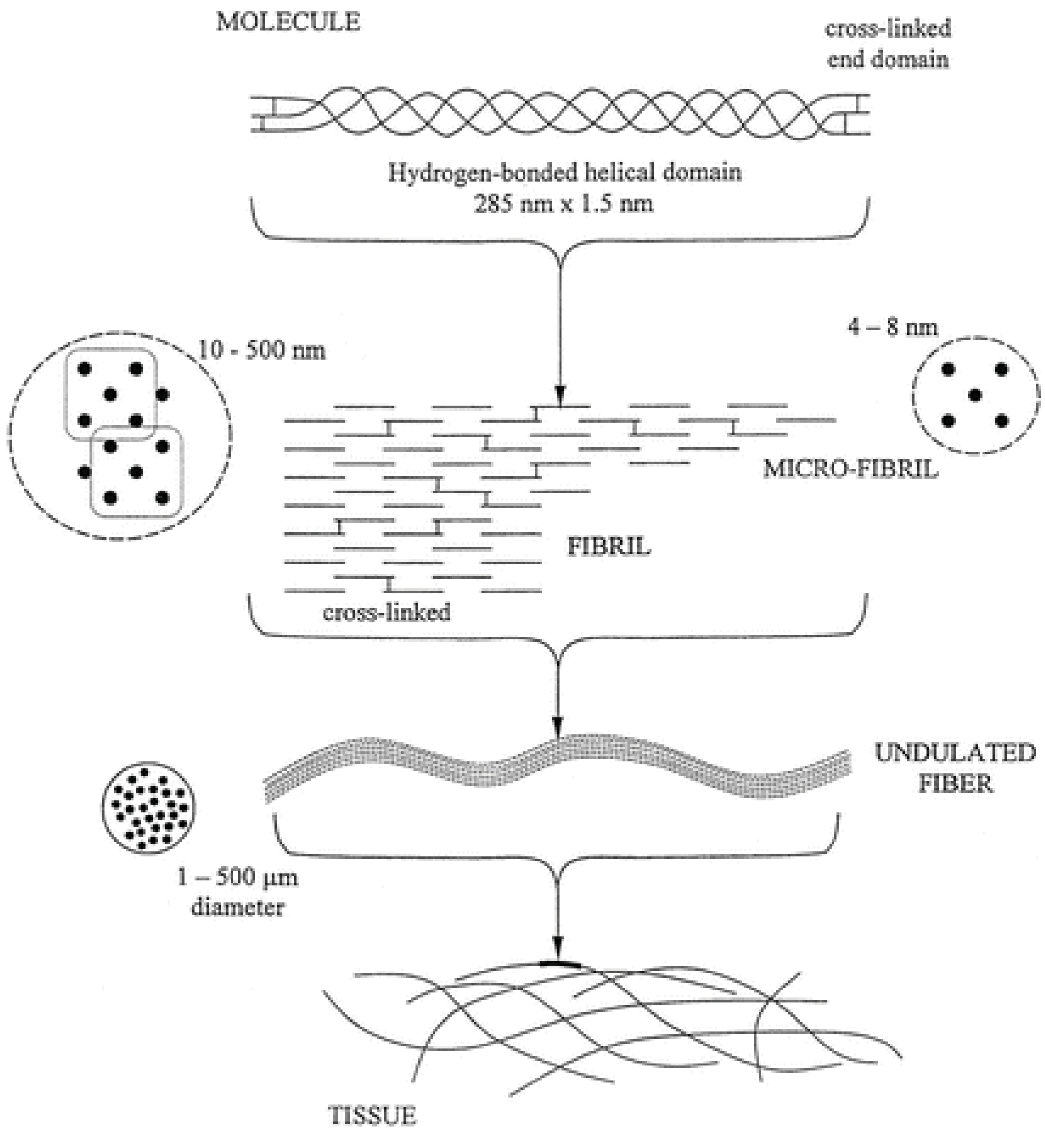
Type IV Collagen: Basement membranes



(Gordon & Hahn, Cell Tiss Res, 2010)

Source: Gordon, Marion K., and Rita A. Hahn. "Collagens." *Cell and Tissue Research* 339, no. 1 (2010): 247-57.

Collagen Structures

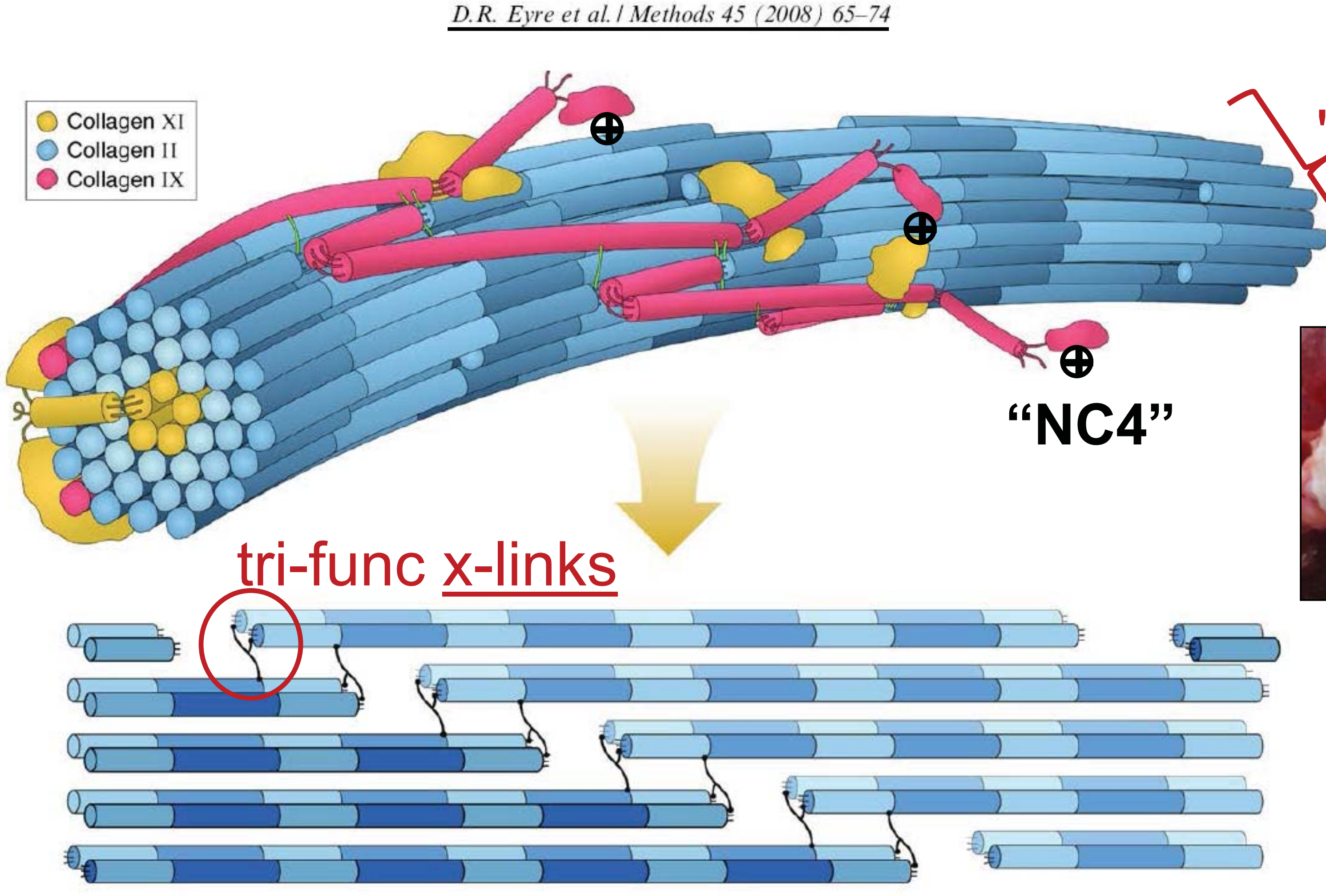


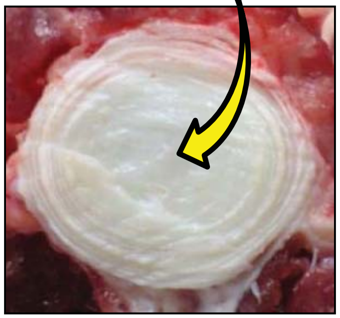
Molecules ↓ **Fibrils** ↓ **Fibers** ↓ **Tissue**

Source: Wright, N. T., and J. D. Humphrey. "Denaturation of Collagen via Heating: An Irreversible Rate Process." *$QQXDO 5HYLHZ RI %LRPHGLFDO (QJLQHHULQJ* 4, no. 1 (2002): 109-28.

