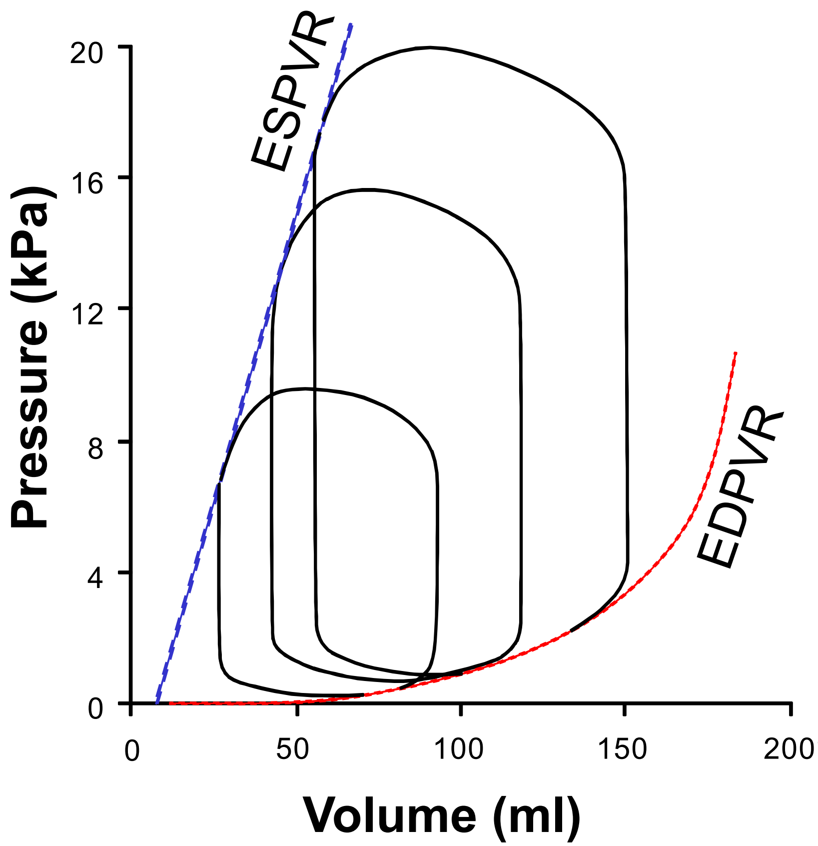
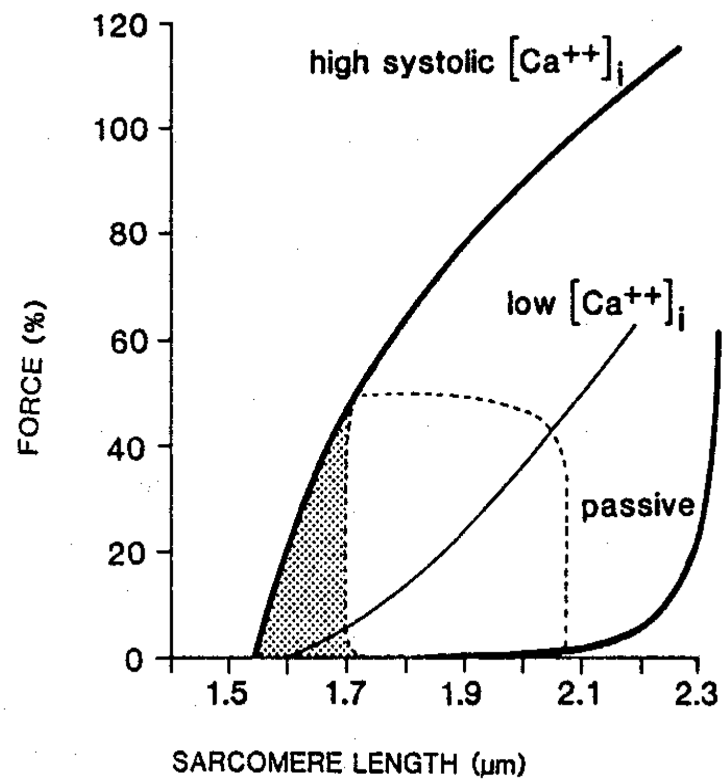
