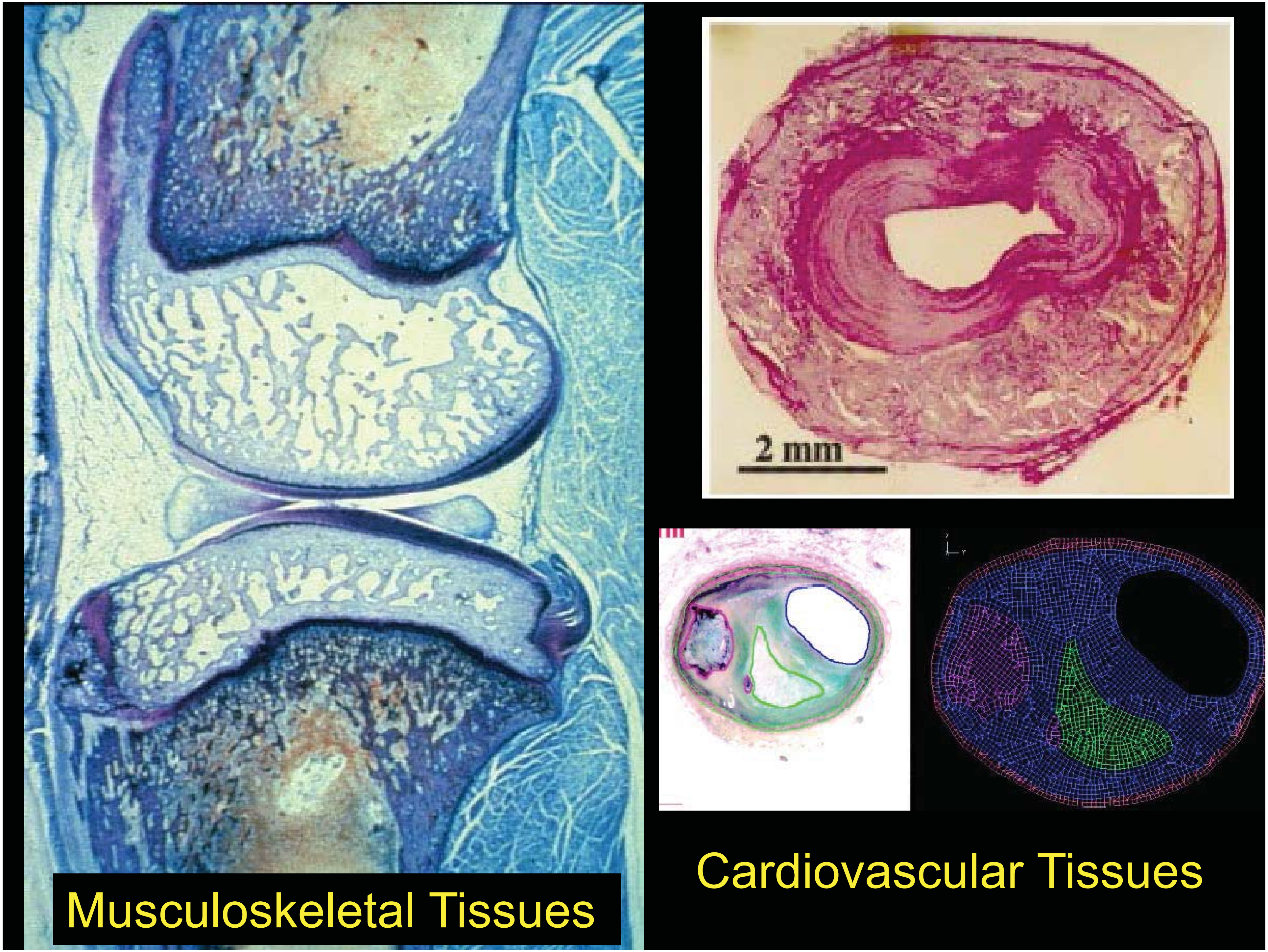
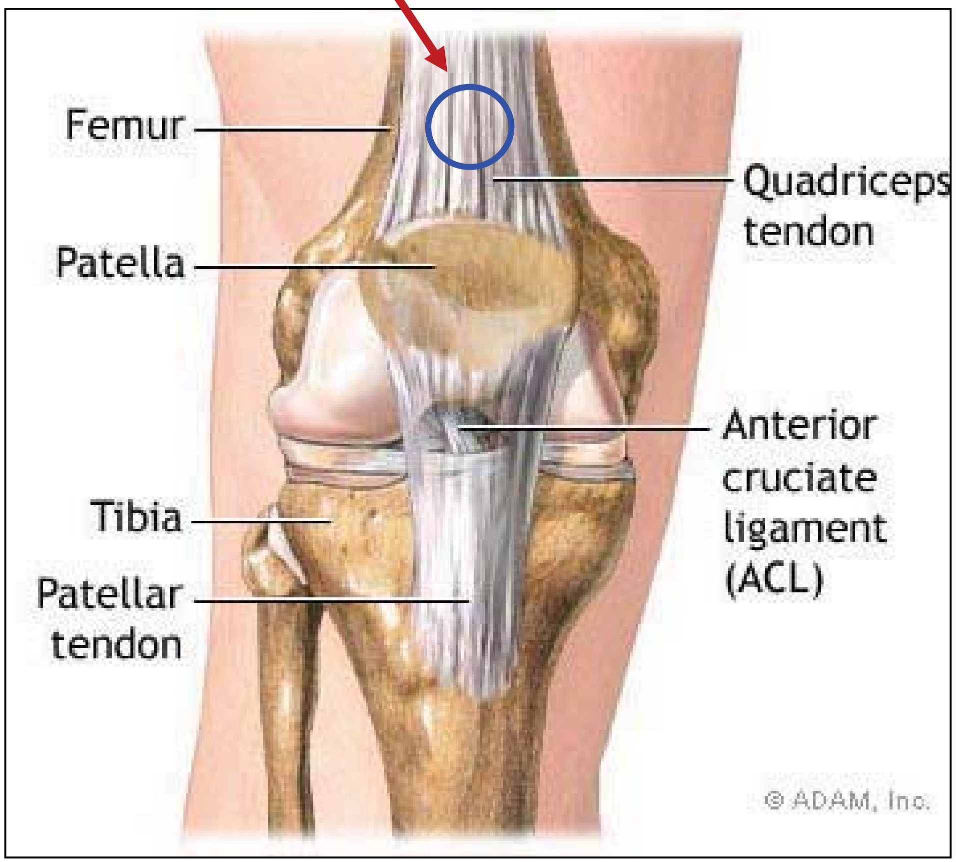


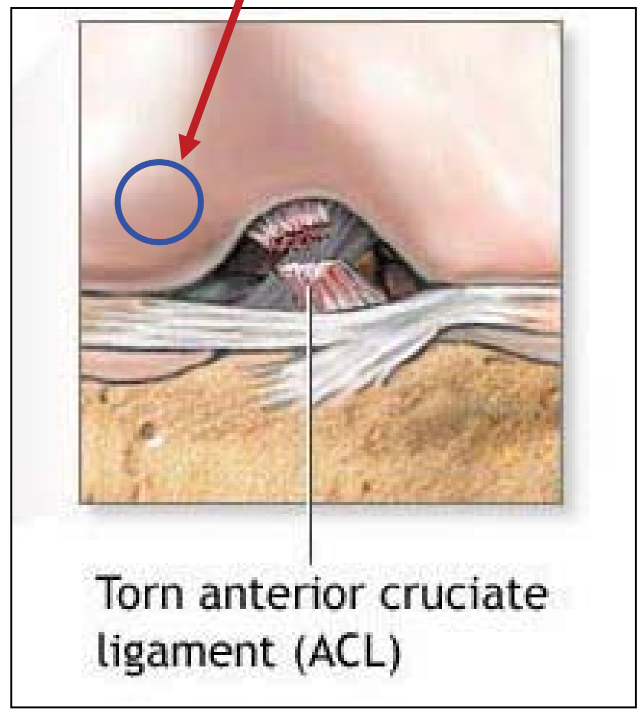
Courtesy of Ernst B. Hunziker. Used with permission.

Musculoskeletal Tissues

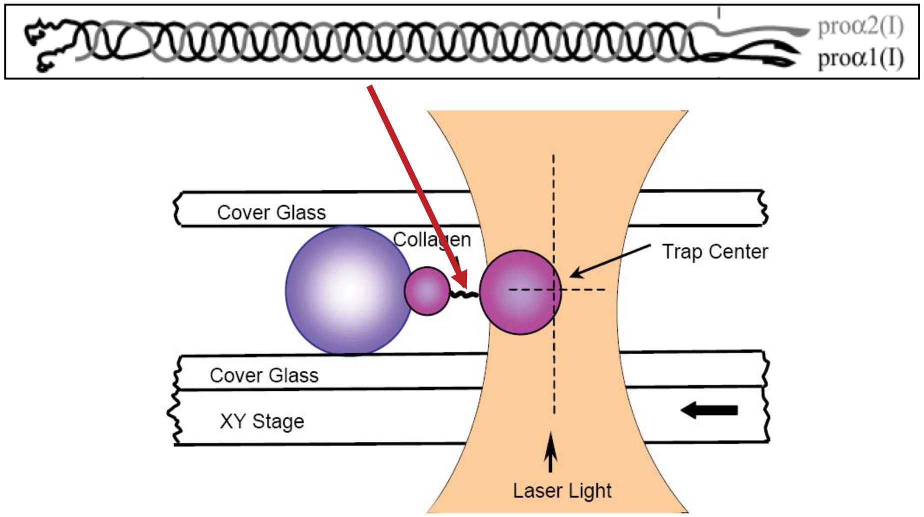
Tension



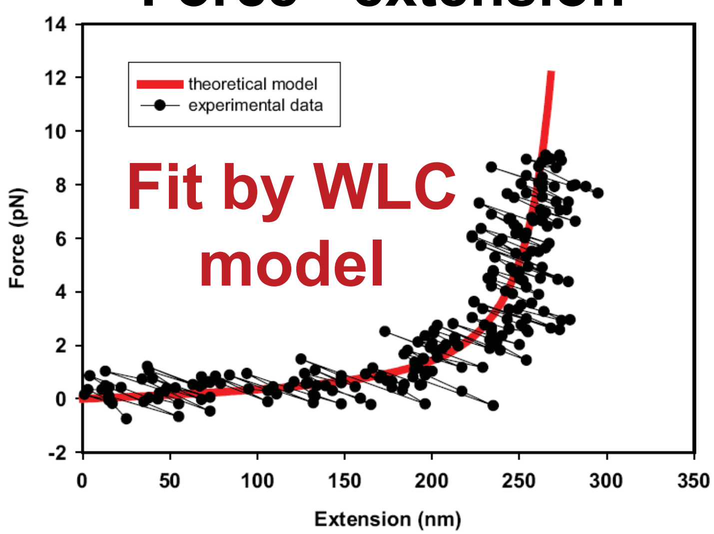
Compression & Shear



Pro-collagen molecule → Tendon

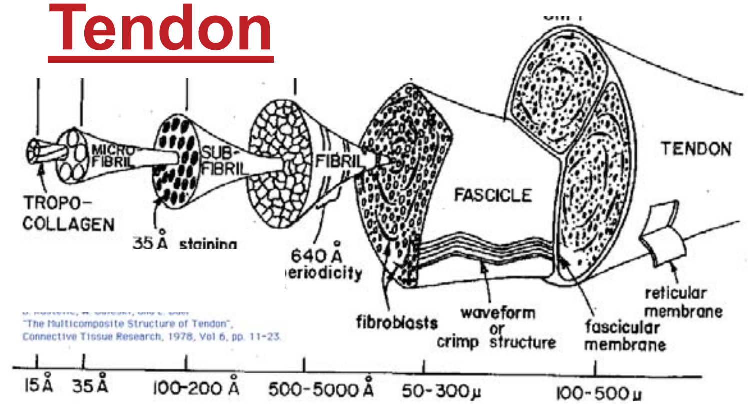


Force - extension

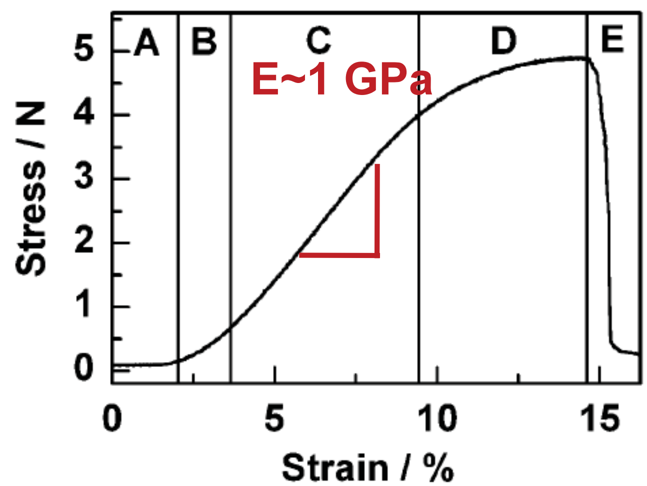


(Sun+, J Biomechanics, 2004)