Wright & Humphrey, Ann Revs BME, 2002

Disc and Cartilage "Type II" collagen is really II-IX-XI combo

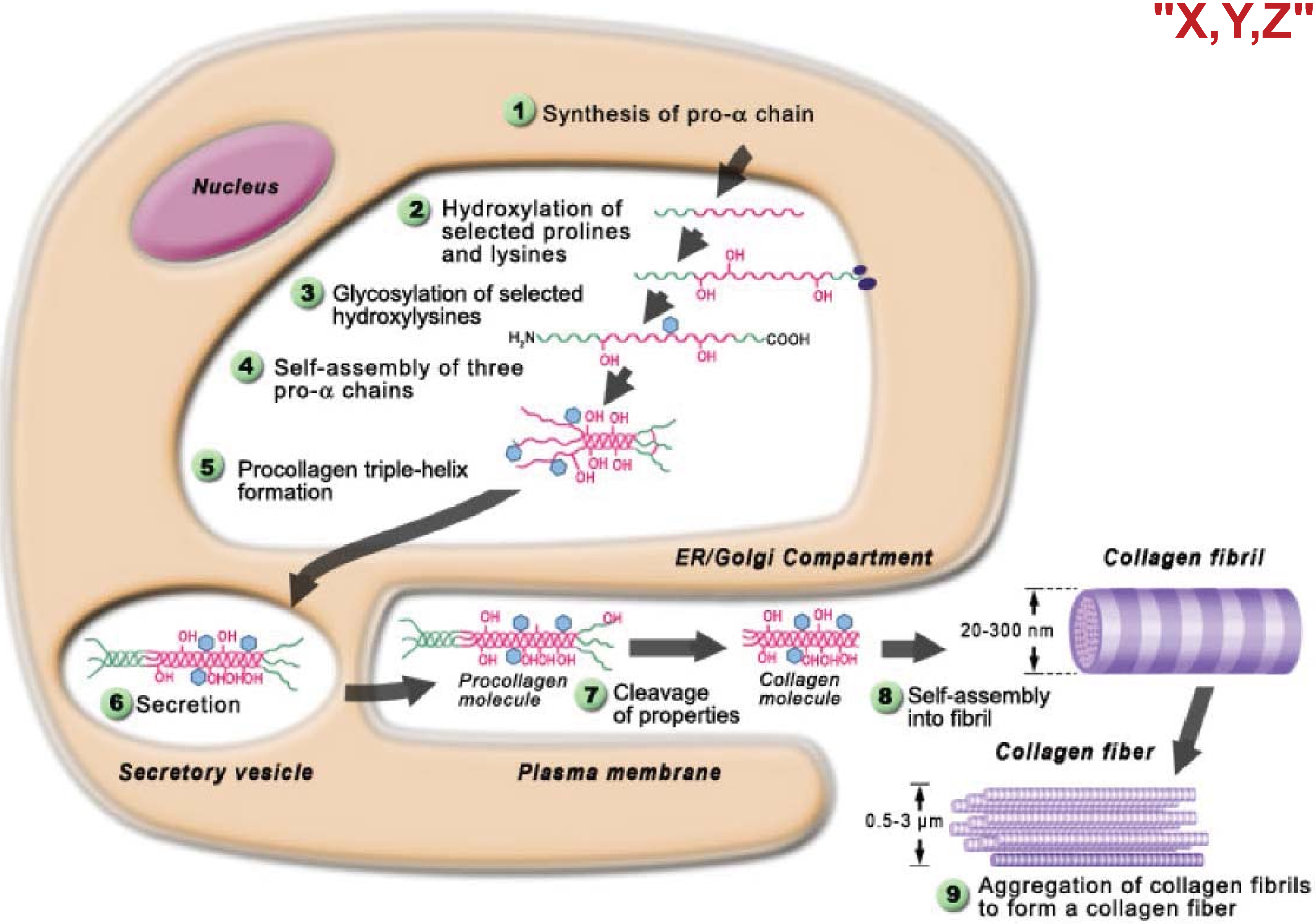




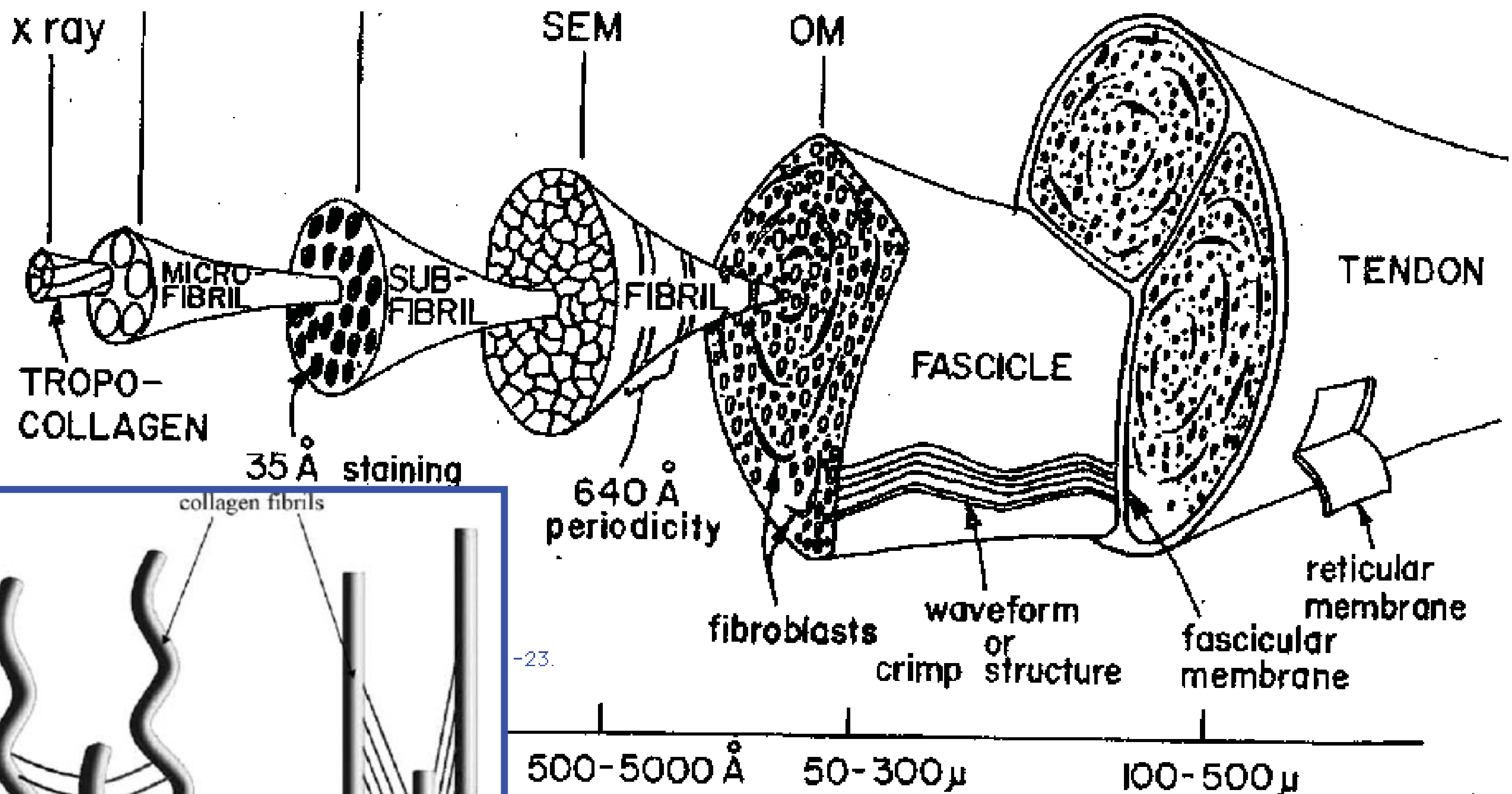
Courtesy of Elsevier, Inc., http://www.sciencedirect.com. Used with permission.
Source: Eyre, David R. "Advances in Collagen Cross-link Analysis." *Methods* 45, no. 1 (2008): 65-74.

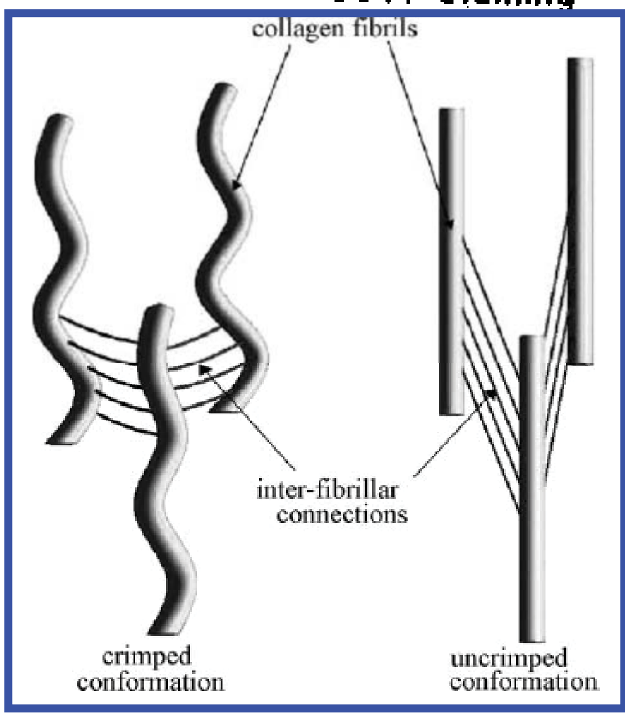
Hierarchical depiction of a heterotypic collagen fibril, emphasizing the internal axial relationships required for mature cross-link formation. Upper: Three-dimensional concept of the type II/IX/XI heterotypic fibril of developing cartilage matrix. Middle: Detail illustrating required nearest neighbor axial relationships for trifunctional intermolecular cross-links to form in collagens of cartilage, bone, and other high-tensile strength tissue matrices. The exact 3D spatial pattern of cross-linking bonds is still unclear for any tissue.

How do cells make collagen molecules and regulate "fibrillogenesis" ? Why do cells in tissue "A" pick collagens "X,Y,Z" ?



Tendon Structure





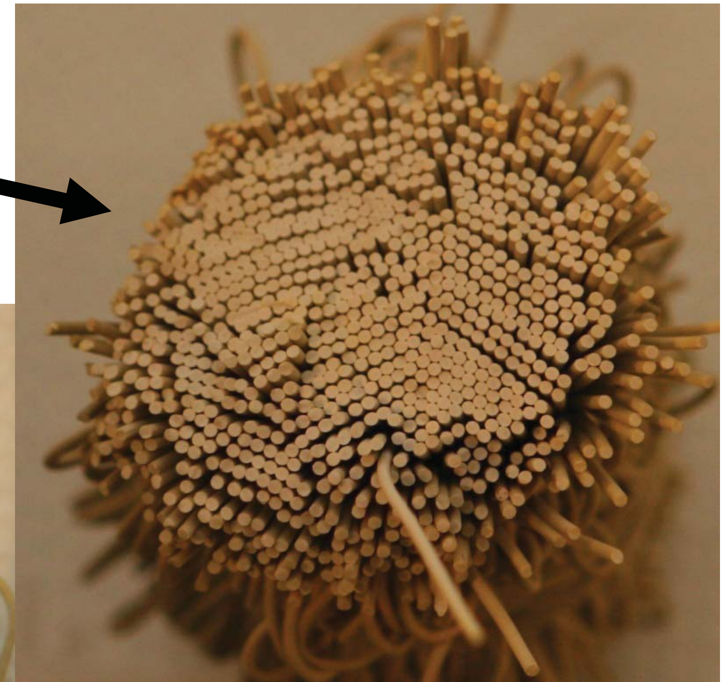
-23.

Source: Kastelic, J., A. Galeski, et al. "The Multicomposite Structure of Tendon." *Connective Tissue Research* 6, no. 1 (1978): 11-23.

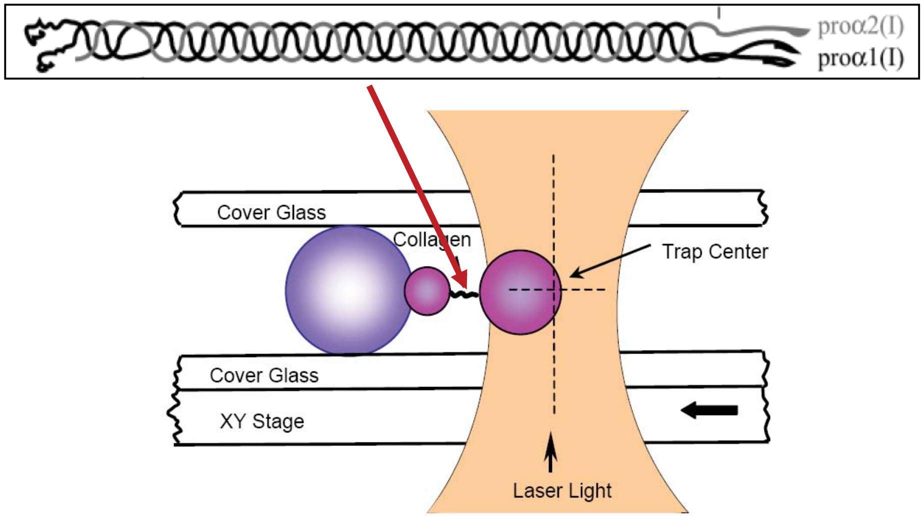
Lots of

Intra-molecular, Inter-molecular, Intra-fibrillar, Inter-fibrillar Crosslinks!!

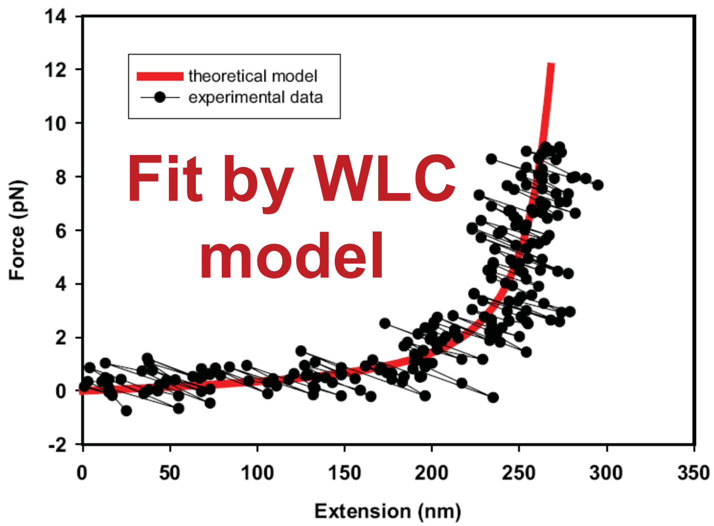
No intra- or inter-molecular crosslinks…..
what were they thinking?