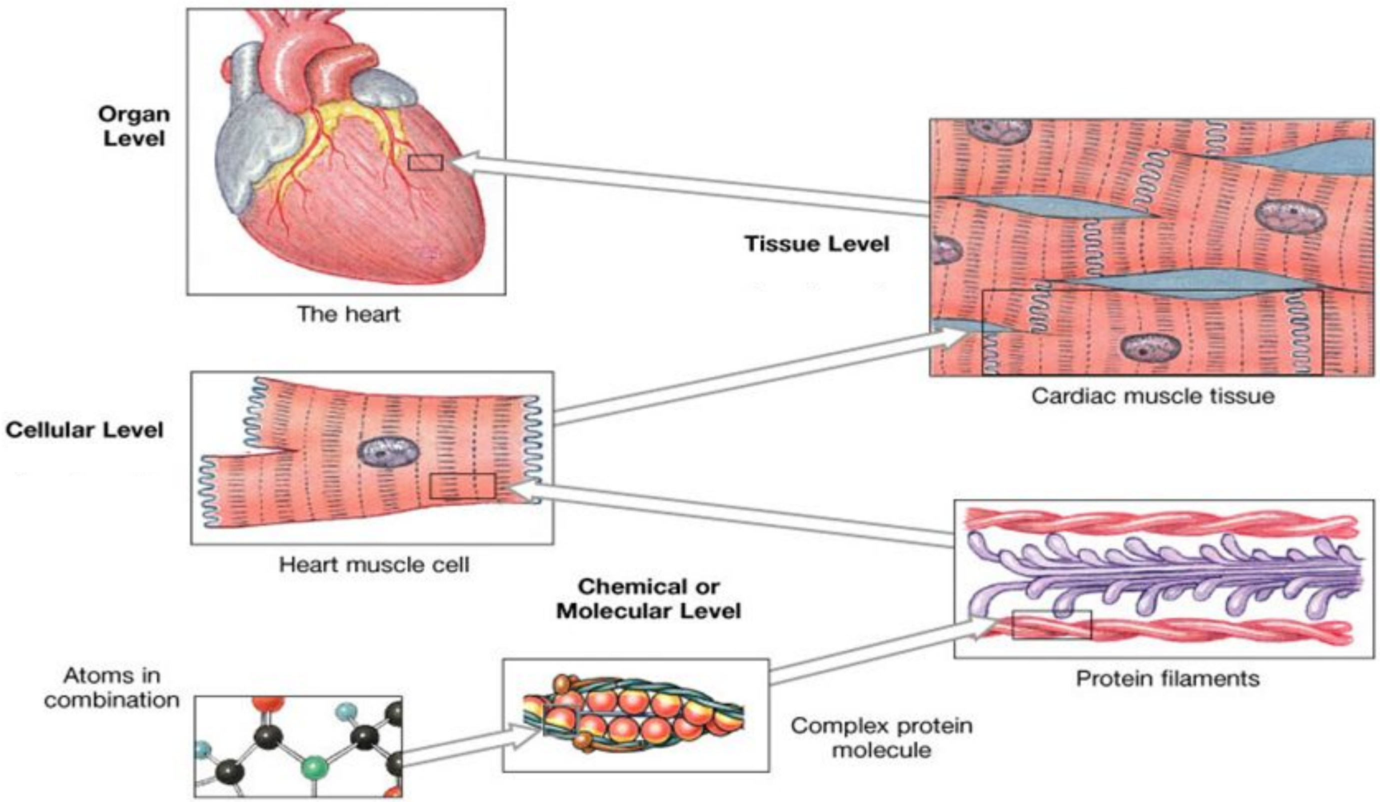
Physiological Basis of the Frank-Starling Mechanism