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



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



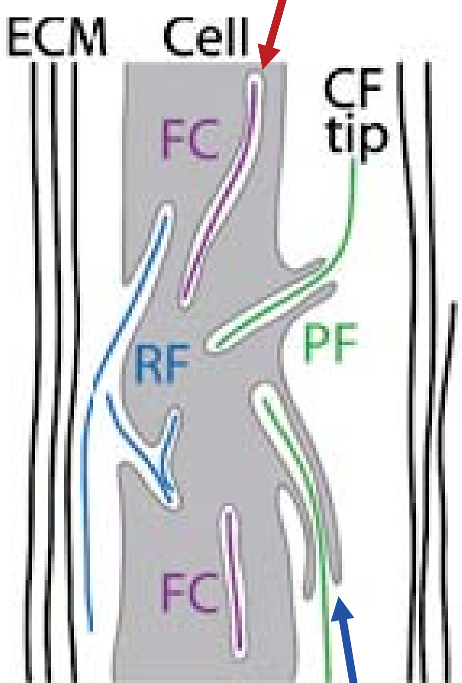
Source: Gutsmann, Thomas. "Force Spectroscopy of Collagen Fibers to Investigate their Mechanical Properties and Structural Organization." *Biophysical Journal* 86, no. 5 (2004): 3186-93.

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

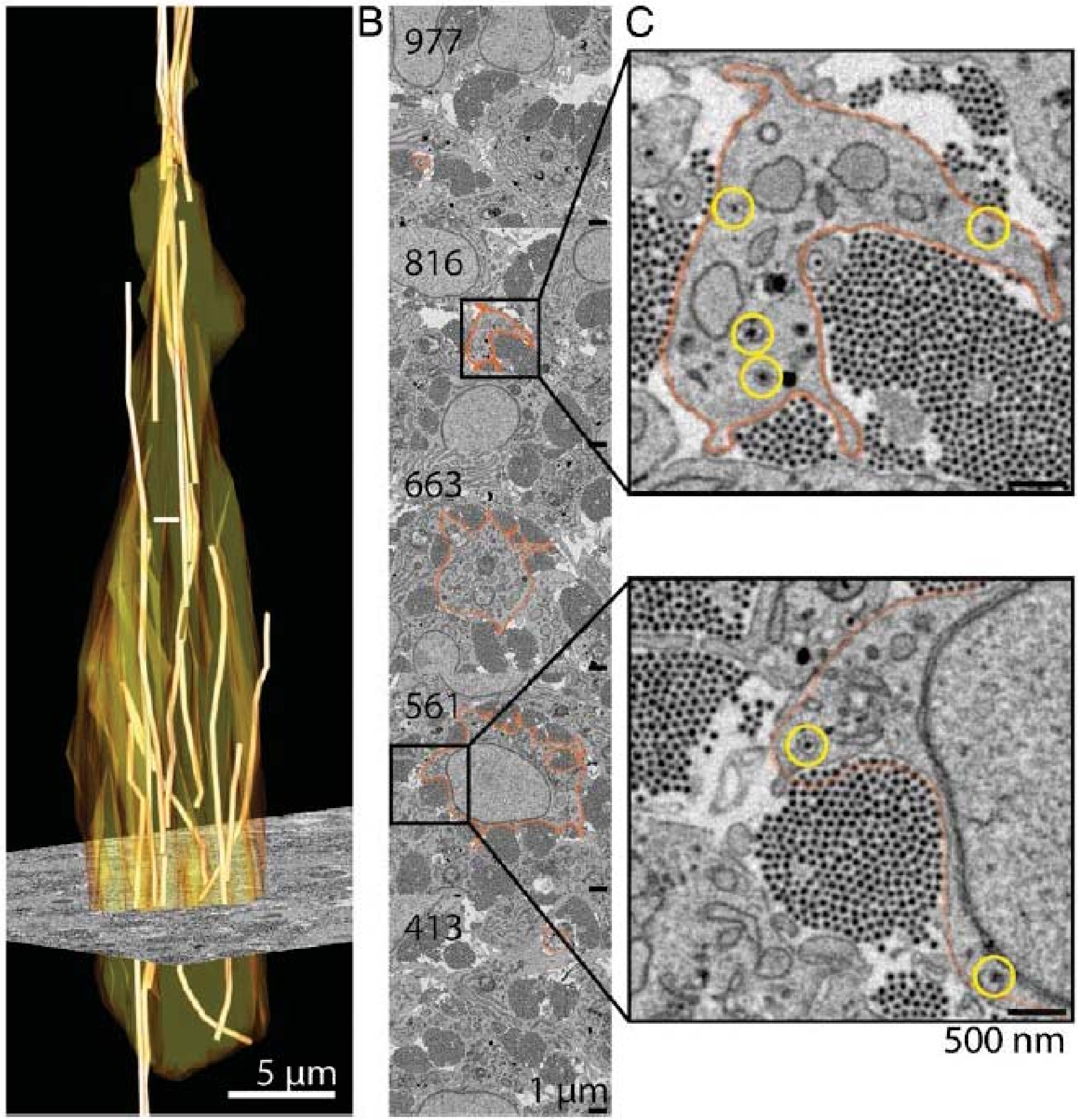
(Gutsmann+, Biophys J, 2004)

Cell ~ 60µm long

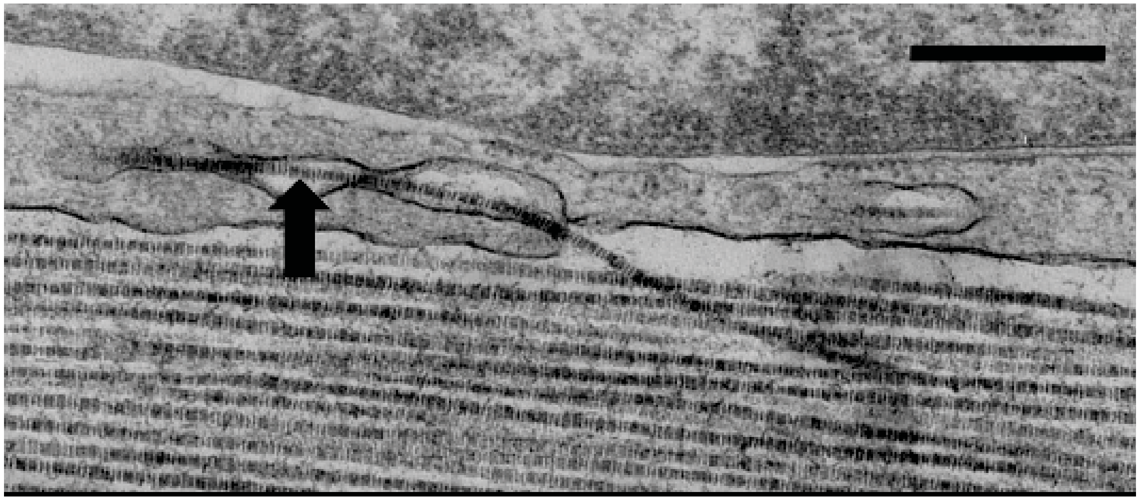
Intracellular Fibricarrier

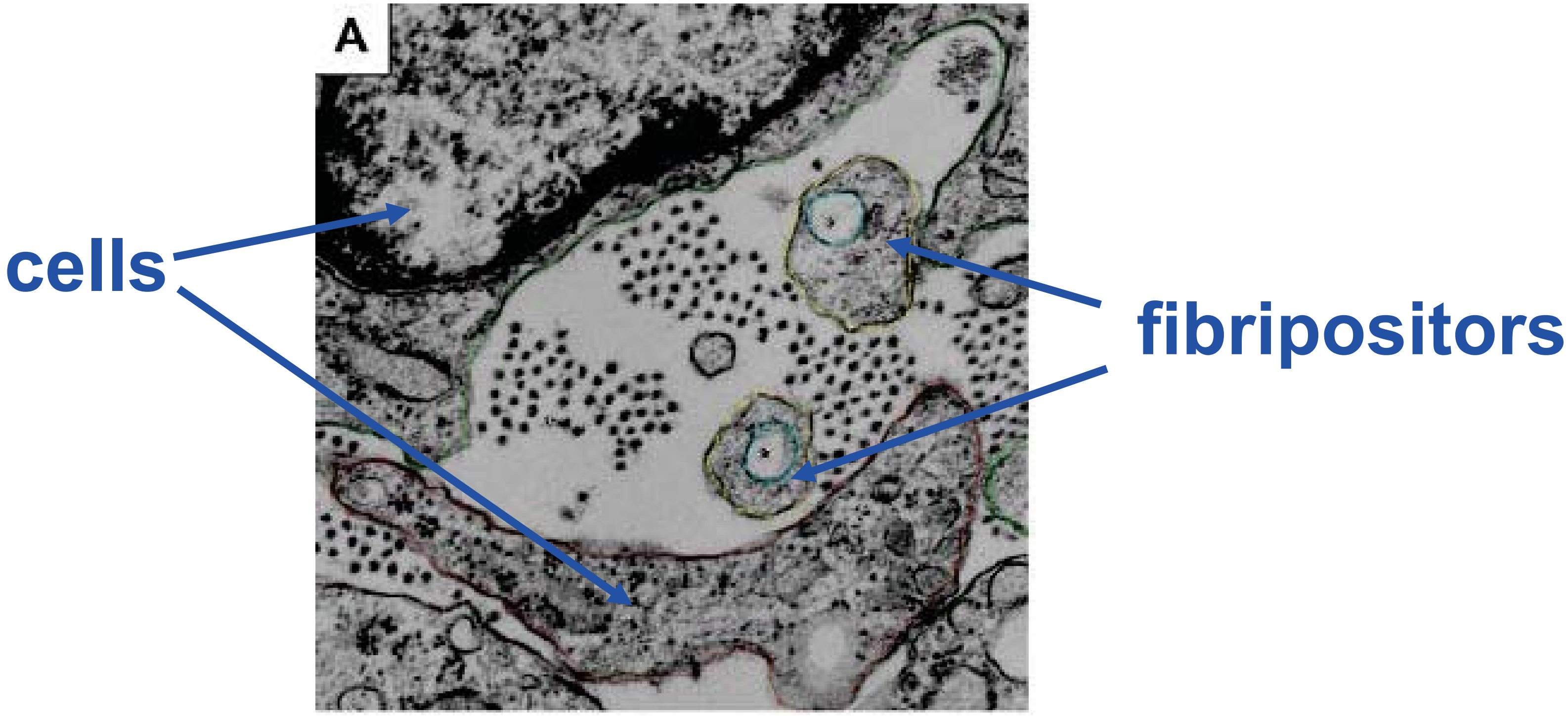


Protruding Fibripositor



Source: Kalson, Nicholas S. et al. "Nonmuscle Myosin II Powered Transport of Newly Formed Collagen Fibrils at the Plasma Membrane." *Proceedings of the National Academy of Sciences* 110, no. 49 (2013): E4743-52.





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.