Pro-collagen molecule →



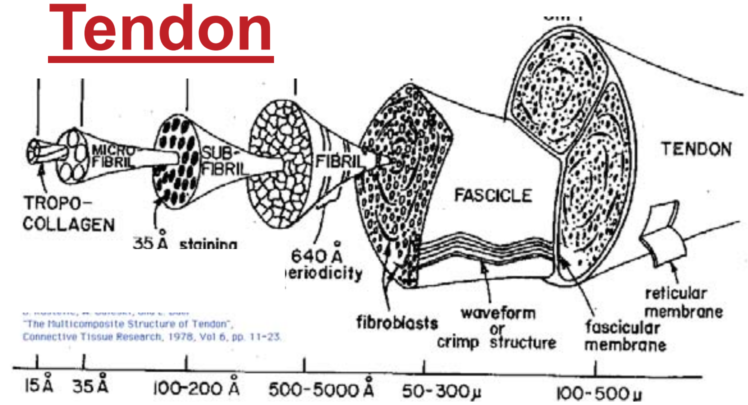
Force - extension



(Sun+, J Biomechanics, 2004)

Source: Sun, Yu-Long, et al. "Stretching Type II Collagen with Optical Tweezers." *Journal of Biomechanics* 37, no. 11 (2004): 1665-9.

Tendon



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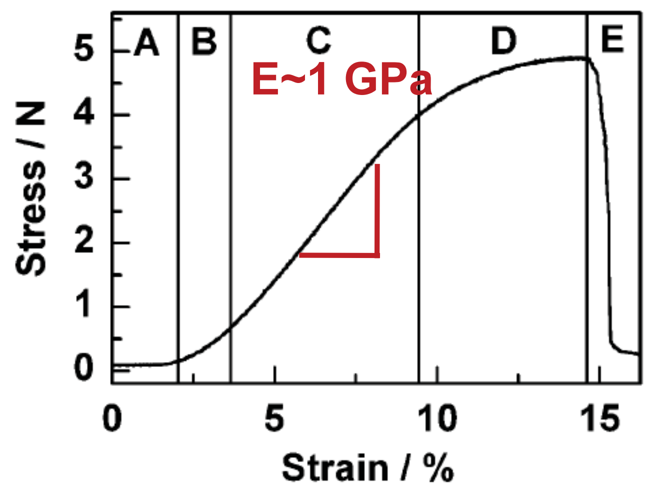


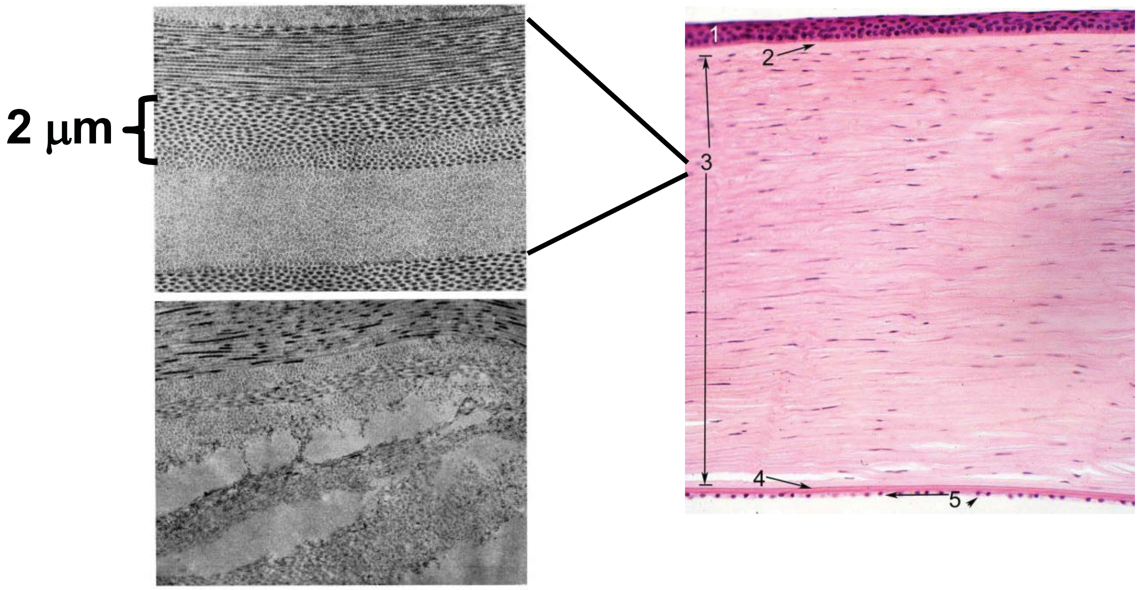
Source: Gutsmann, Thomas. "Force Spectroscopy of Collagen Fibers to Investigate their Mechanical Properties and Structural Organization." *Biophysical Journal* 86, no. 5 (2004): 3186-93.

Stress vs strain curve of a rat tail tendon: (A-B) Toe - heel region, **(C) linear region**, (D) plateau, (E) rupture of the tendon.

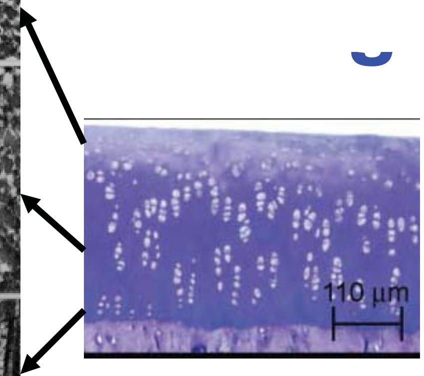
(Gutsmann+, Biophys J, 2004)

Cornea

Cornea collagen architecture

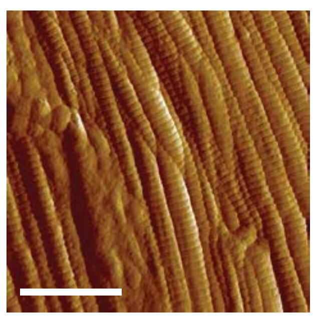


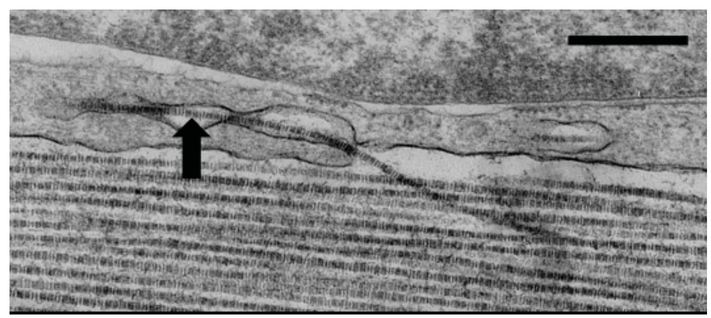
Collagen (type II)



Tendon

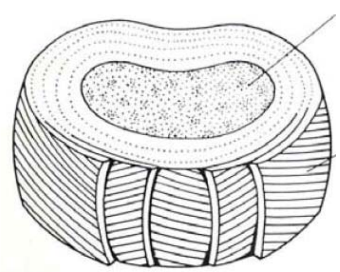
imaged by AFM

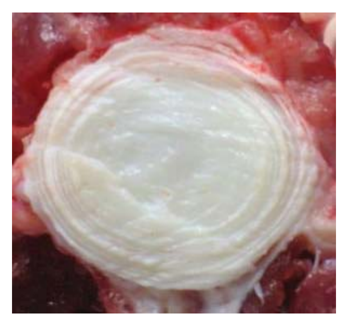




Tendon collagen fibrils (~28 nm) secreted and organized by tendon fibroblast

Collagen architecture of the disc

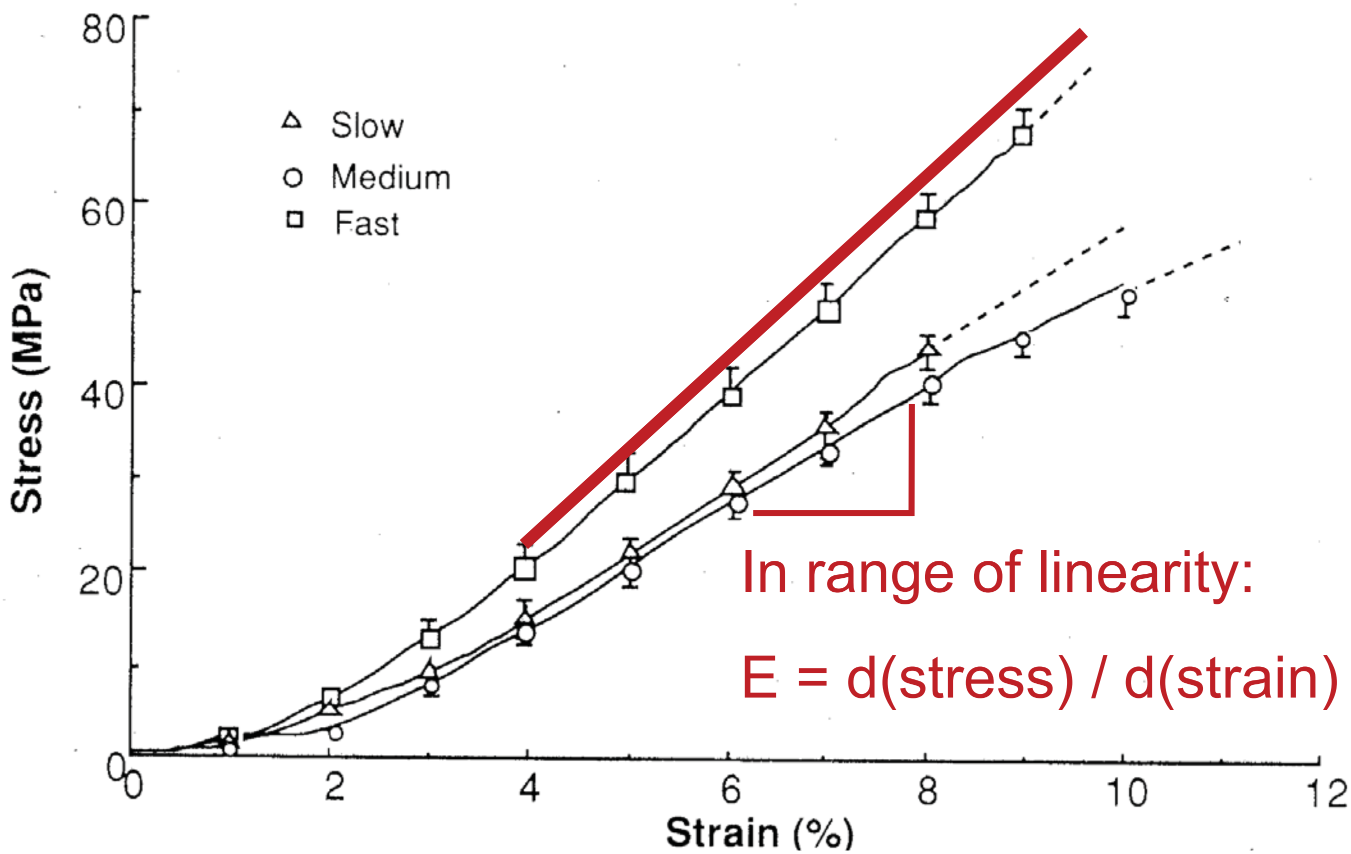




Human intervertebral

Disc

Young's Modulus of Ligament (ACL) ≈ 1 GPa



In range of linearity:

E = d(stress) / d(strain) ~ 10^9 Pa

Macro –Tissue – scale Measurement

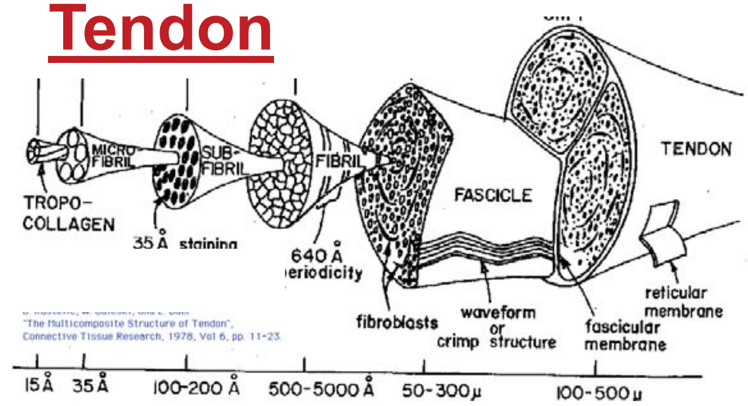
Danto MI, Woo SL-Y (1993) The mechanical properties of skeletally mature rabbit anterior cruciate ligament and patellar tendon over a range of strain rates. J Orthop Res 11:58–67

Source: Danto, Michael I., and Savio L.Y. Woo. "The Mechanical Properties of Skeletally Mature Rabbit Anterior Cruciate Ligament and Patellar Tendon Over a Range of Strain Rates." *Journal of Orthopaedic Research* 11, no. 1 (1993): 58-67.