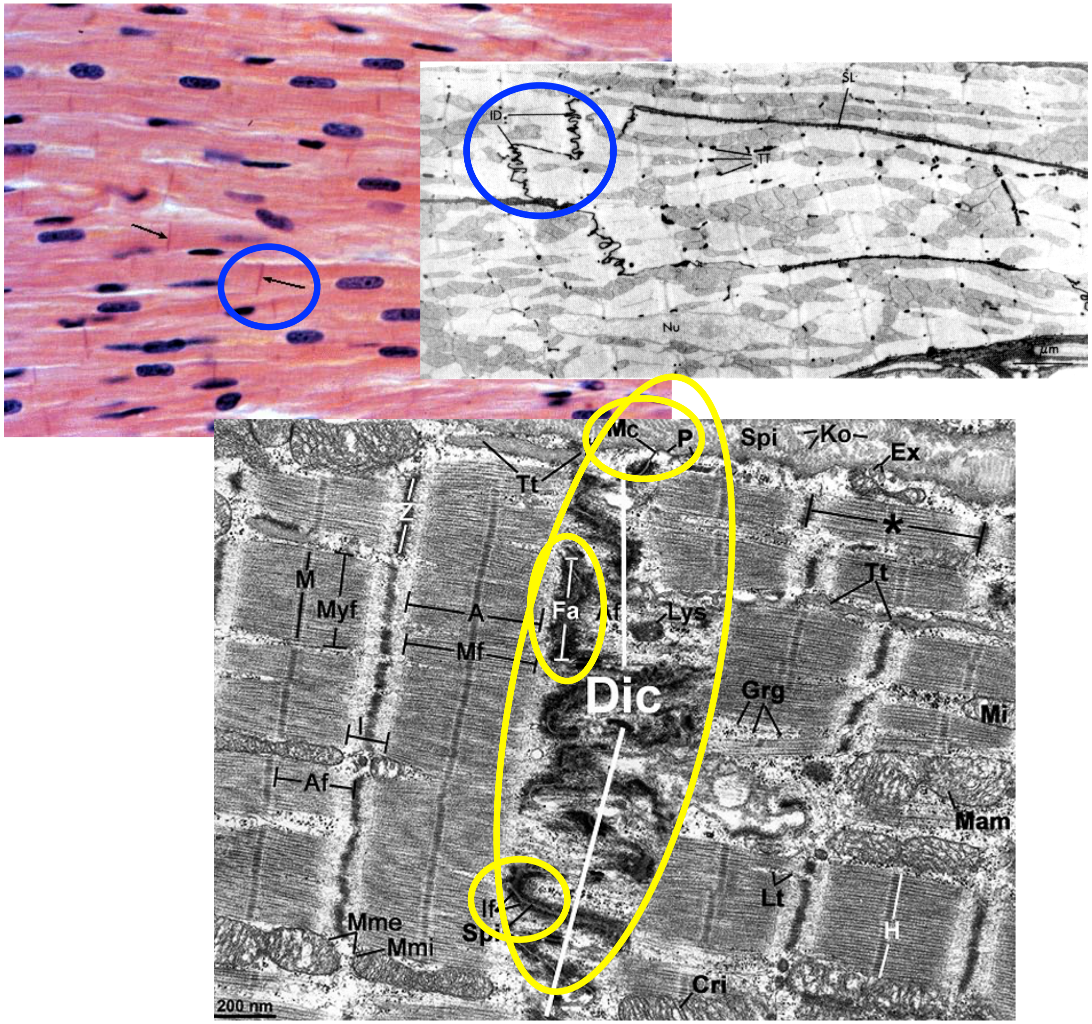


Levels of Organization



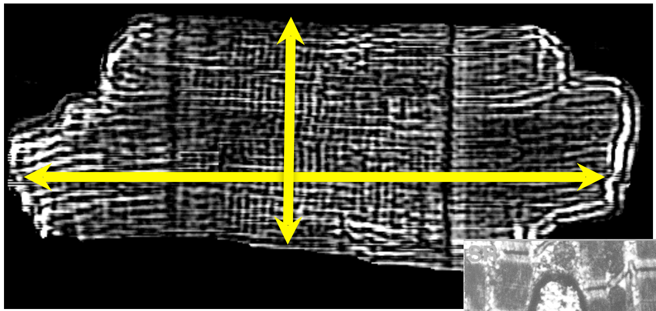
Physiology M.Ilker Gelisen MD

Cardiac Myocyte Connections

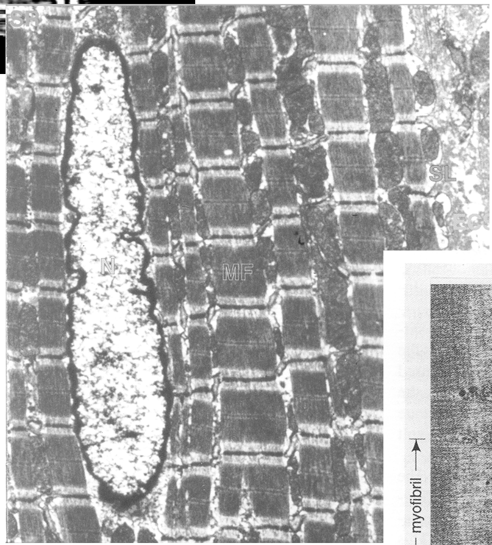


- Myocytes connect to an average of 11 other cells (half end-to-end and half side-to-side)
- Functional *syncytium*
- Myofiber angle *dispersion* (about 12-15º)
- Intercalated discs
  - gap junctions
    - connexons
      - connexins
  - adherens junctions (fascia adherens)
    - anchoring sites for sarcomeres (actin)
  - desmosomes (macula adherens)
    - connect cytoskeleton (intermediate filaments) between cells.