J. Visualized Experiments 2010

Video Article

Preparation of Rat Tail Tendons for Biomechanical and Mechanobiological Studies

Amélie Bruneau, Nadia Champagne, Paule Cousineau-Pelletier, Gabriel Parent, Eve Langelier
Groupe PERSEUS, Faculté de Génie Département de génie mécanique, Université de Sherbrooke

Correspondence to: Eve Langelier at Eve.Langelier@Usherbrooke.ca

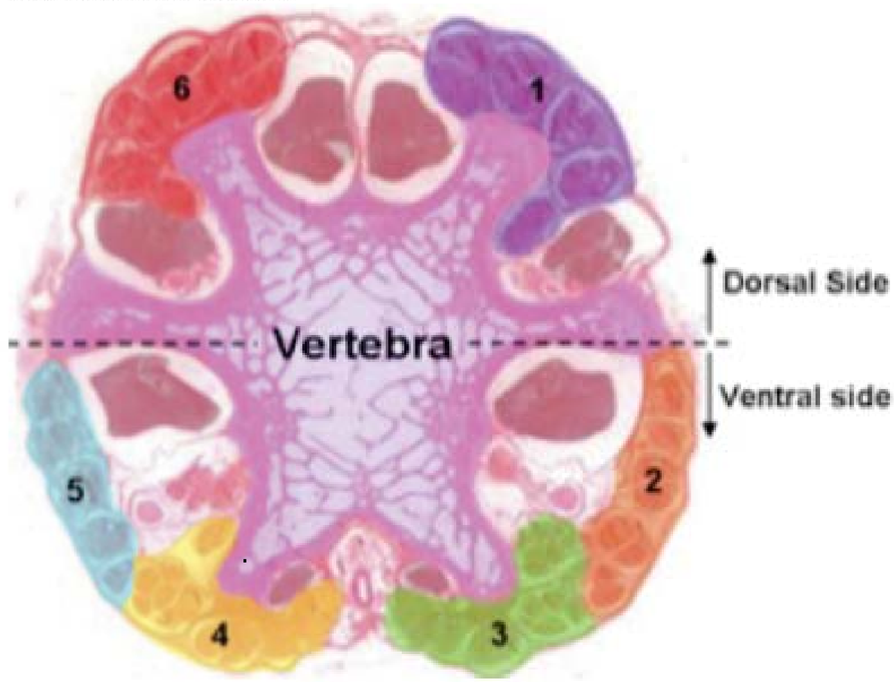
Part 1: Extraction

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")



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

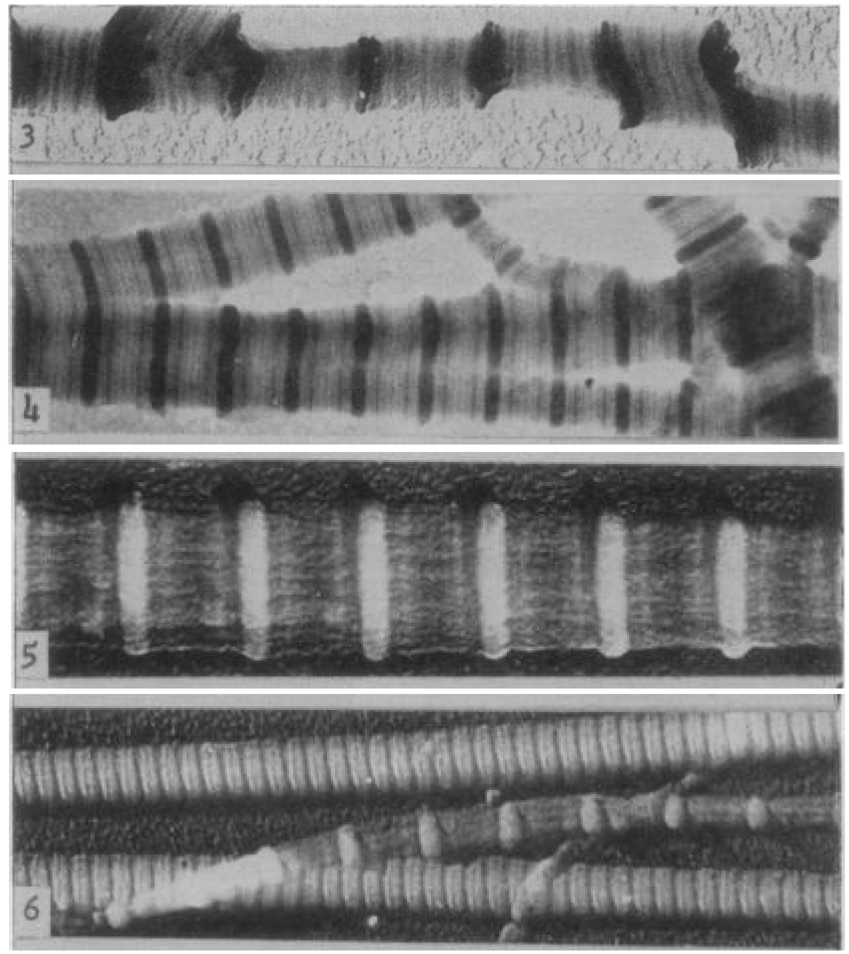
BY JOHN H. HIGHBERGER, JEROME GROSS AND FRANCIS O. SCHMITT

RESEARCH DIVISION, UNITED SHOE MACHINERY CORPORATION, BEVERLY, MASSACHUSETTS; MEDICAL CLINIC OF THE MASSACHUSETTS GENERAL HOSPITAL;† AND BIOLOGY DEPARTMENT, MASSACHUSETTS INSTITUTE OF TECHNOLOGY

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.

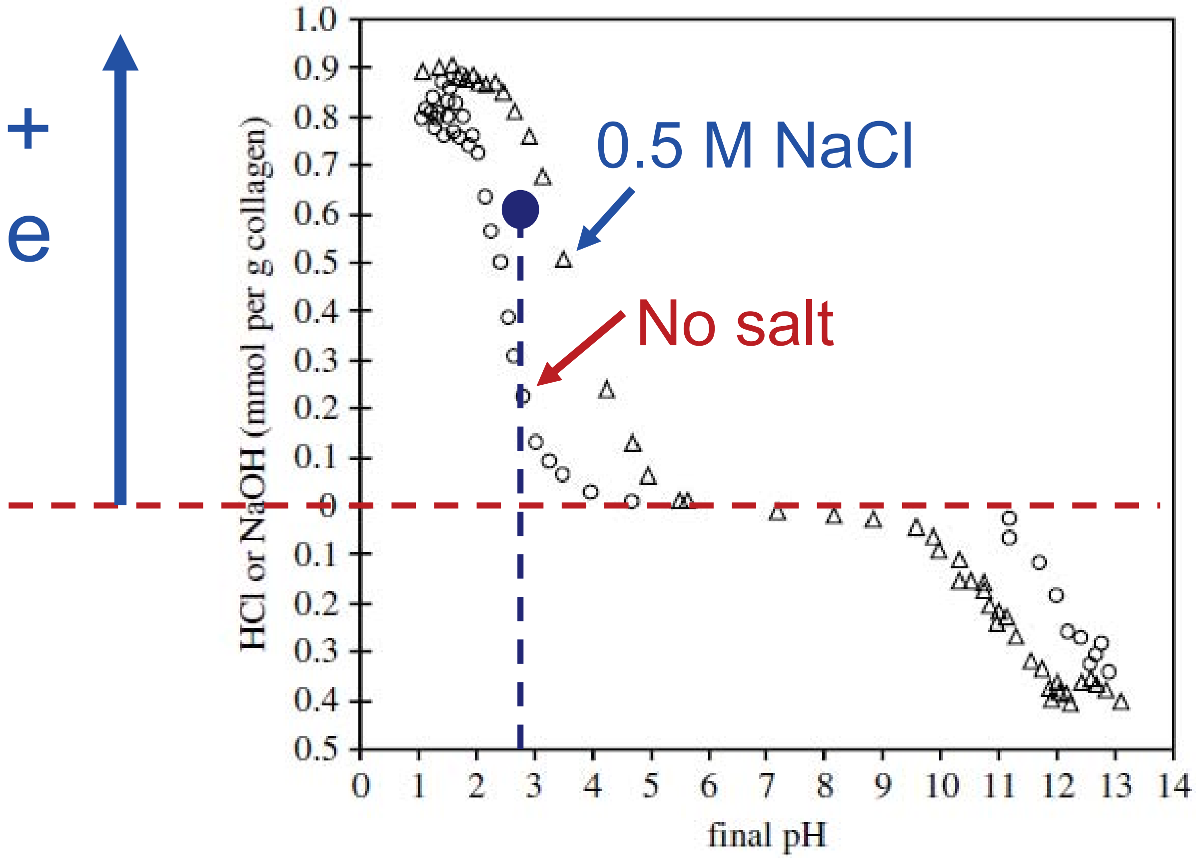
Rat Tail Tendon Collagen

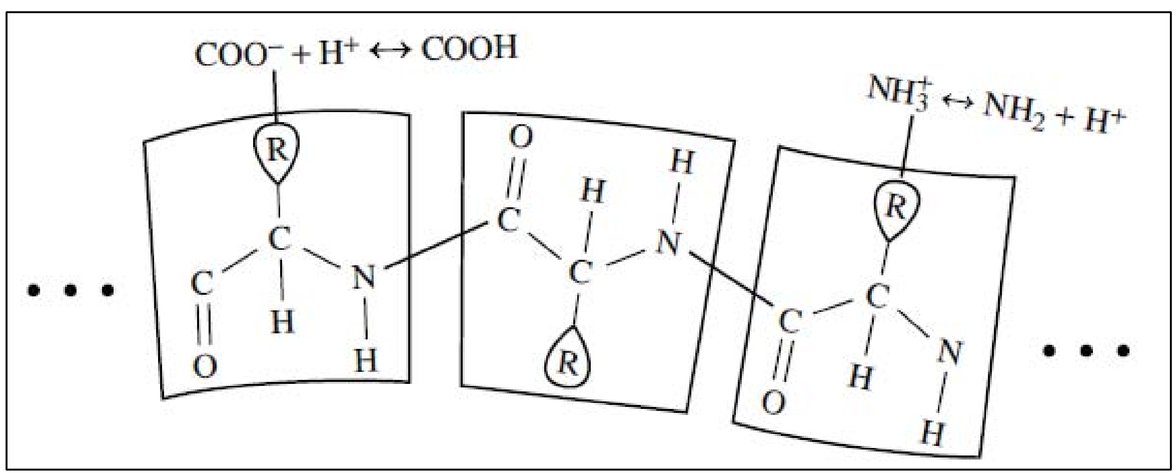


Highberger, John H., et al. "The Interaction of Mucoprotein with Soluble Collagen: An Electron Microscope Study." *3URFHHGLQJV RI WKH 1DWLRQDO $FDGHP\ RI 6FLHQFHV RI WKH 8QLWHG 6WDWHV RI $PHULFD* 37, no. 5 (1951): 286.

pH titration of Collagen (type I)