Photograph by Christophe Pallot/Agence Zoom © Getty Images $OO ULJKWV UHVHUYHG 7KLV FRQWHQW LV excluded
RXU &UHDWLYH &RPPRQV OLFHQVH)RU PRUH LQIRUPDWLRQ VHH KWWS RFZ PLW HGX KHOS IDT IDLU XVH .

- How do cells make fibrils from procollagen??
- How are collagen fibrils laid down and oriented??
- What is process during tissue embryogenesis ??
- What about mature tissue *after injury*: how do tendons / ligaments heal ??

Tendon



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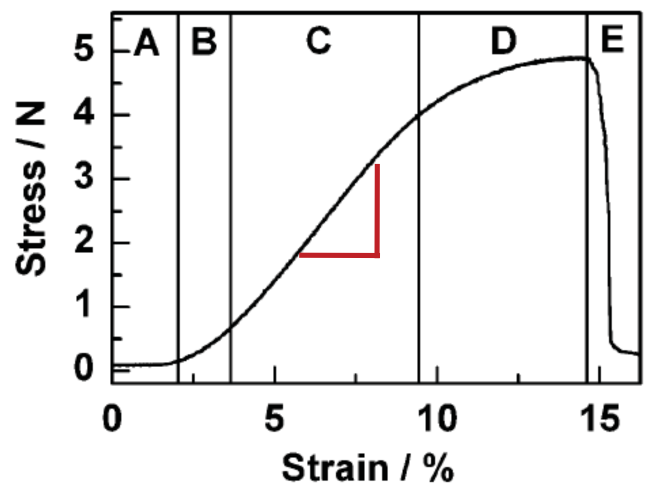

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Stress vs strain curve of a rat tail tendon: (A-B) Toe - heel region, **(C) linear region**, (D) plateau, (E) rupture of the tendon.

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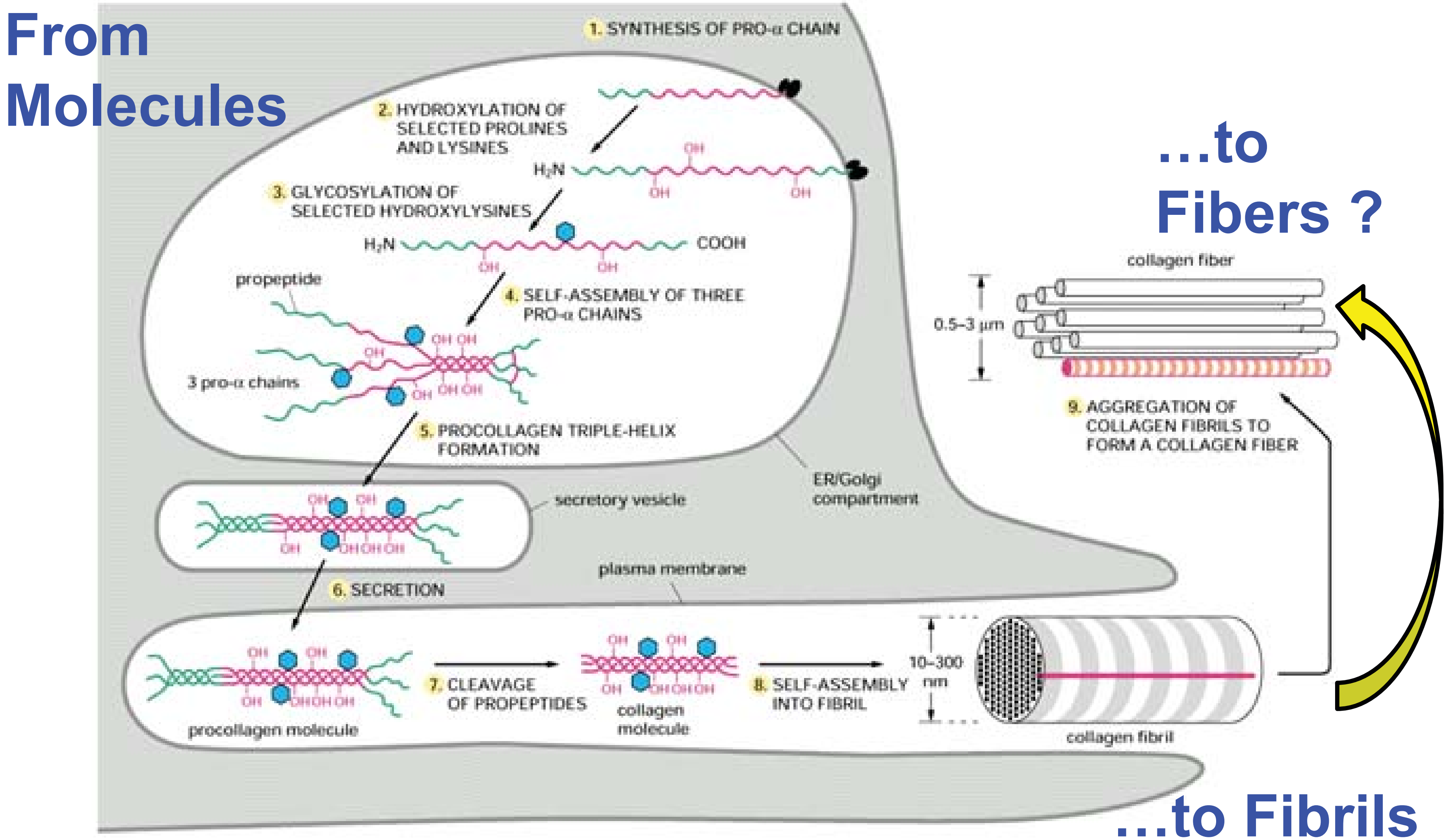


Figure 19-43. The intracellular and extracellular events involved in the formation of a collagen fibril. Note that collagen fibrils are shown assembling in the extracellular space contained within a large infolding in the plasma membrane. As one example of how the collagen fibrils can form ordered arrays in the extracellular space, they are shown further assembling into large collagen fibers, which are visible in the light microscope. The covalent cross-links that stabilize the extracellular assemblies are not shown.

Coalignment of plasma membrane channels and protrusions (fibripositors) specifies the parallelism of tendon

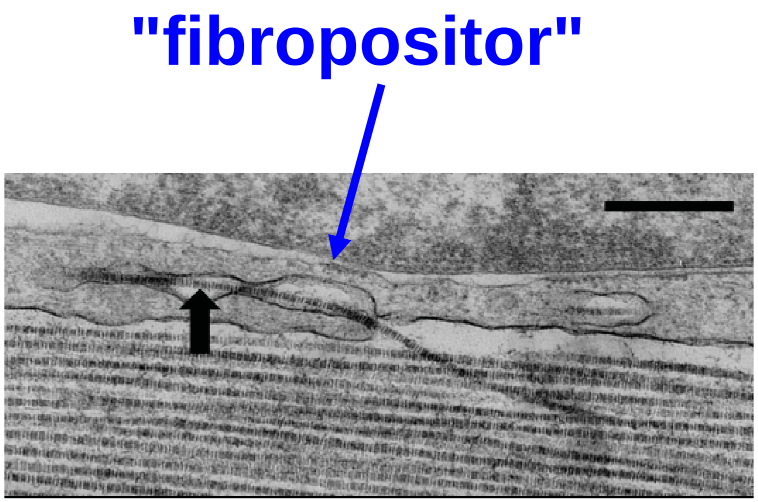
J Cell Biol 2004

Elizabeth G. Canty, Yinhui Lu, Roger S. Meadows, Michael K. Shaw, David F. Holmes, and Karl E. Kadler

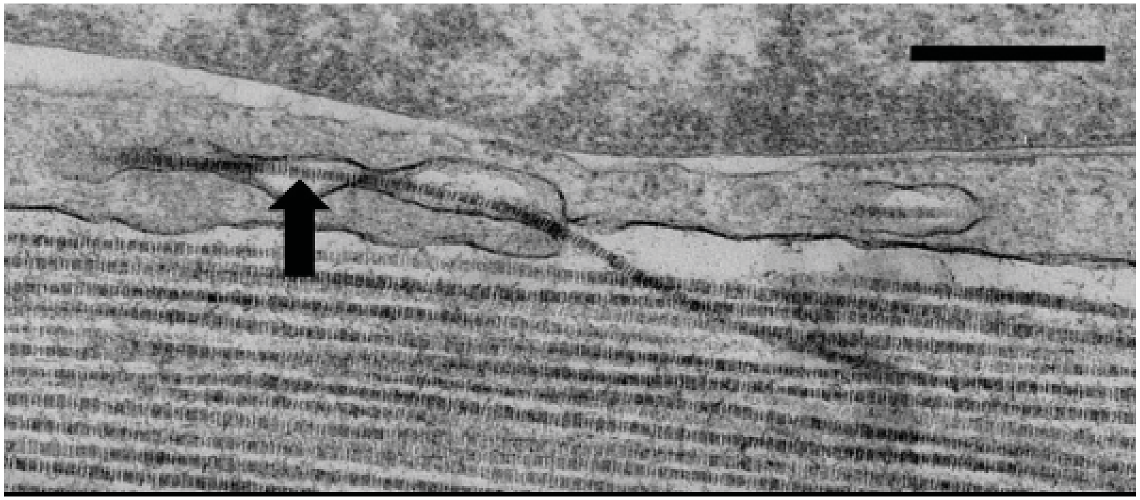
Wellcome Trust Centre for Cell-Matrix Research, School of Biological Sciences, University of Manchester, Manchester M13 9PT UK