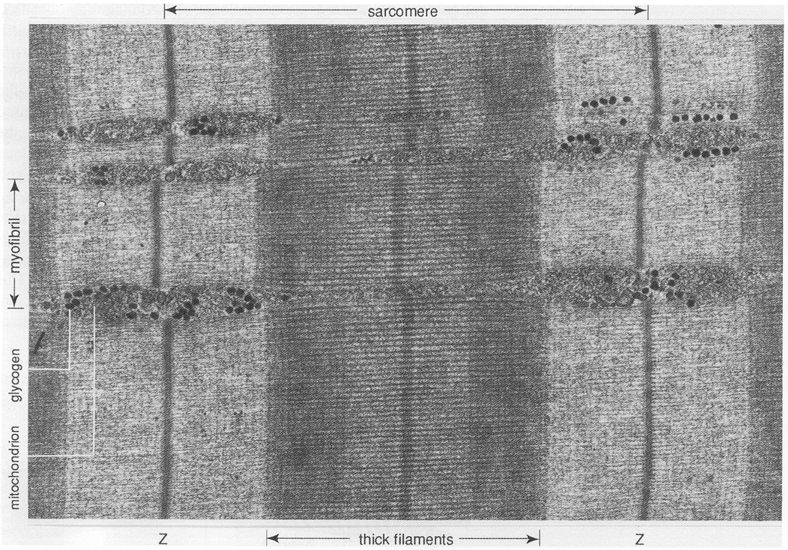
Myocytes



- Rod-shaped
- Striated
- 80-100 μm long
- 15-25 μm diameter



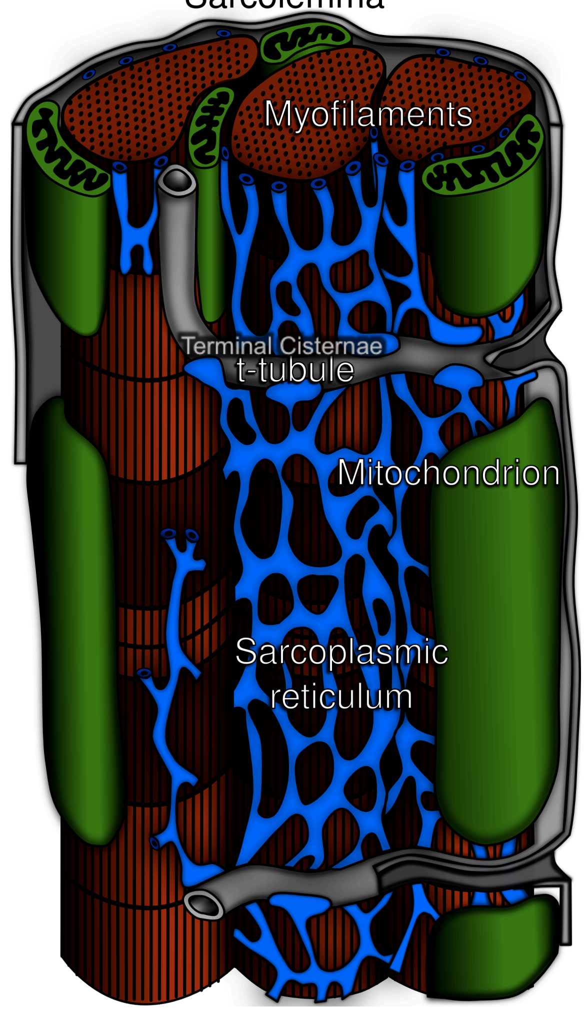
- Sarcolemma
- Mitochondria (M) ~30%
- Nucleus (N)
- Myofibrils (MF)
- Sarcoplasmic Reticulum and T-tubule network



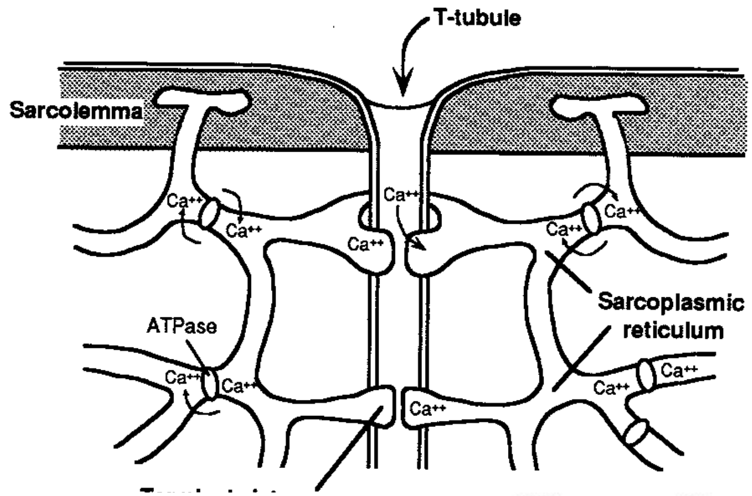
- Slow cardiac myosin isoforms
- Cardiac troponin
- Titin