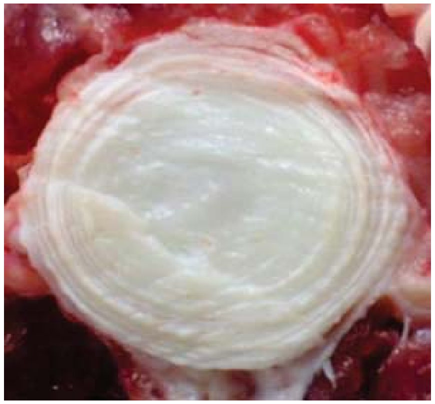
Net + Charge

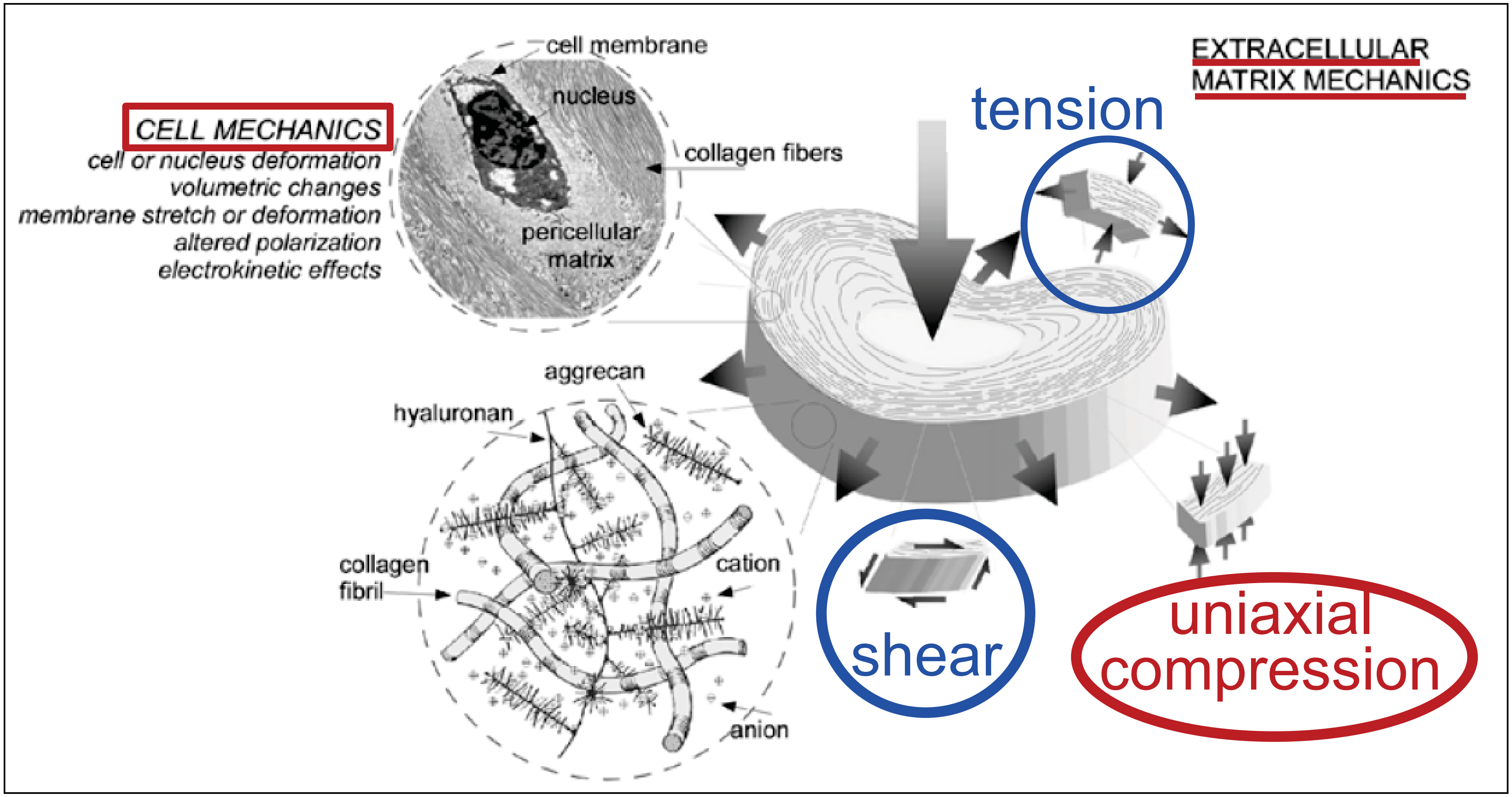




Mechanobiology of the Intervertebral Disc and Relevance to Disc Degeneration

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





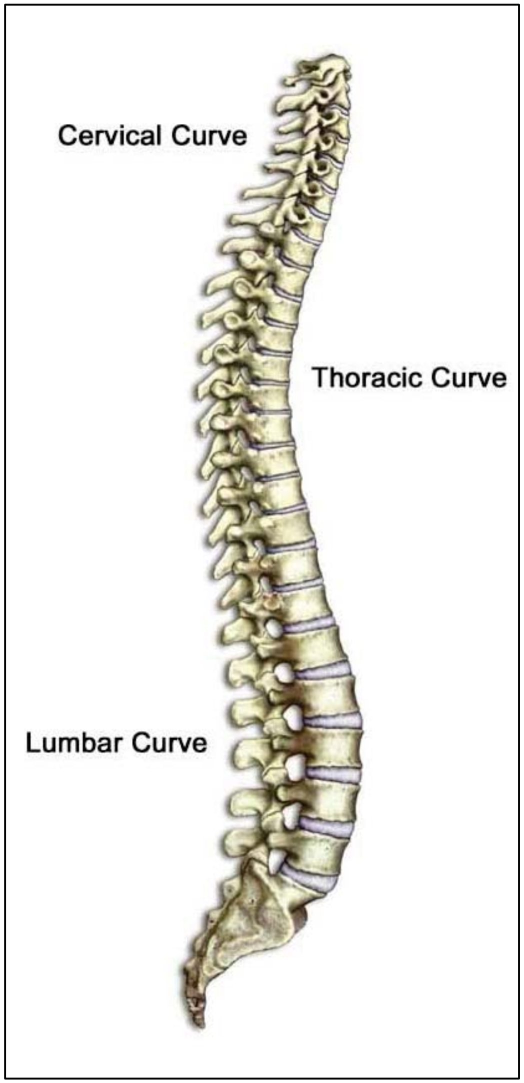
Source: Setton, Lori A., and Jun Chen. "Mechanobiology of the Intervertebral Disc and Relevance to Disc Degeneration." *The Journal of Bone & Joint Surgery* 88, no. suppl 2 (2006): 52-7.

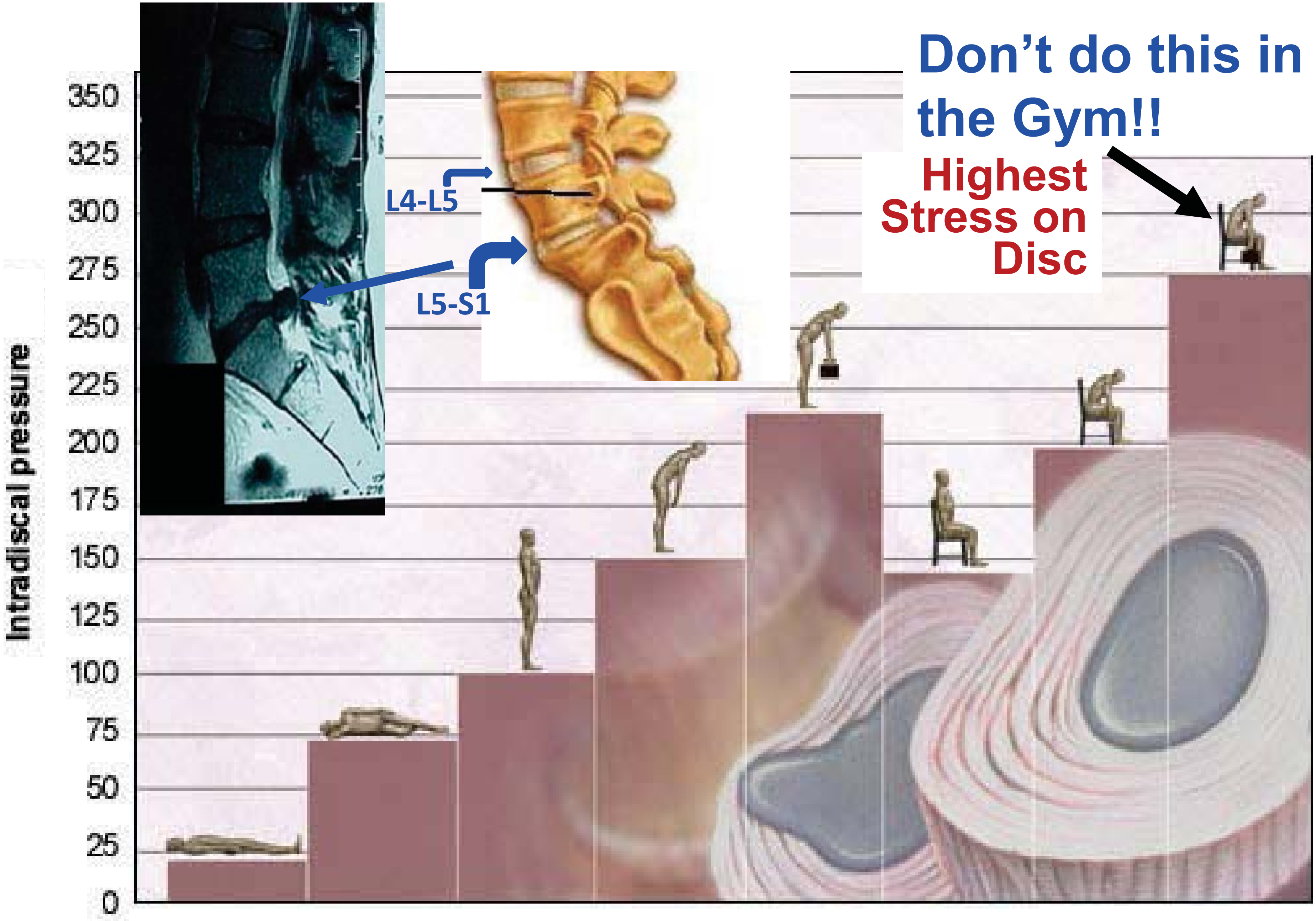
(J Bone & Joint Surg, 2006)

Intervertebral Disc

- Intervertebral disk make up 20-30% of the height of the spine; disc thickness varies from 3mm in cervical region, 5mm in thoracic region to 9 mm in the lumbar region.

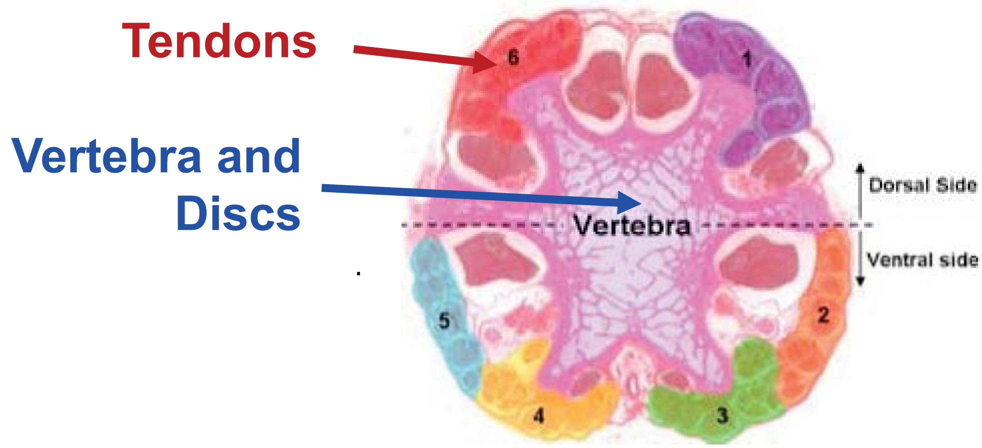
- Ratio between the vertebral body height and the disk height will dictate the mobility between the vertebra –
  - Highest ratio in cervical region allows for motion
  - Lowest ratio in thoracic region limits motion