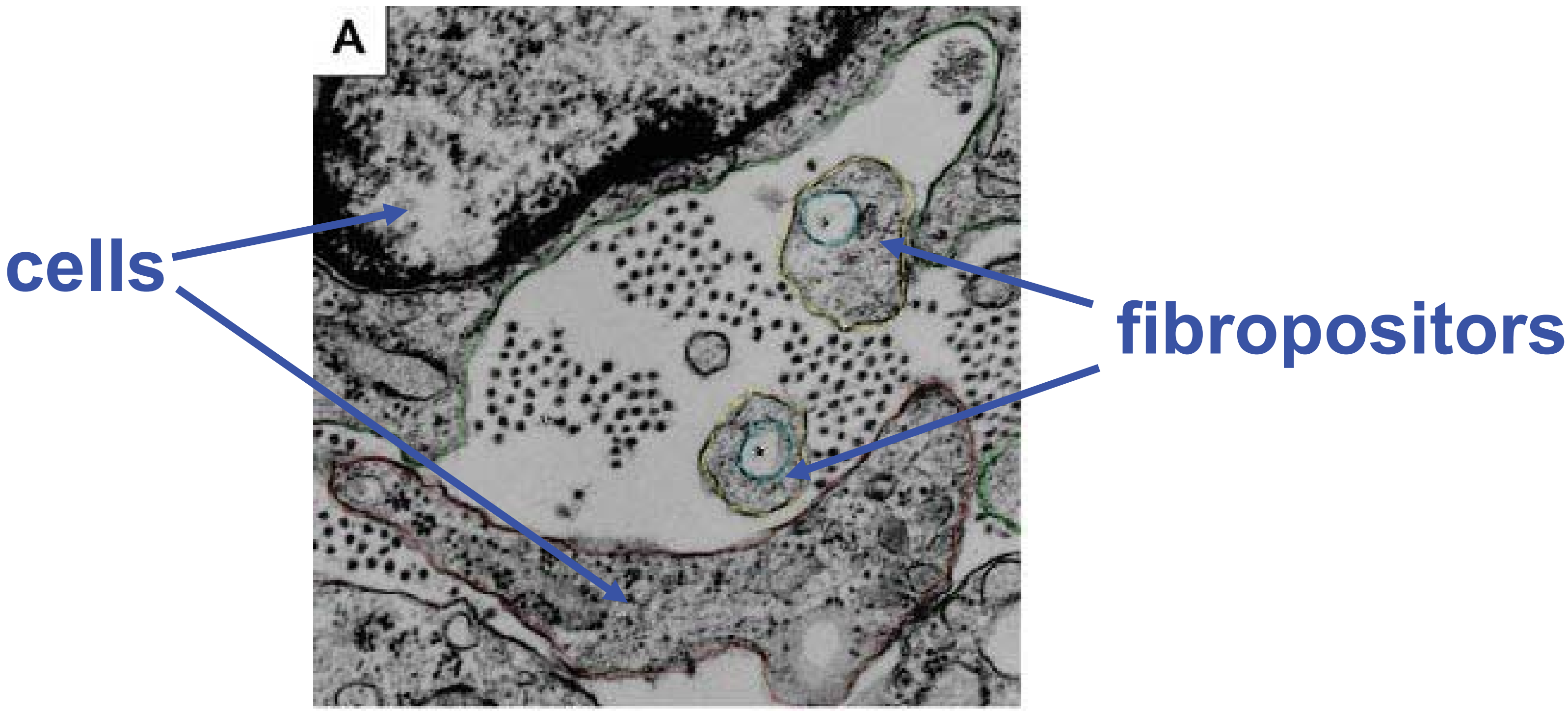
The functional properties of tendon require an extracellular matrix (ECM) rich in elongated collagen fibrils in parallel register. We sought to understand how embryonic fibroblasts elaborate this exquisite arrangement of fibrils. We show that procollagen processing and collagen fibrillogenesis are initiated in Golgi to plasma membrane carriers (GPCs). These carriers and their cargo of 28-nm-diam fibrils are targeted to previously unidentified plasma membrane (PM) protrusions (here designated "fibripositors") that are parallel to the tendon axis and project into parallel channels between cells. The base of the fibripositor lumen (buried several microns within the cell) is a nucleation site

Fibripositors are absent at postnatal stages when fibrils increase in diameter by accretion of extracellular collagen, thereby maintaining parallelism of the tendon. Thus, we show that the parallelism of tendon is determined by the late secretory pathway



Source: Canty, Elizabeth G. "Coalignment of Plasma Membrane Channels and Protrusions (fibripositors) specifies the Parallelism of Tendon." *The Journal of Cell Biology* 165, no. 4 (2004): 553-63.





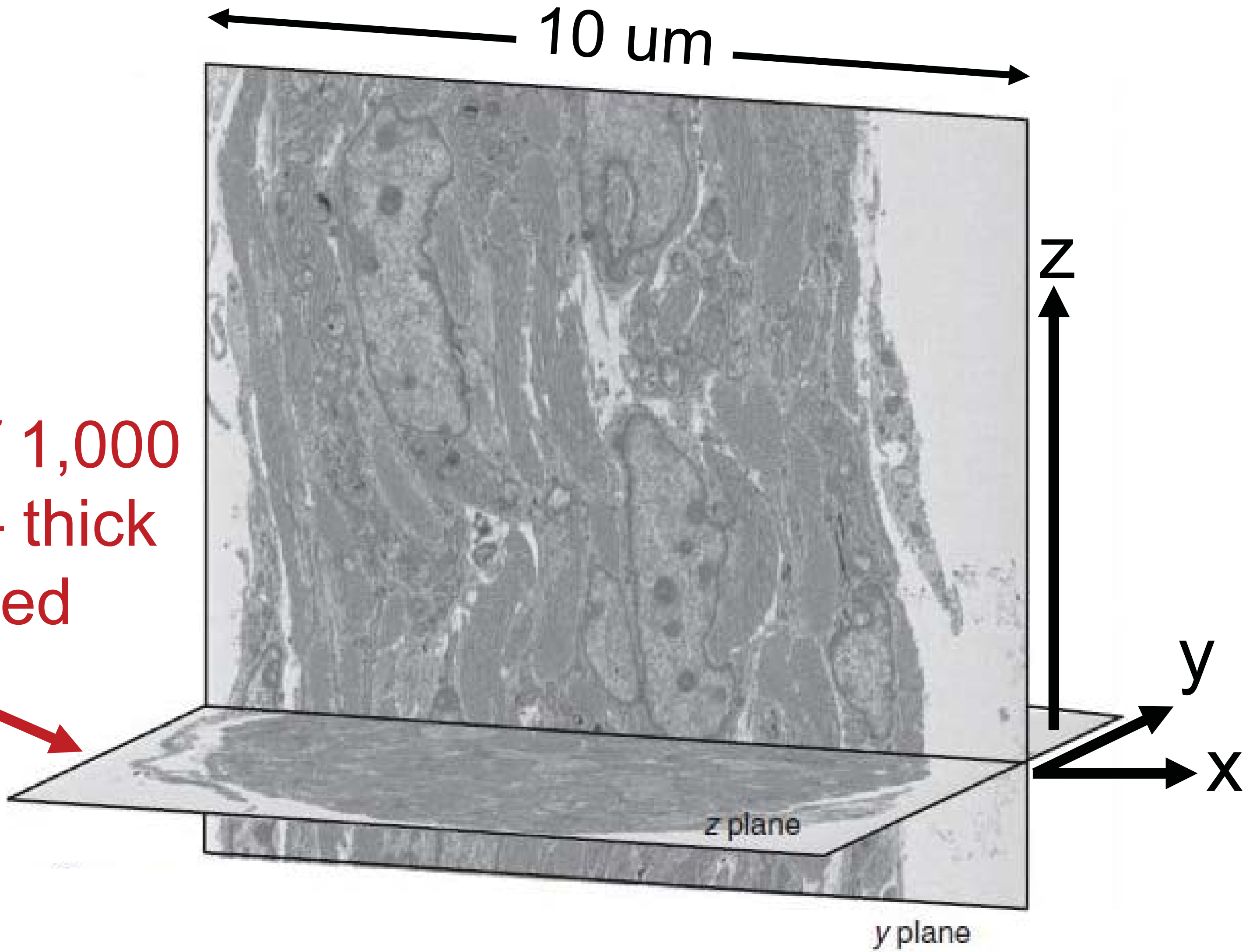
Source: Canty, Elizabeth G. "Coalignment of Plasma Membrane Channels and Protrusions (fibripositors) specifies the Parallelism of Tendon." *The Journal of Cell Biology* 165, no. 4 (2004): 553-63.

Serial Block Face -- Scanning Electron Microscopy

(“SBF-SEM”)

Embryonic Mouse Tail Tendon

Stacks of 1,000 100 nm – thick microtomed slices



Starborg / Kadler+, 1446 | VOL.8 NO.7 | 2013 | NATURE PROTOCOLS

Source: Starborg, Tobias., et al. "Using Transmission Electron Microscopy and 3View to Determine Collagen Fibril Size and Three-dimensional Organization." *1DWXUH 3URWRFRO* 8, no. 7 (2013): 1433-48.

Nonmuscle myosin II powered transport of newly formed collagen fibrils at the plasma membrane

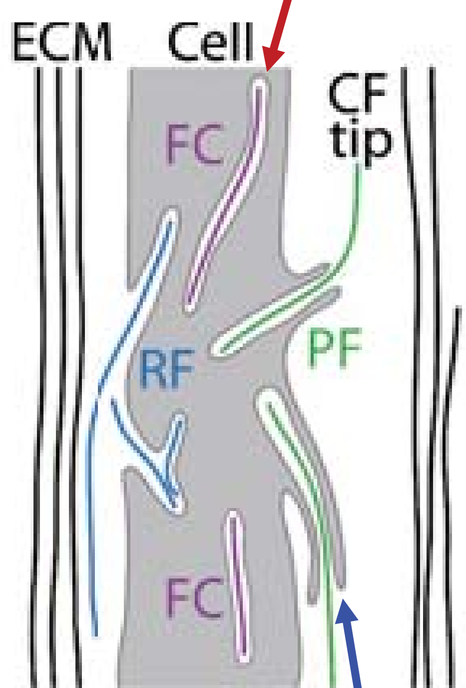
Nicholas S. Kalson[a,1], Tobias Starborg[a,1], Yinhui Lu[a], Aleksandr Mironov[b], Sally M. Humphries[a], David F. Holmes[a], and Karl E. Kadler[a,2]

PNAS 2013

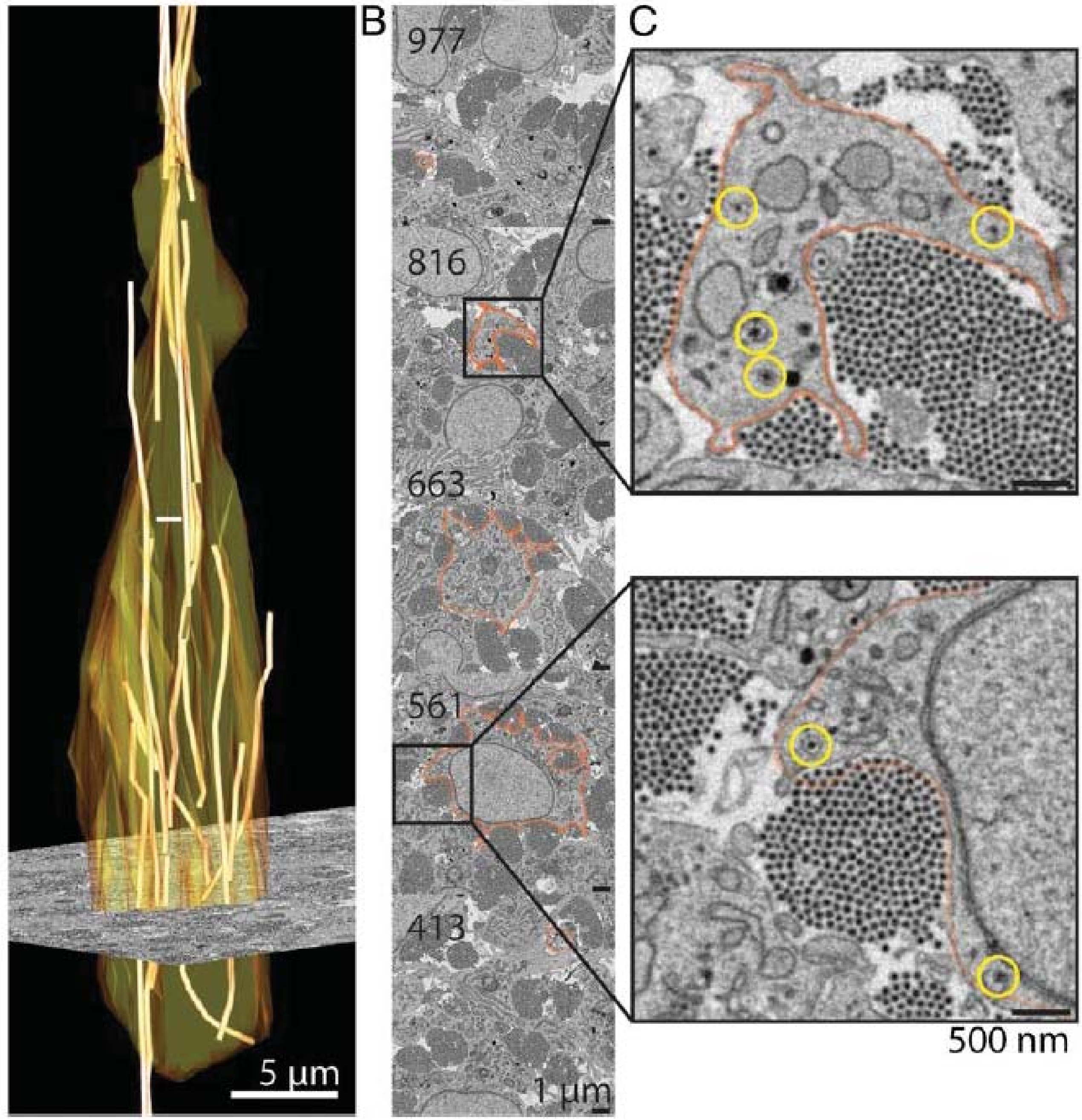
- Collagen fibrils are >1mm long; they are the longest, largest, most size-pleomorphic protein in vertebrates; knowing how cells transport collagen fibrils: key to tissue morphogenesis.
- We identified newly formed collagen fibrils being transported at the surface of embryonic tendon cells in vivo by using SBF-SEM of the cell-matrix interface.
- Newly formed fibrils: ~1 to ~30µm. The shortest (1–10µm) occurred in intracellular fibricarriers; the longest (~30µm) occurred in plasma membrane fibripositors.
- Non-muscle myosin II (NMII)powers transport of new collagen fibrils at the plasma membrane; NMII-dependent cell-force model is the basis for the creation and dynamics of fibripositor structures for making collagen rich tissues.