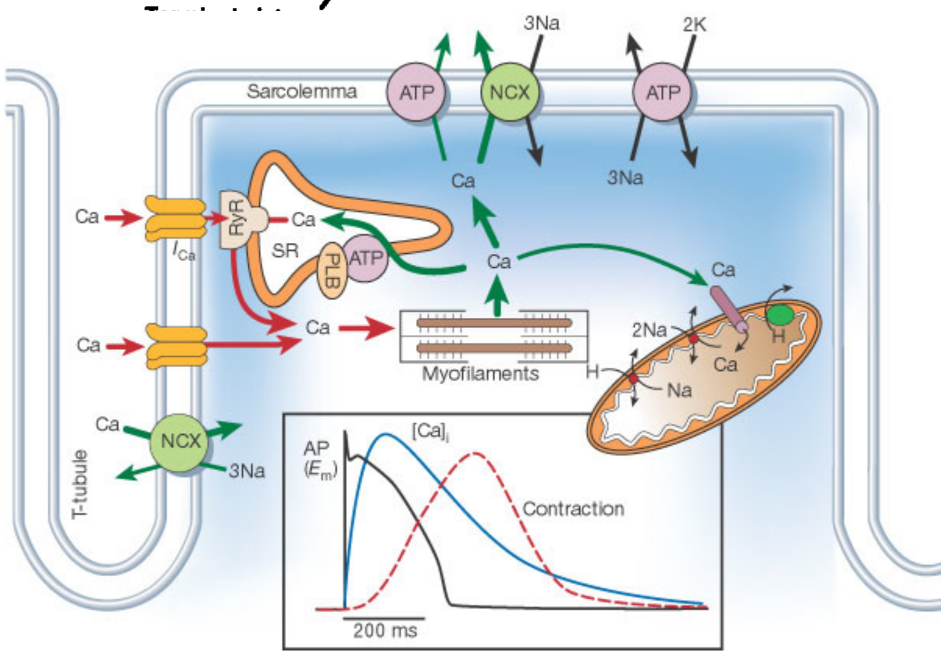
The Muscle Fiber (Myocyte)

- Sarcolemma (plasma membrane)
- Sarcoplasm (cytosol)
- Sarcoplasmic Reticulum (ER)
- Mitochrondria (ATP powerhouse)
- Transverse (T) Tubule
- Junctional SR