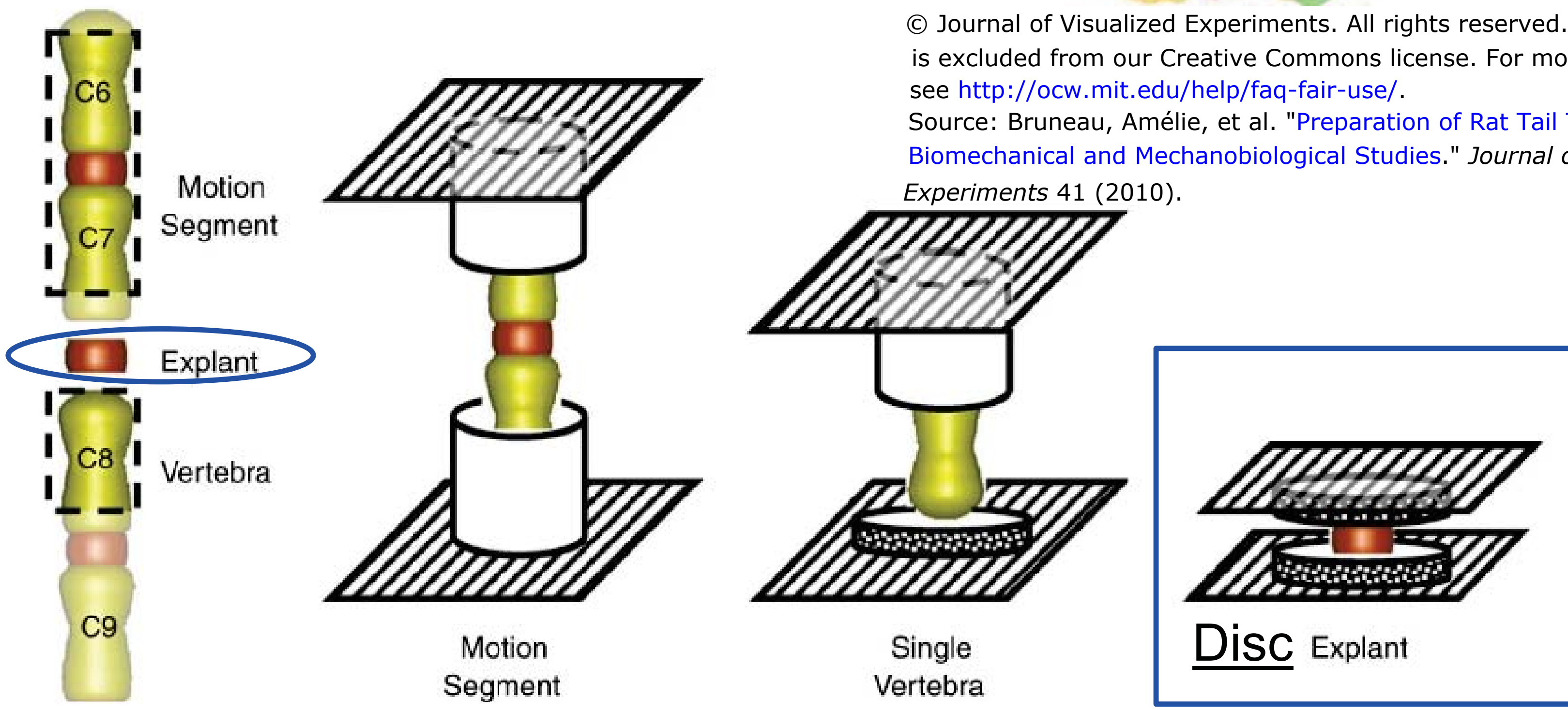

Don't do this in the Gym!!
Highest Stress on Disc
L4-L5
L5-S1
Intradiscal pressure
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0

“Unconfined Compression” of intervertebral disc

(Animal Model: rat tail)

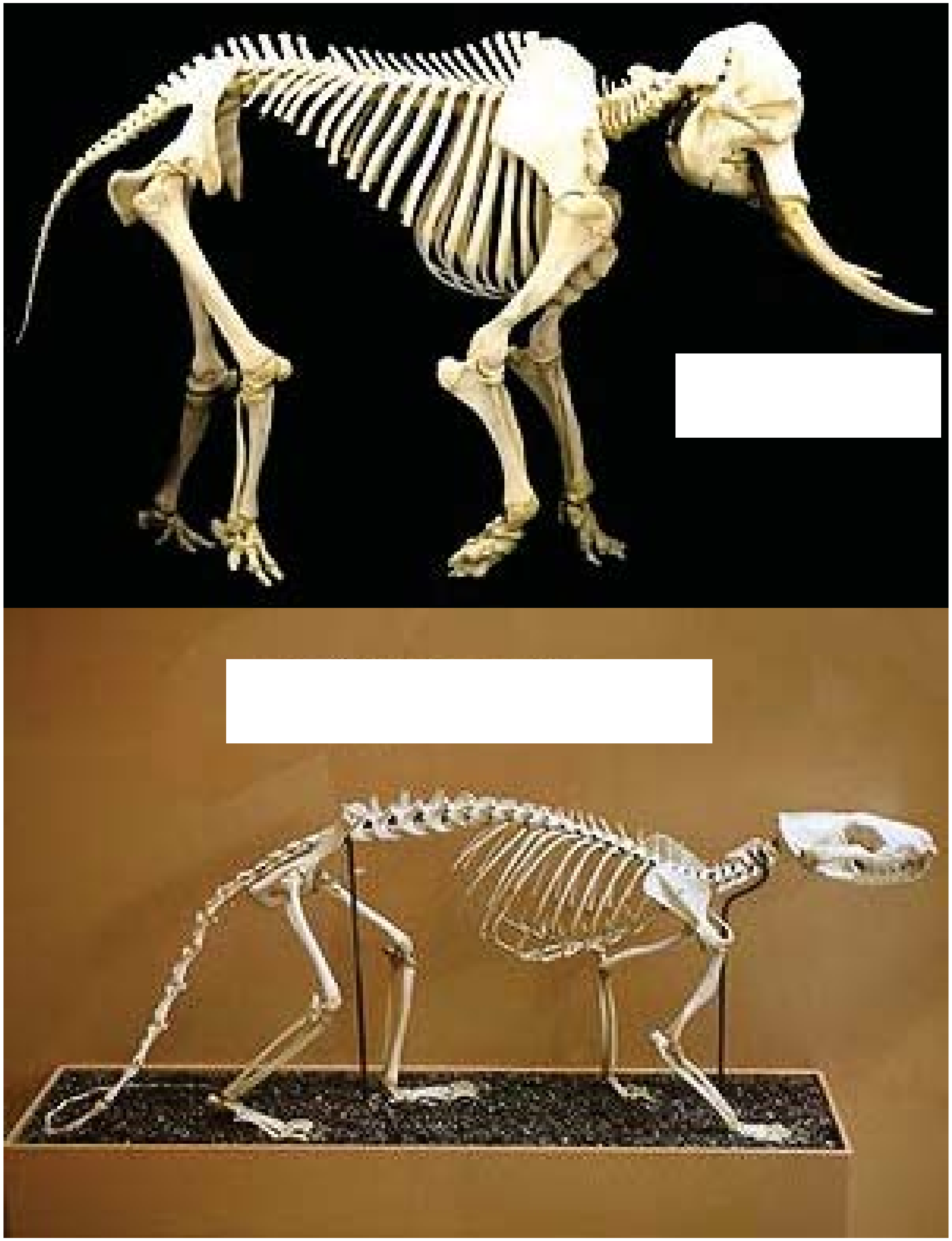


Source: Bruneau, Amélie, et al. "Preparation of Rat Tail Tendons for Biomechanical and Mechanobiological Studies." *Journal of Visualized Experiments* 41 (2010).



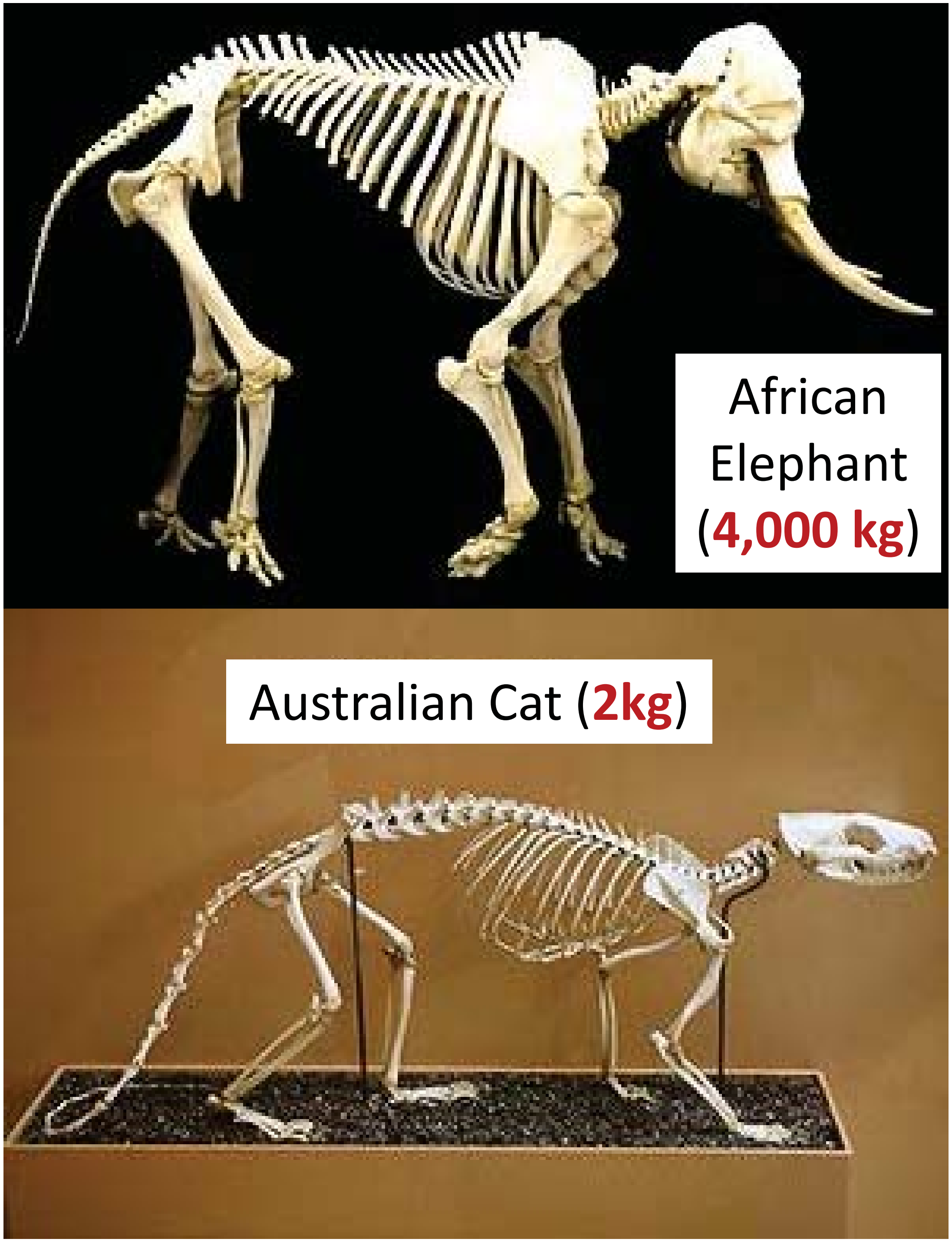
Source: MacLean, Jeffrey J., et al. "Role of Endplates in Contributing to Compression Behaviors of Motion Segments and Intervertebral Discs." *Journal of Biomechanics* 40, no. 1 (2007): 55-63.

(MacLean+, J Biomechanics, 2007)



Courtesy of Cliff on flickr. License: CC BY.