Cell ~ 60μm long

Intracellular Fibricarrier

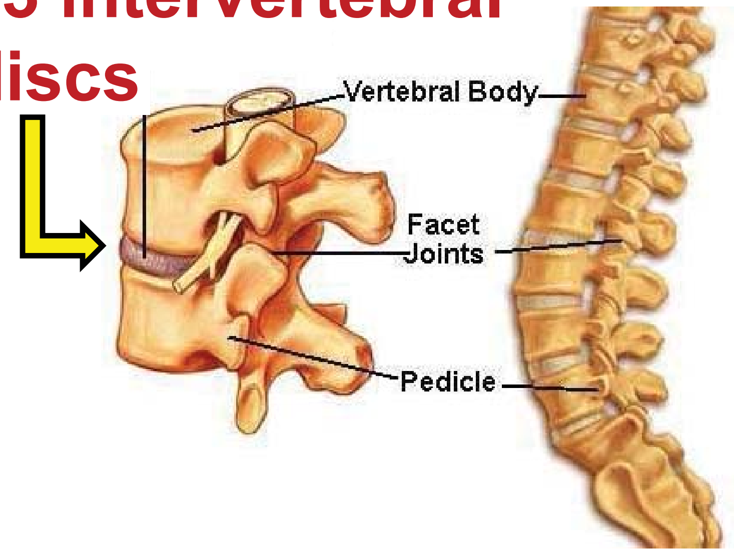


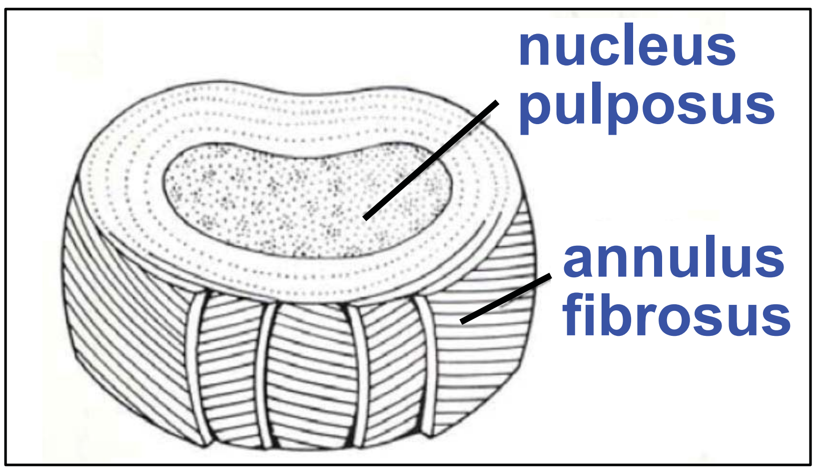
Protruding Fibripositor



Disc Extracellular Matrix Composition

23 intervertebral discs





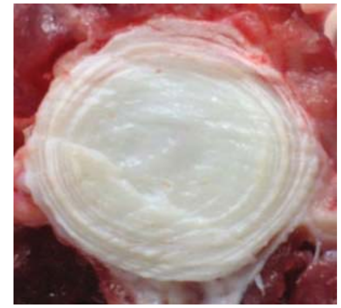
Collagens	Proteoglycans
Fibrillar	**Aggregating**
Type I AF	NP Aggrecan
Type II NP	AF Versican
Type III	NP Hyaluronan
Type V AF	NP Link protein
Type XI NP	**Fibril-associated**
Fibril-associated	AF Decorin
Type IX NP	AF Biglycan
Type XII	Fibromodulin
Type XIV	Lumican
Pericellular	**Pericellular**
Type VI	Perlecan
Type X	

(Peter Roughley, Spine, 2004)

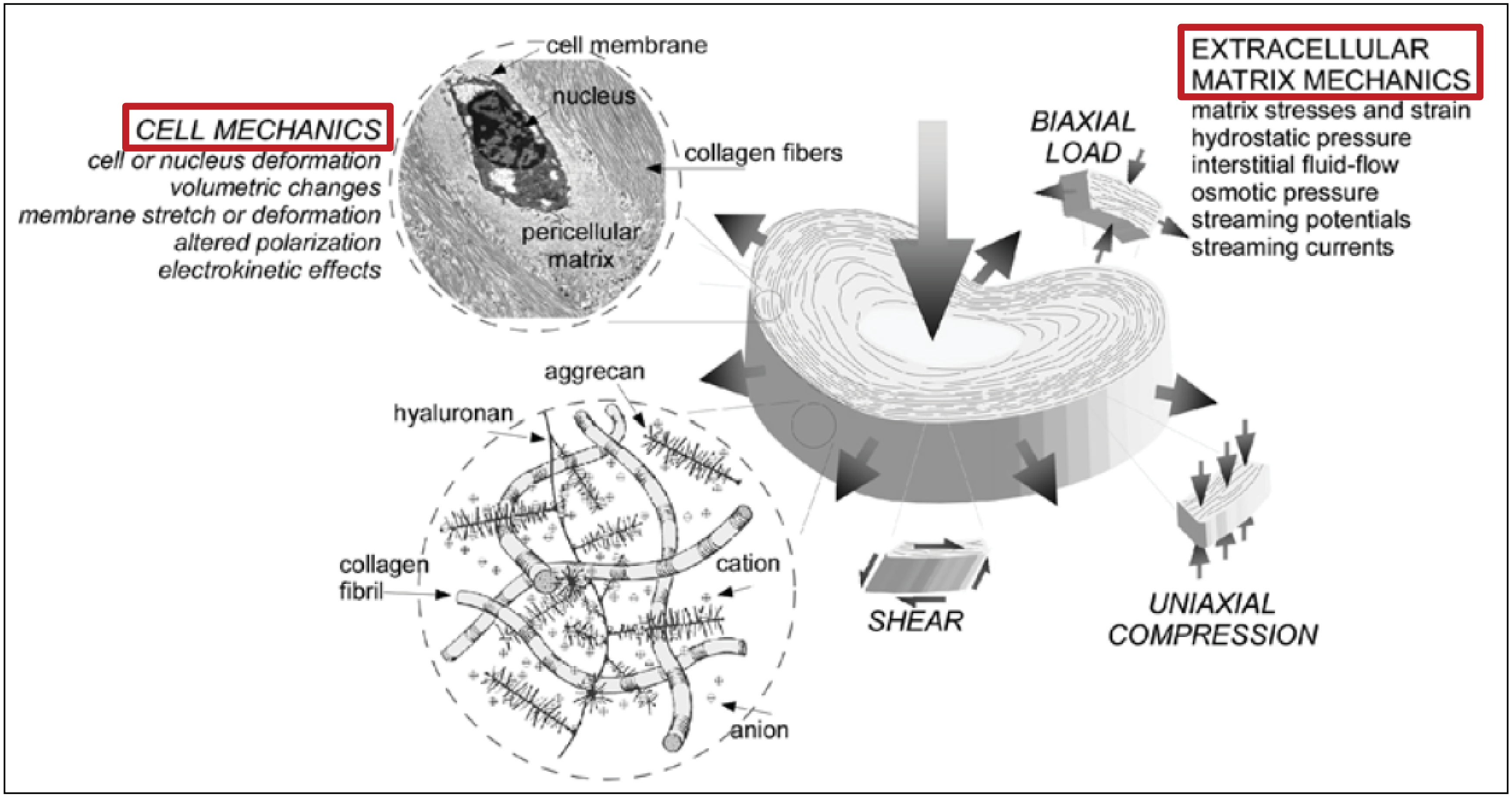
Source: Roughley, Peter J. "Biology of Intervertebral Disc Aging and Degeneration: Involvement of the Extracellular Matrix." *Spine* 29, no. 23 (2004): 2691-9.

Mechanobiology of the Intervertebral Disc and Relevance to Disc Degeneration

By Lori A. Setton, PhD, and Jun Chen, PhD



(Fig 7.1)



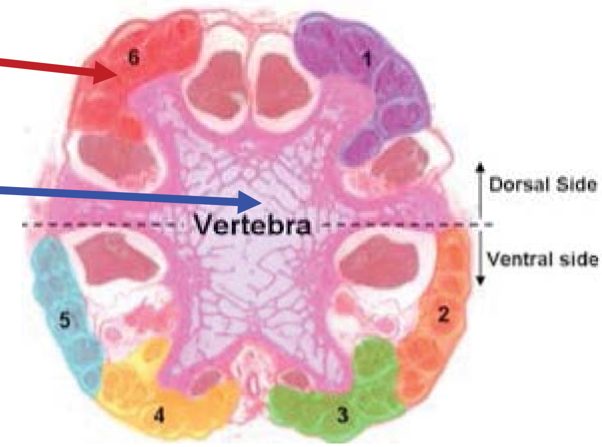
Source: Setton, Lori A., and Jun Chen. "Mechanobiology of the Intervertebral Disc and Relevance to Disc Degeneration." *7KH -RXUQDO RI %RQH -RLQW 6XUJHU* 88, no. suppl 2 (2006): 52-57.