Excitation-Contraction Coupling

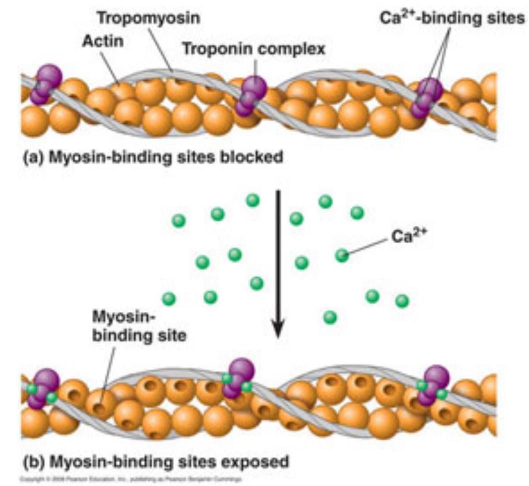


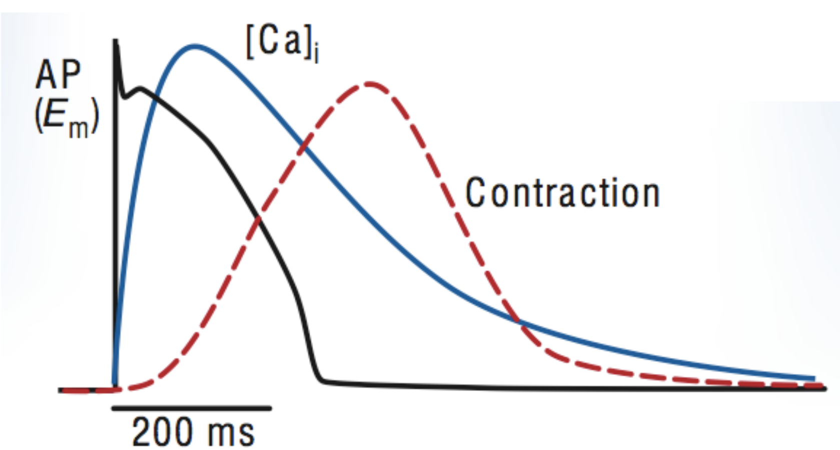


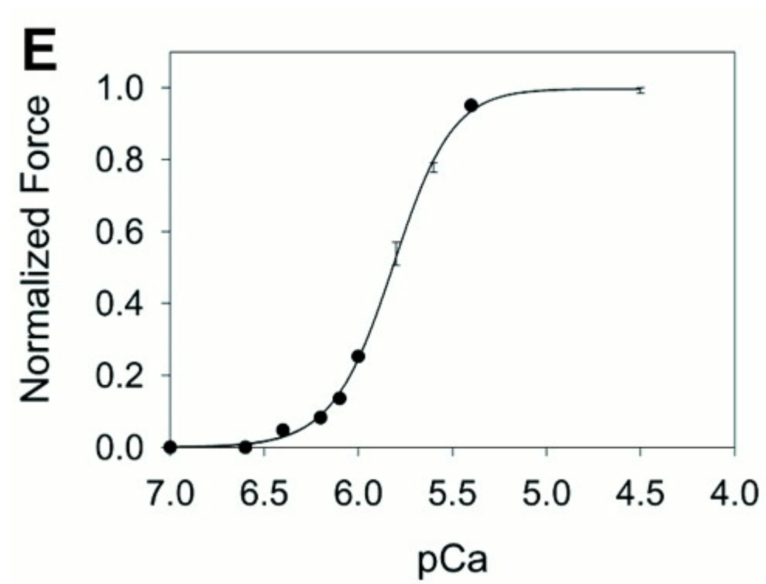
- L-type Calcium current
- Calcium-induced calcium release from the sarcoplasmic reticulum via ryanodine receptors
- Na+/Ca2+ exchange
- Sarcolemmal Ca2+ pump
- SR Ca2+ ATP-dependent pump

Myofilament Activation

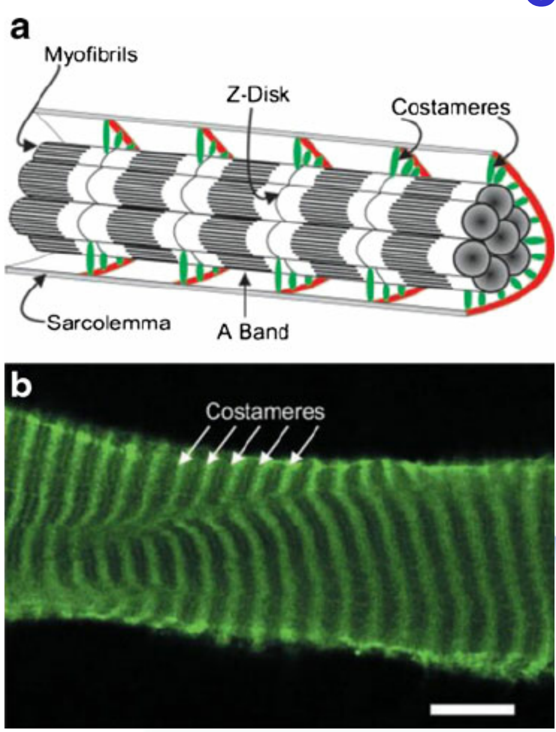
- Force-development is triggered by the intracellular [Ca^{2+}] transient.
- Ca^{2+} binds to troponin-C sites, which are separated by neighboring troponin-C sites every 7 actin monomers
- Nearest neighbor cooperative activation