Scaling of body size



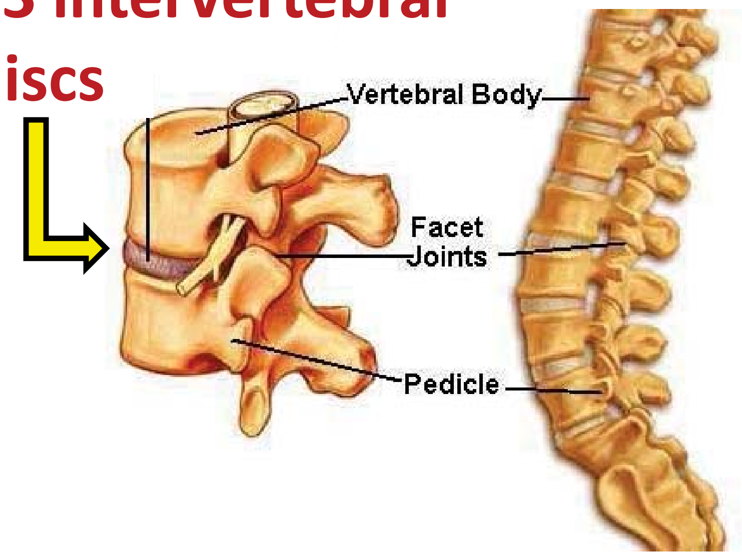
Scaling of body size

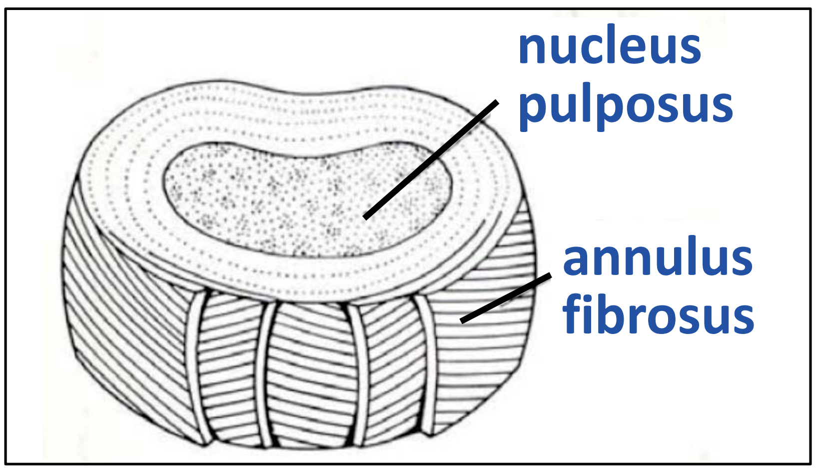
Perfect Isometric scaling of organisms:

- Volume-based properties change proportionally to the body mass
- Surface area-based properties change with mass to the power 2/3
- Length-based properties change with mass to the 1/3 power

Disc Extracellular Matrix Composition

23 intervertebral discs





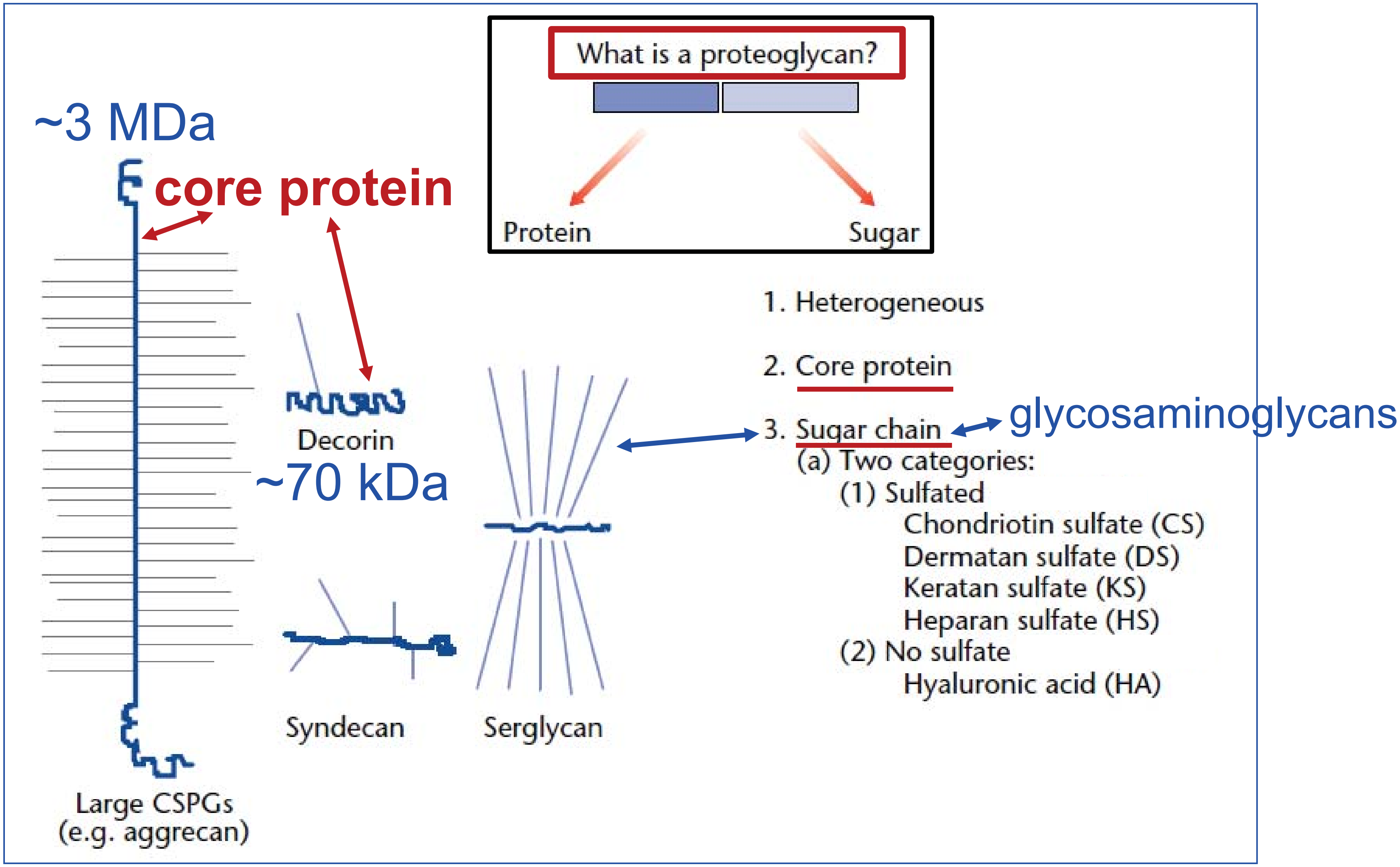
Collagens		Proteoglycans	
Fibrillar			**Aggregating**
Type I	AF	NP	Aggrecan
Type II	NP	AF	Versican
Type III		NP	Hyaluronan
Type V	AF	NP	Link protein
Type XI	NP		**Fibril-associated**
Fibril-associated		AF	Decorin
Type IX	NP	AF	Biglycan
Type XII			Fibromodulin
Type XIV			Lumican
Pericellular			**Pericellular**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

Proteoglycans: Resist Compressive Stress

Nancy B Schwartz, *University of Chicago, Illinois, USA* Encyclop Life Sci, 2009



Source: Schwartz, Nancy B. "Proteoglycans." eLS (2009).

PROTEOGLYCAN SUPERFAMILY

- **ECM molecules with (a) Core protein & (b) Glycosaminoglycan (GAG) chains**

- **"Sub-families" include**

  - Extracellular • **Large Aggregating (Aggrecan)**
    • Small Leucine-Rich PG (SLRPs)
  - Cell Surface (e.g., glycocalyx HSPGs)

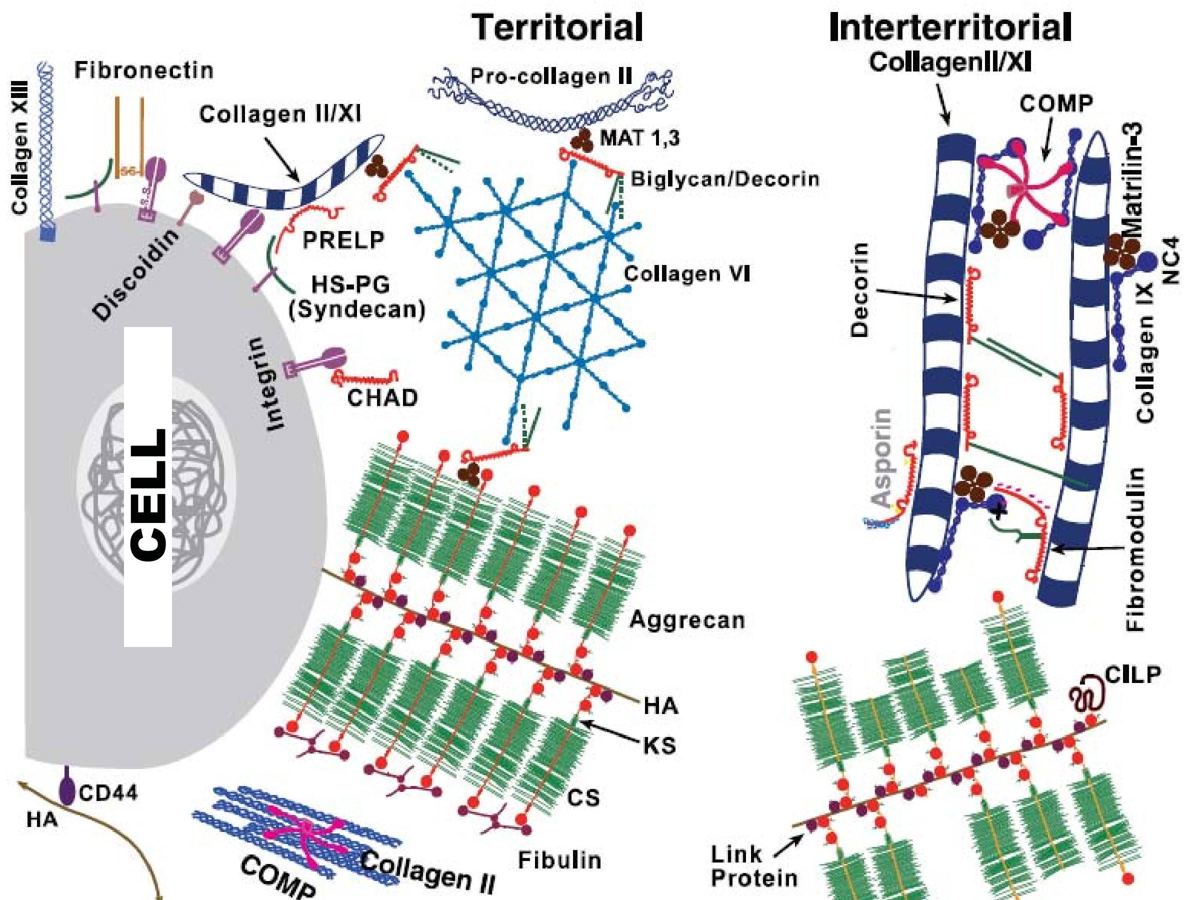
Lander, J Cell Biol, 2000: "….PGs have been credited with controlling: cell division, adhesion, spreading, migration, chemoattraction, axon guidance, matrix assembly, lipoprotein uptake, extracellular proteolysis, and viral entry….