(J Bone & Joint Surg, 2006)

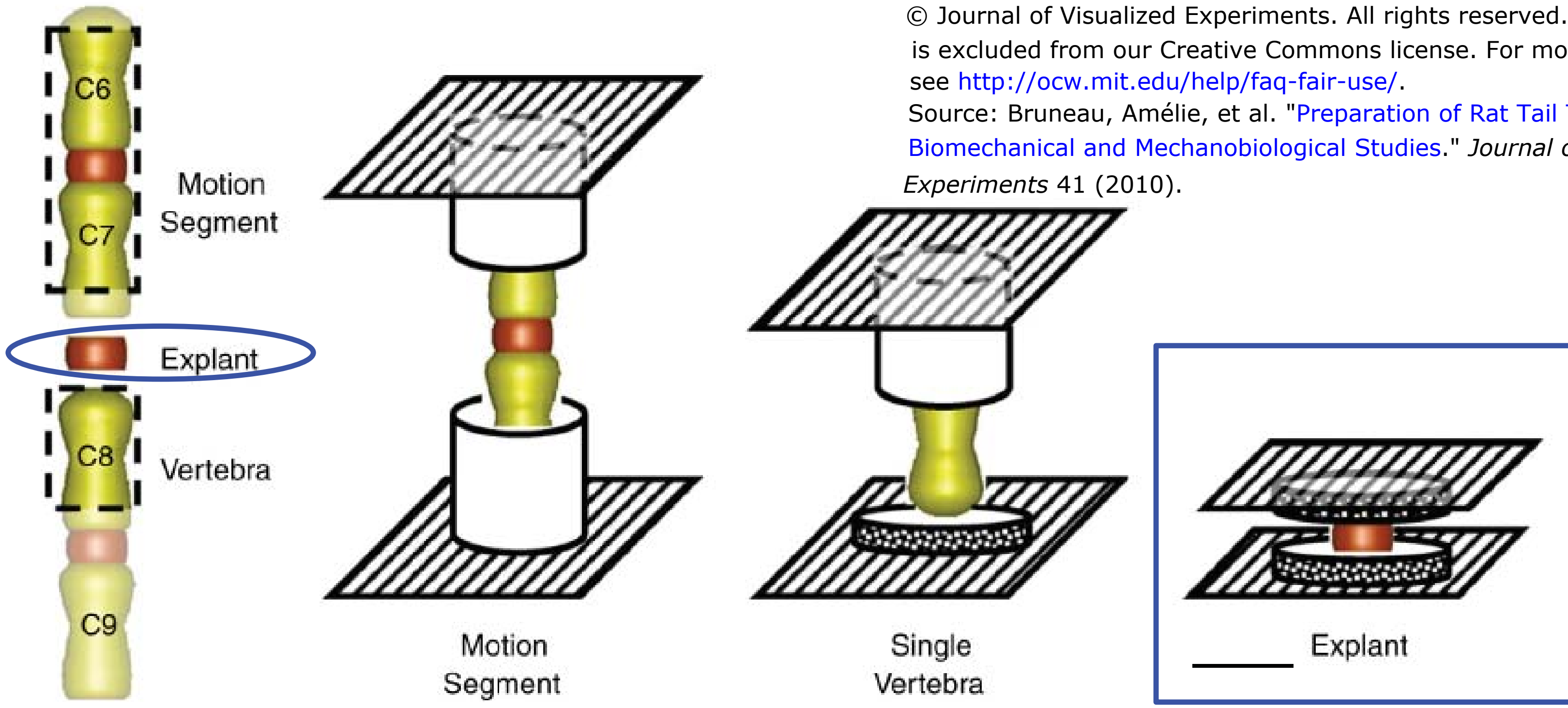
“Creep-Compression” of intervertebral disc (rat tail)

Tendons

Vertebra and Discs

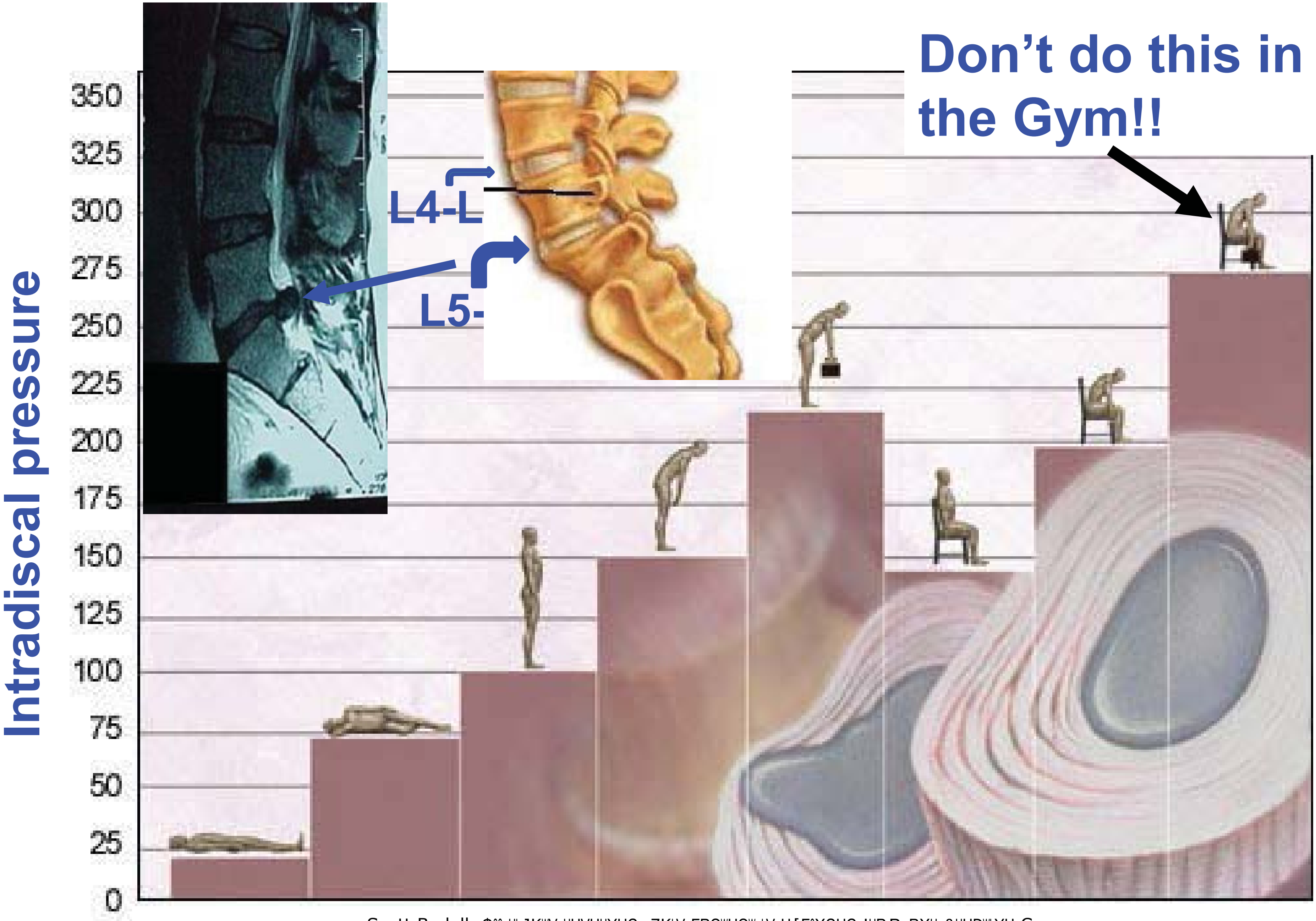


Source: Bruneau, Amélie, et al. "Preparation of Rat Tail Tendons for Biomechanical and Mechanobiological Studies." *Journal of Visualized Experiments* 41 (2010).



Source: MacLean, Jeffrey J., et al. "Role of Endplates in Contributing to Compression Behaviors of Motion Segments and Intervertebral Discs." *-RXUQDO RI %LRPHFKDQLFV* 40, no. 1 (2007): 55-63.

(MacLean+, J Biomechanics, 2007)

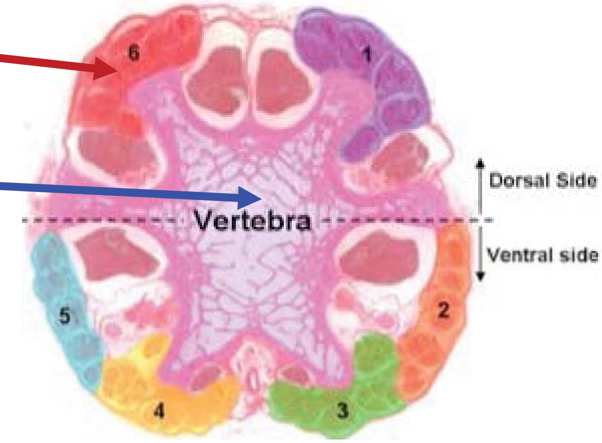


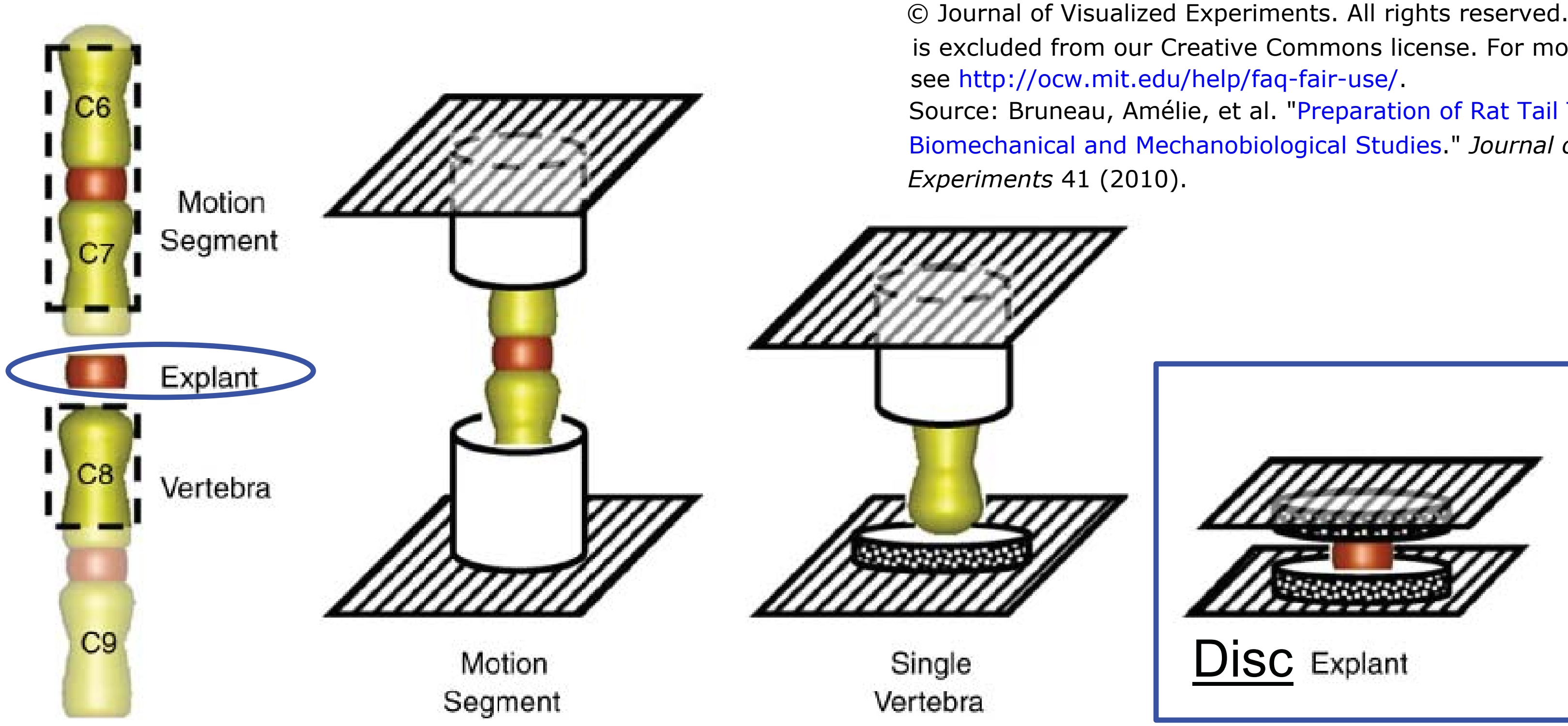
(original data from Alf Nachemson et al., JBJS, 1964)