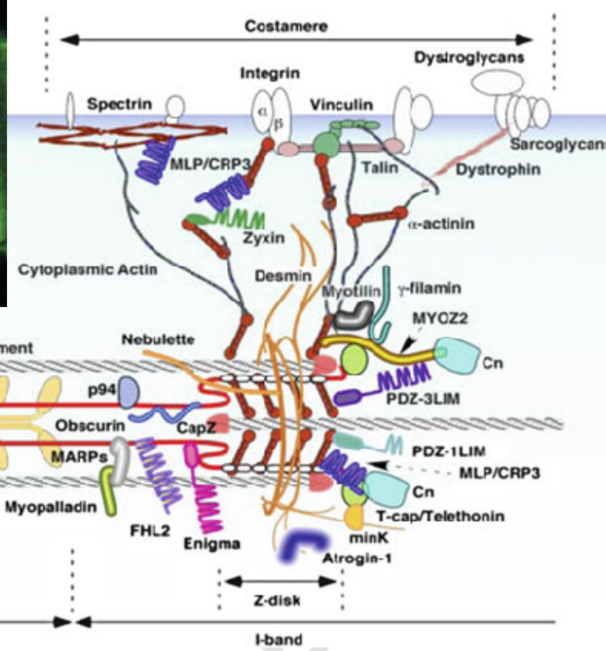


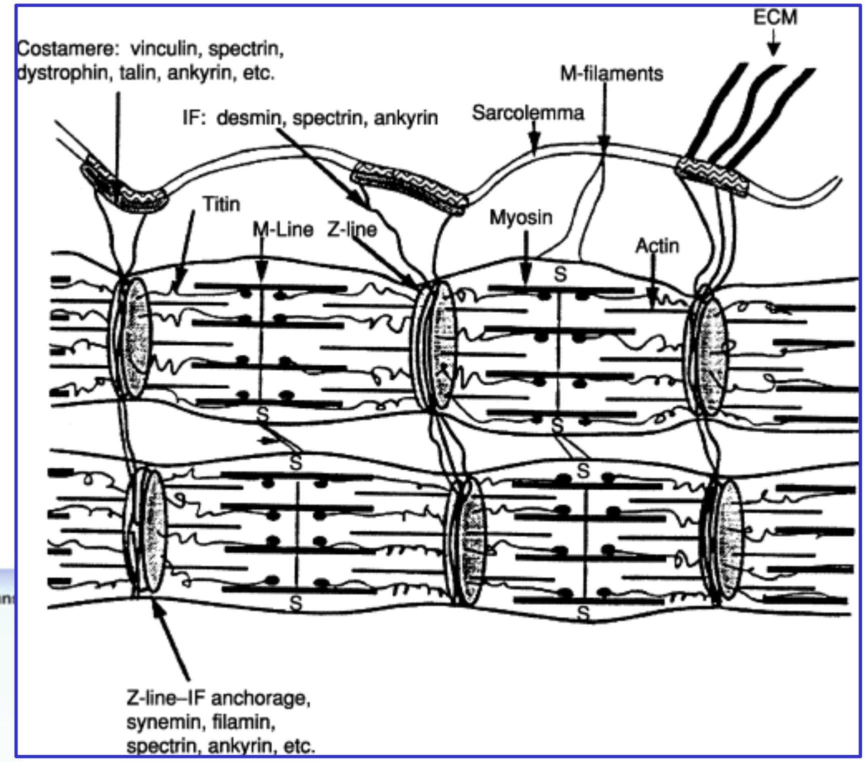




Costameres

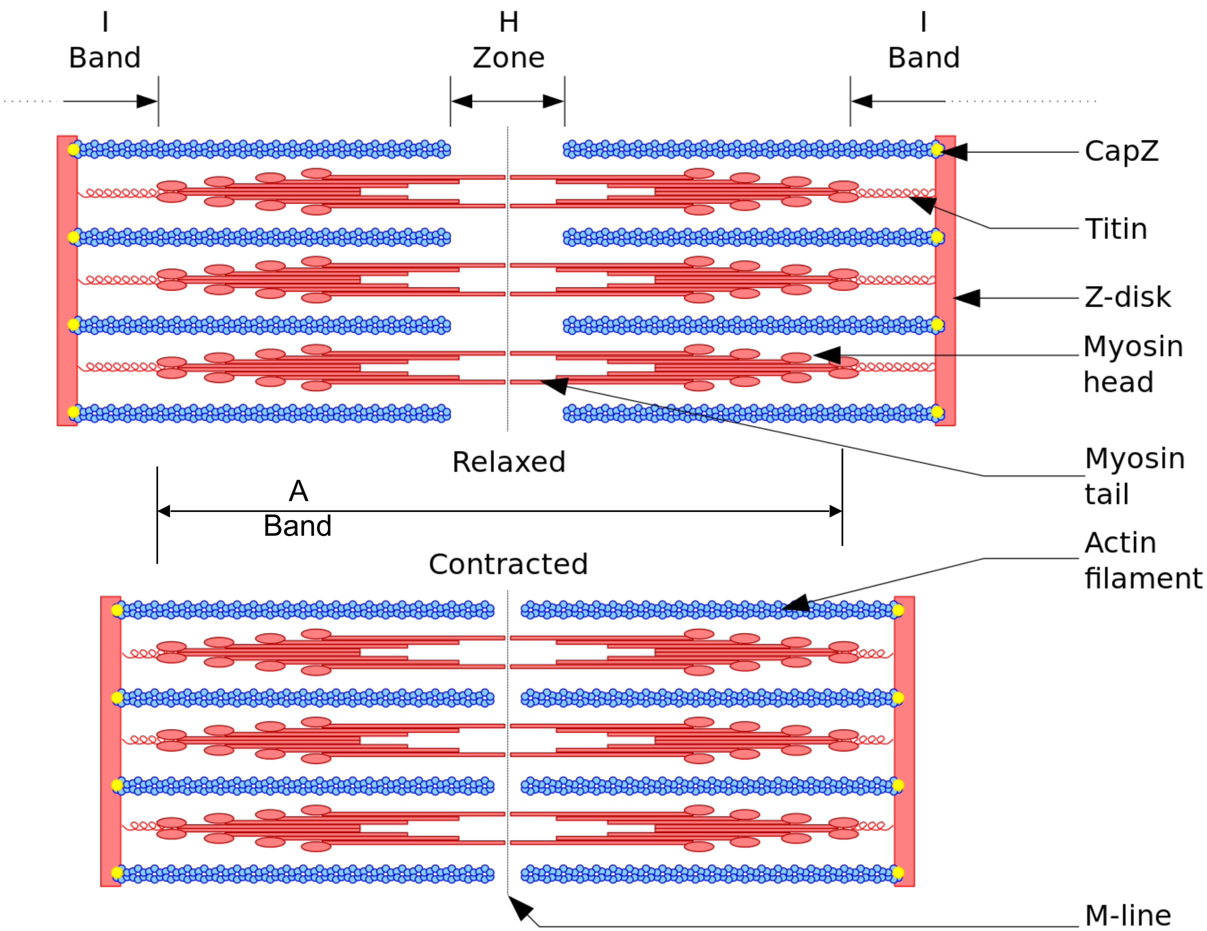

a
Myofibrils
Z-Disk
Costameres
Sarcolemma
A Band
b
Costameres


Costamere
Dystroglycans
Integrin
Spectrin
Vinculin
Sarcoglycans
MLP/CRP3
Talin
Dystrophin
α-actinin
Zyxin
Cytoplasmic Actin
Desmin
Myotilin
γ-filamin
MYOZ2
Nebulette
Thin Filament
Obscurin
FHL2
Titin
p94
Cn
PDZ-3LIM
Thick Filament