Proteoglycans and more – from molecules to biology

Int. J. Exp Pathol, 2009

Dick Heinegård

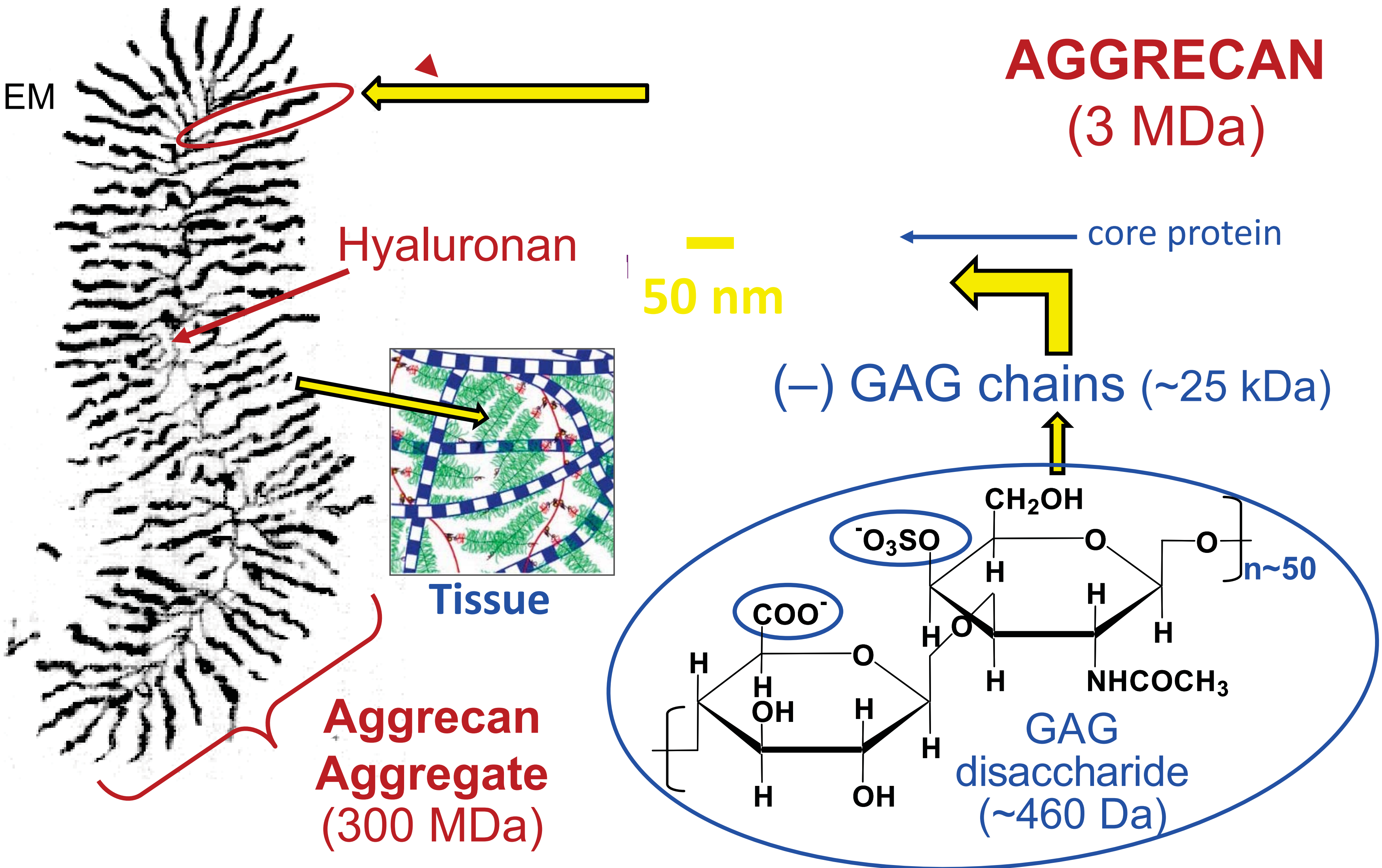
Department of Clinical Sciences, Section for Rheumatology, Molecular Skeletal Biology, Lund University, Lund, Sweden

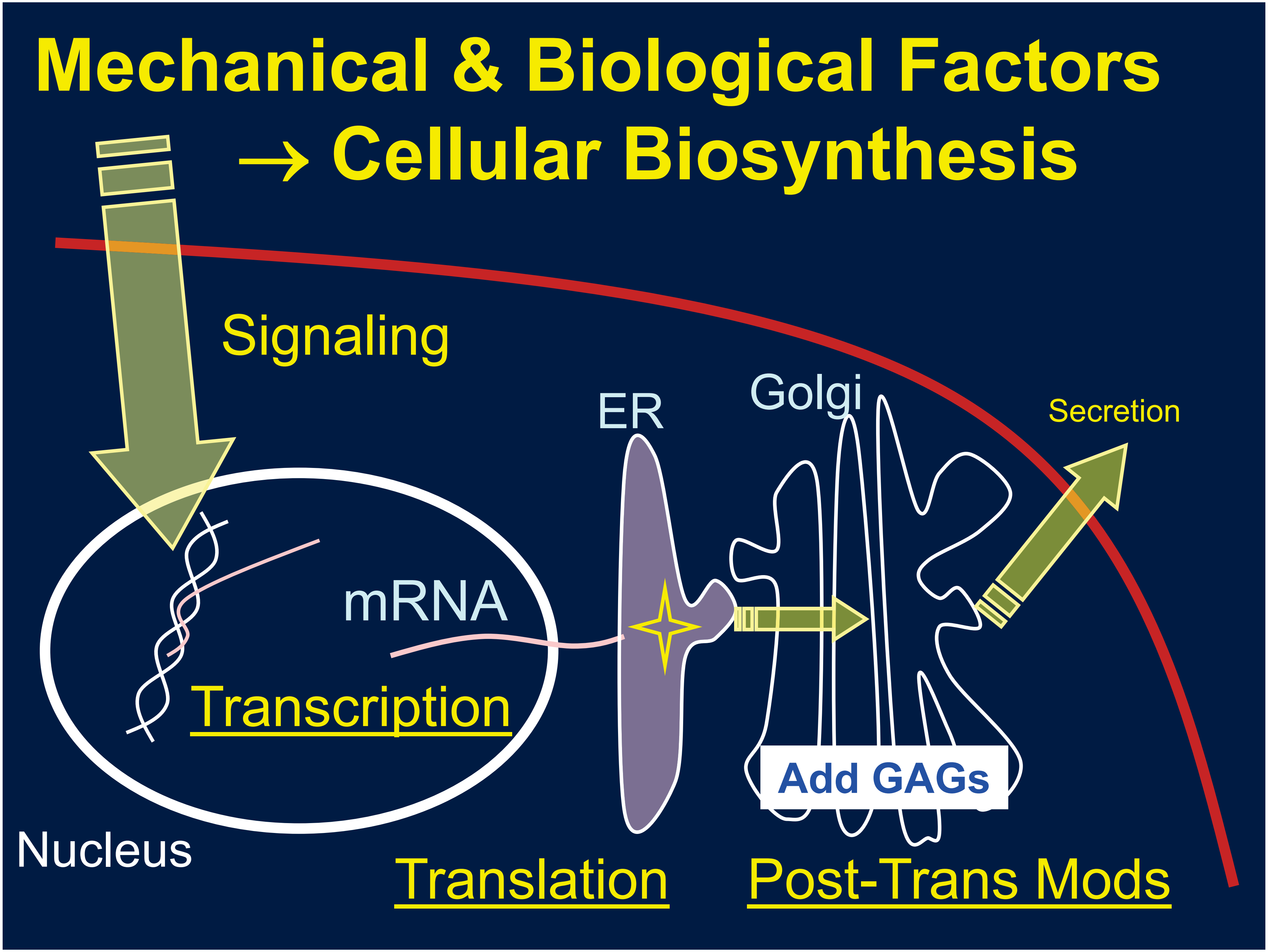


Source: Heinegård, Dick, and Tore Saxne. "The Role of the Cartilage Matrix in Osteoarthritis." *Nature Reviews Rheumatology* 7, no. 1 (2011): 50-56.

"In this article the organization and functional details of the extracellular matrix, with particular focus on cartilage, are described."

AGGRECAN: Resists "Static" Compression & Fluid Flow ("Dynamic" Compression)



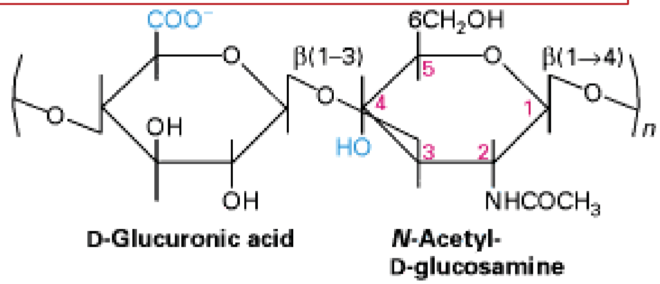


Mechanical & Biological Factors
→ Cellular Biosynthesis
Signaling
Golgi
ER
Secretion
mRNA
Transcription
Add GAGs
Nucleus
Translation
Post-Trans Mods

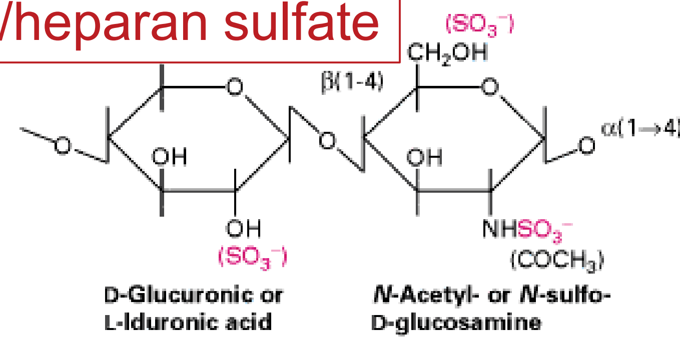
Family of Glycosaminoglycans (GAG Chains):

→ Glycosylation of 'Core Protein' (addition of sugar moieties)

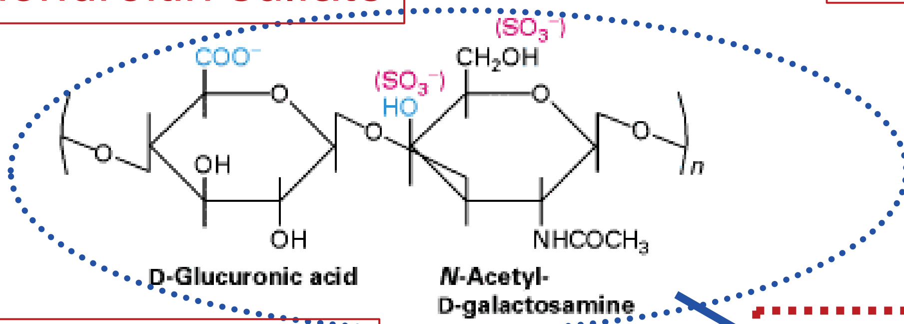
Hyaluronan (hyaluronic acid)