"Creep-Compression" of intervertebral disc (rat tail)

Tendons

Vertebra and Discs





(MacLean+, J Biomechanics, 2007)

THE INTERACTION OF MUCOPROTEIN WITH SOLUBLE COLLAGEN; AN ELECTRON MICROSCOPE STUDY*

By John H. Highberger, Jerome Gross and Francis O. Schmitt

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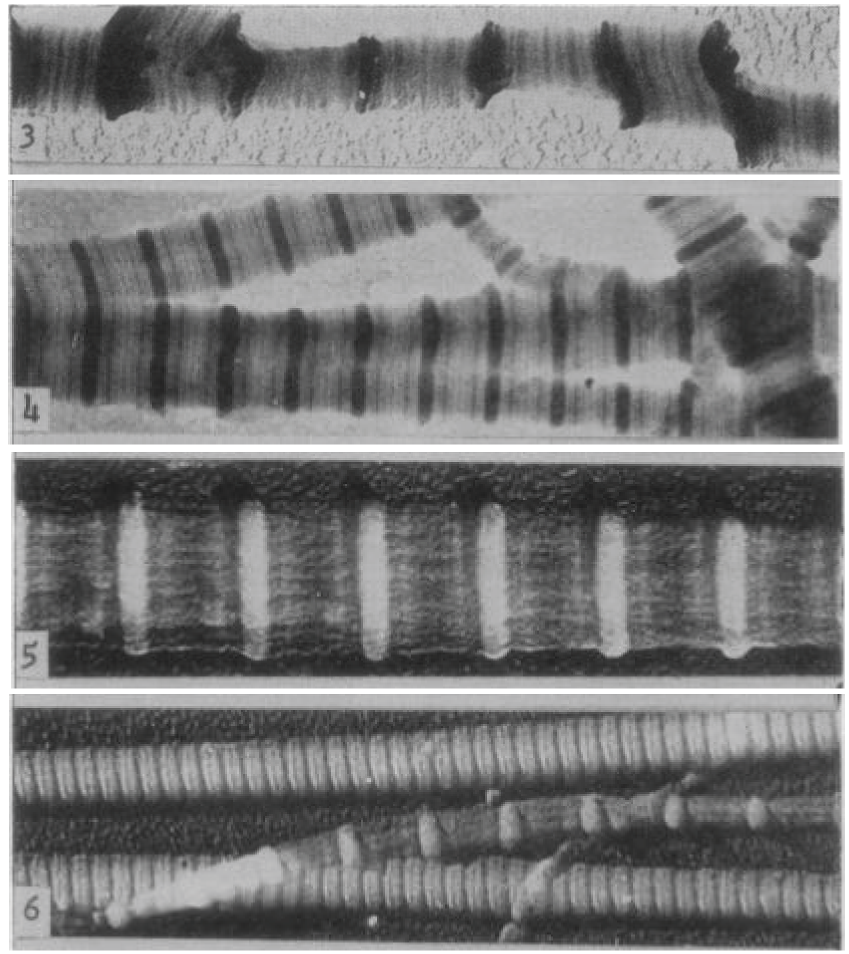
PNAS 1951

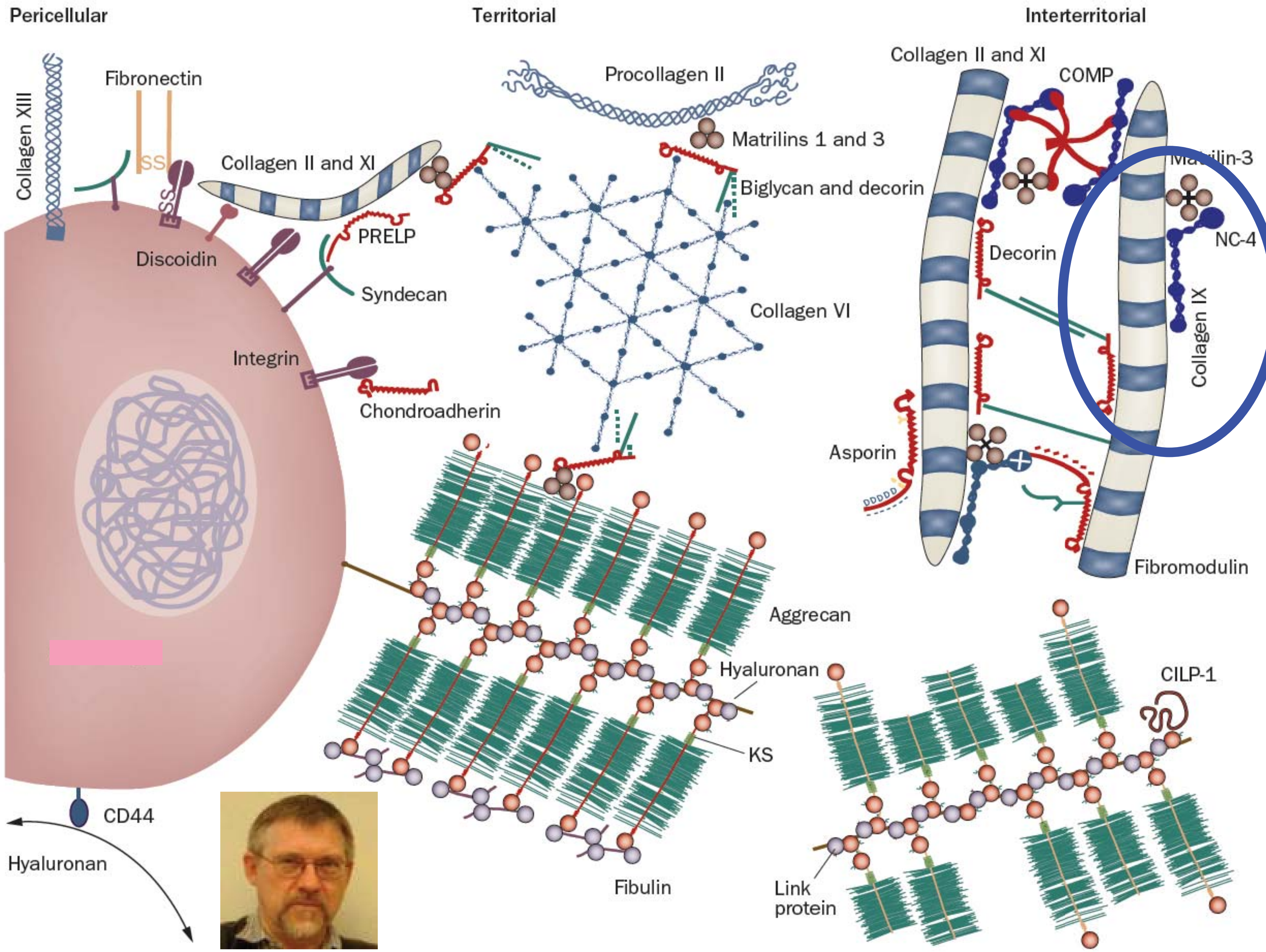
The collagen of certain forms of connective tissue, such as rat tail tendon and the fish swin bladder (ichthyocol), dissolve in dilute acid to yield a clear, relatively viscous solution. When NaCl is added to such a solution to a concentration of 0.2–1.0%, or if the solution be neutralized, a fibrous precipitate of collagen is produced.[1–3] Electron microscope studies have demonstrated that the reconstituted fibrils show the axial period and intra-period fine structure typical of native collagen fibrils although the acid filtrate contains only very thin filaments.[4, 5] The process by which the thin filaments in the acid filtrate aggregate laterally to produce the typical collagen structure is of interest not only from the physical chemical point of view but also because a better understanding of the phenomenon may provide clues as to the mechanism of fibrogenesis *in vivo*. Investigations of the process of fibril reconstitution from acid filtrates of collagen by the addition of salt have been made in these laboratories[6] and will be reported in detail elsewhere. For the present it may be noted that the type of fibril structure observed in the electron microscope (axial repeating patterns of about 650 A, 220 A, or no apparent pattern) depends upon the concentrations of salt and collagen. The experiments described in this paper suggest that other factors may also be of importance in the process of reconstitution.



Rat Tail Tendon Collagen

Highberger, John H., et al. "The Interaction of Mucoprotein with Soluble Collagen: An Electron Microscope Study." 3URFHHGLQJV RI WKH 1DWLRQDO $FDGHP\ RI 6FLHQFHV RI WKH 8QLWHG 6WDWHV RI $PHULFD 37, no. 5 (1951): 286.

Cells Synthesize 100s of Extracellular Matrix Macromolecules



(Dick Heinegård, Nature Revs. Rheumatology 2010)

Source: Heinegård, Dick, and Tore Saxne. "The Role of the Cartilage Matrix in Osteoarthritis." *Nature Reviews Rheumatology* 7, no. 1 (2011): 50-56.

J. Visualized Experiments 2010

Video Article

Preparation of Rat Tail Tendons for Biomechanical and Mechanobiological Studies

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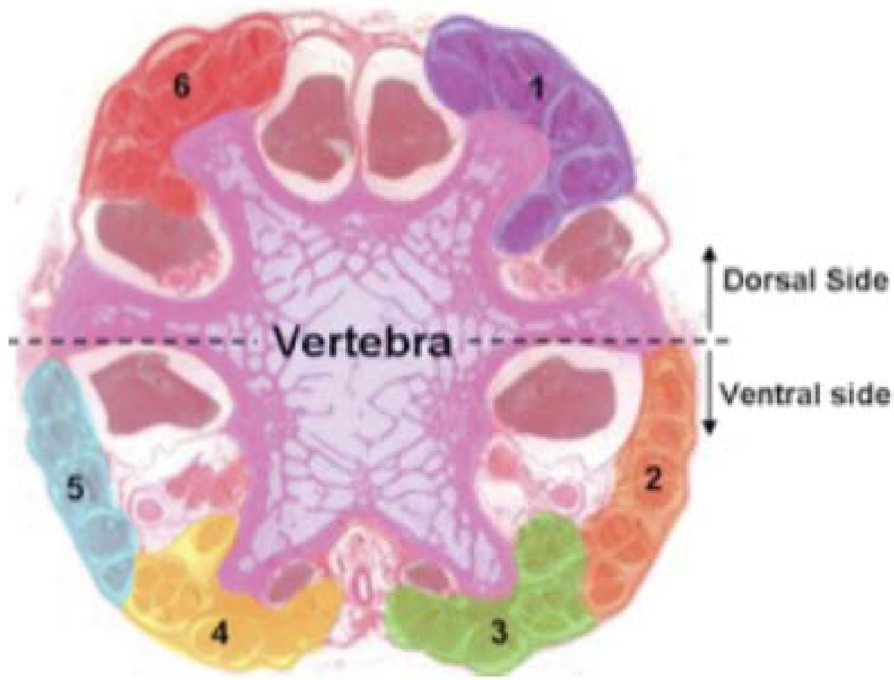
Part 1: Extraction

After resection, the tail is carefully manipulated by its extremities to avoid damaging the tissues.
are carried out in cold saline solution.

1A) Materials:

- Cold saline solution (D-PBS)
- Crushed ice
- Surface protector
- Cutting board
- Individual manipulation plates
- 2 500 ml dishes
- 2 2L glass dishes
- Adhesive tape
- 1 Tweezers
- 1 Forceps
- 1 Tweezers stand
- 1 Pair of surgical shears
- 1 Scalpel
- 1 Pair of surgical scissors

Figure 1. Individual manipulation with orientation identification ("P" for "proximal")



20.310J / 3.053J / 6.024J / 2.797J Molecular, Cellular, and Tissue Biomechanics
Spring 2015