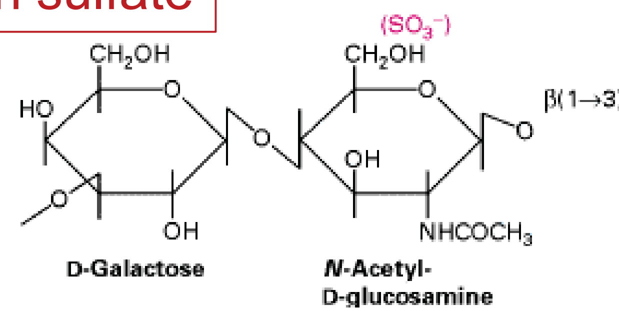
Heparin/heparan sulfate



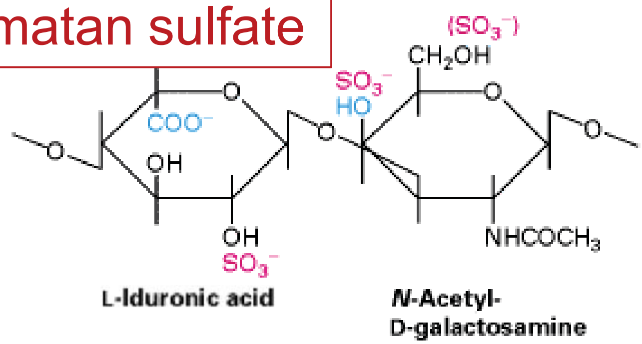
Chondroitin sulfate

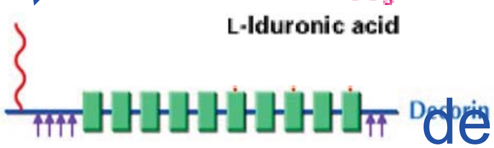


Keratan sulfate



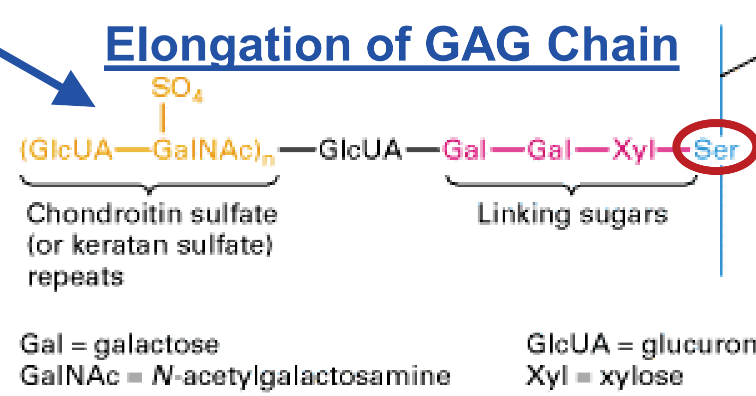
Dermatan sulfate





decorin

Elongation of GAG Chain



aggrecan

Aggrecan Monomer

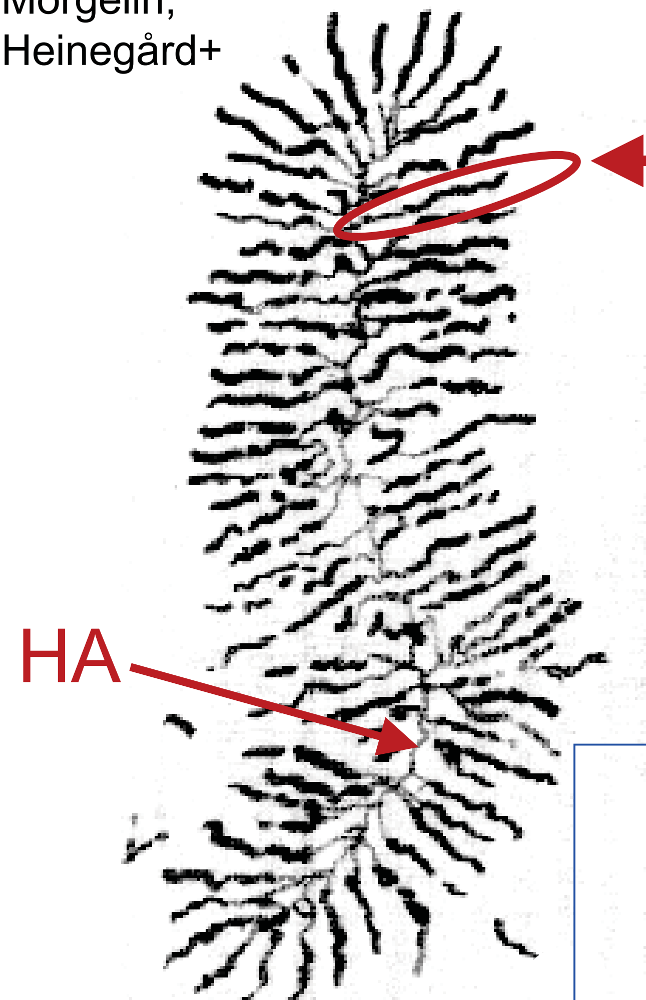
(Bovine Fetal Epiphyseal)

(Ng+, J. Struc Biol, 2003)

EM:

Buckwalter, Rosenberg+

Mörgelin, Heinegård+



"Aggregate"

GAG Chains

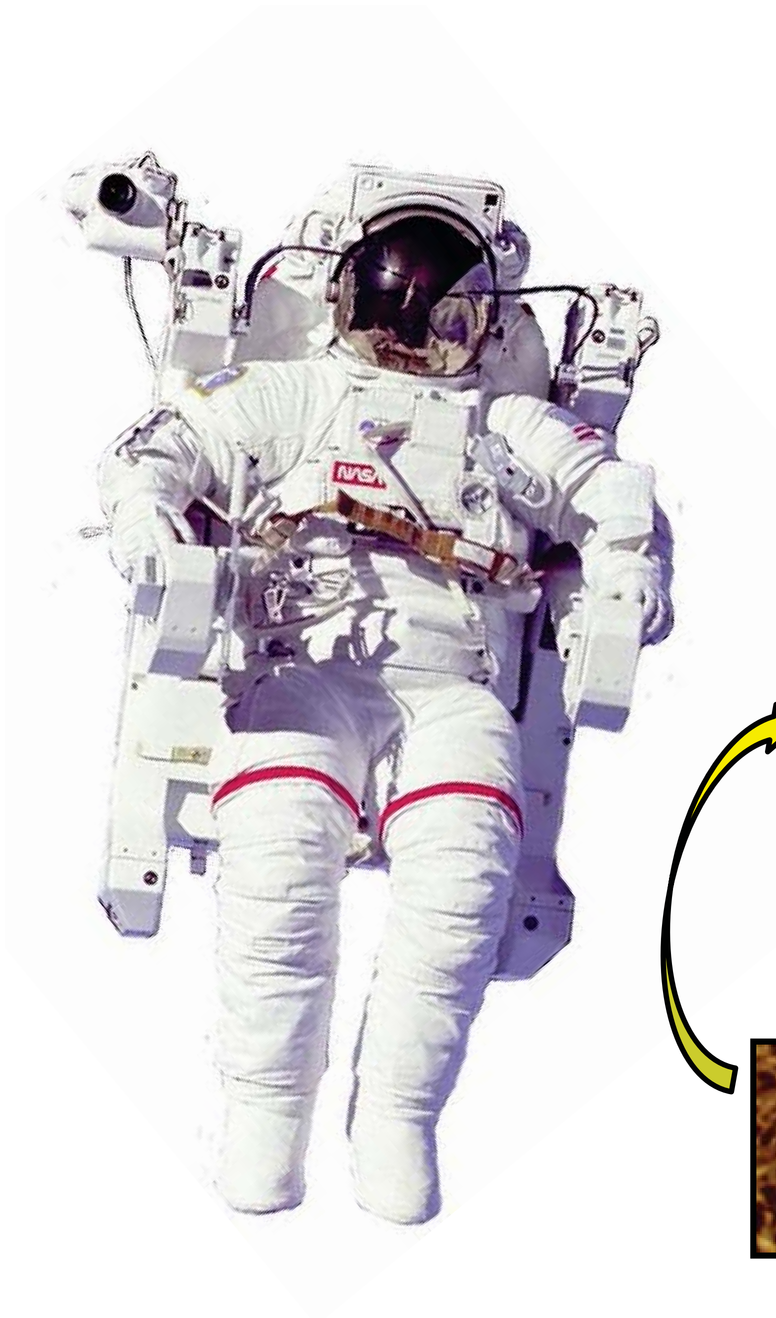
Core Protein

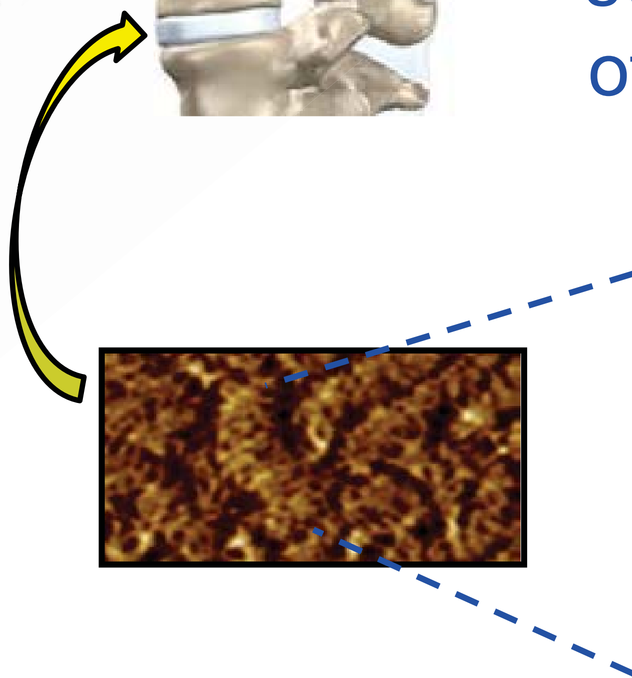
50 nm

(Tapping mode AFM)

...why does the cell make such a ***complicated****,* ***charged*** *molecule to resist compressive loads??*

(cells in cartilage, disc, tendon, many other tissues)



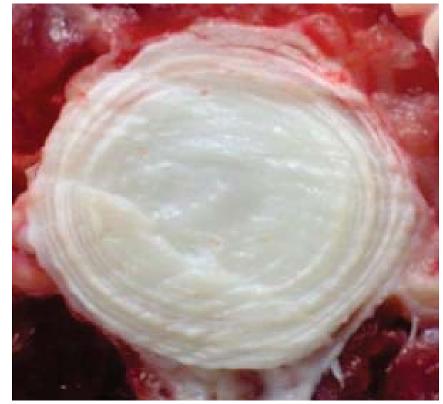


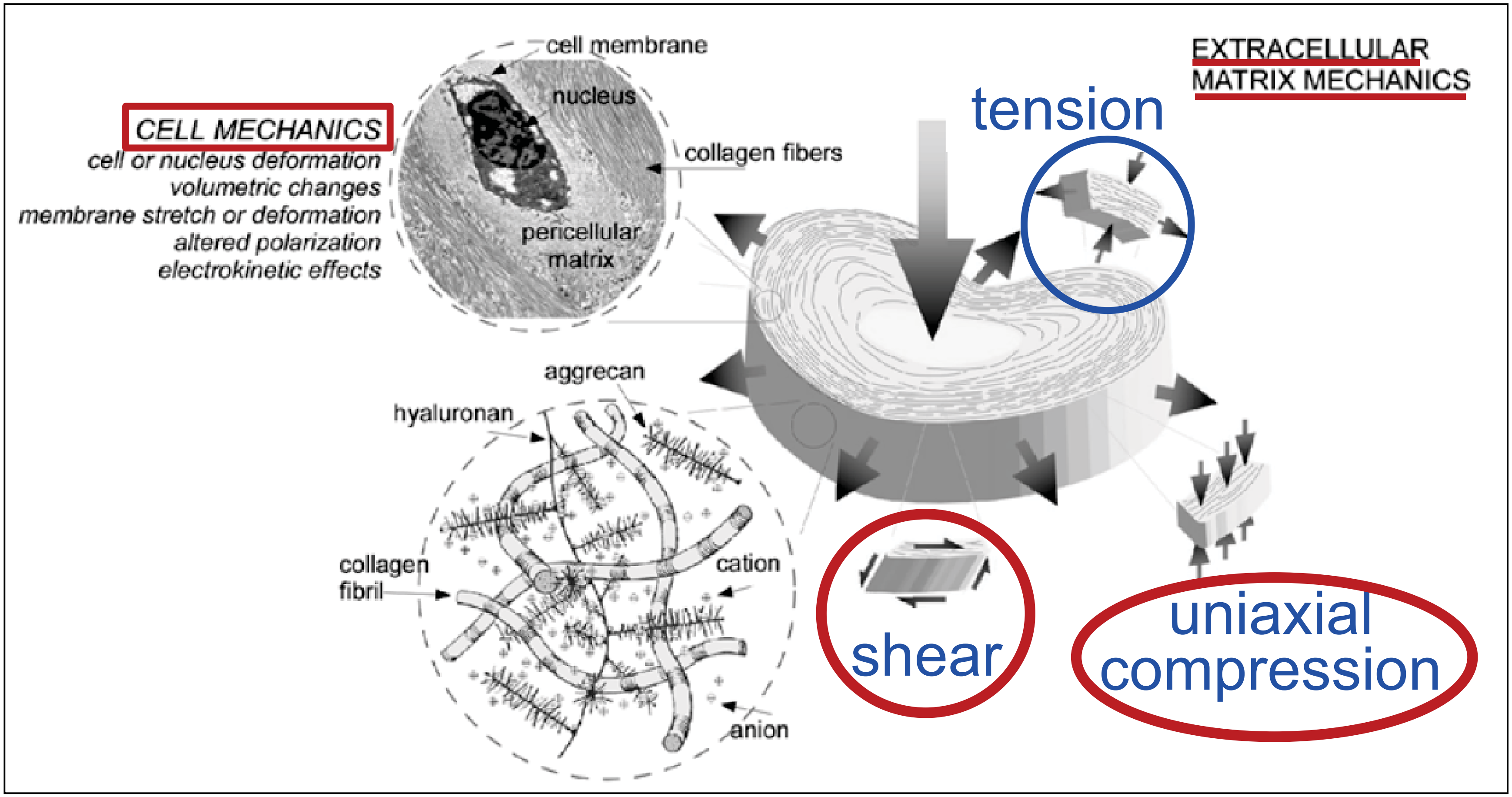
Astronauts gain
≃2 inches in height
during space flight:
<u>swelling of the
intervertebral discs
under 0-gravity</u>:

"swelling pressure"
of highly charged
aggrecan !!

Mechanobiology of the Intervertebral Disc and Relevance to Disc Degeneration

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





Source: Setton, Lori A., and Jun Chen. "Mechanobiology of the Intervertebral Disc and Relevance to Disc Degeneration." *The Journal of Bone & Joint Surgery* 88, no. suppl 2 (2006): 52-7.

(J Bone & Joint Surg, 2006)

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