Obscurin
CapZ
PDZ-1LIM
p62
MARPs
MLP/CRP3
nbr1
Cn
MURFs
Myopalladin
T-cap/Telethonin
FHL2
Enigma
minK
Atrogin-1
M-disk
H-band
Z-disk
A-band
I-band

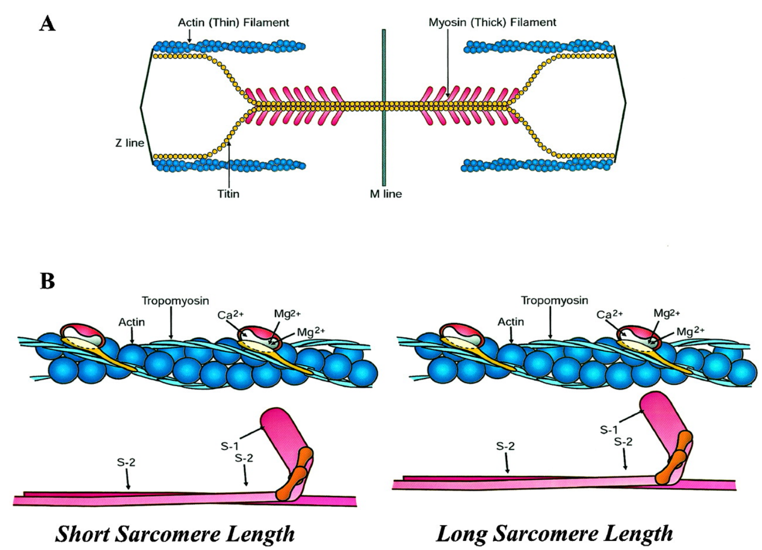

ECM
Costamere: vinculin, spectrin,
dystrophin, talin, ankyrin, etc.
M-filaments
IF: desmin, spectrin, ankyrin
Sarcolemma
Titin
M-Line
Z-line
Myosin
Actin
S
Z-line–IF anchorage,
synemin, filamin,
spectrin, ankyrin, etc.

The Sarcomere

Diastole



Systole

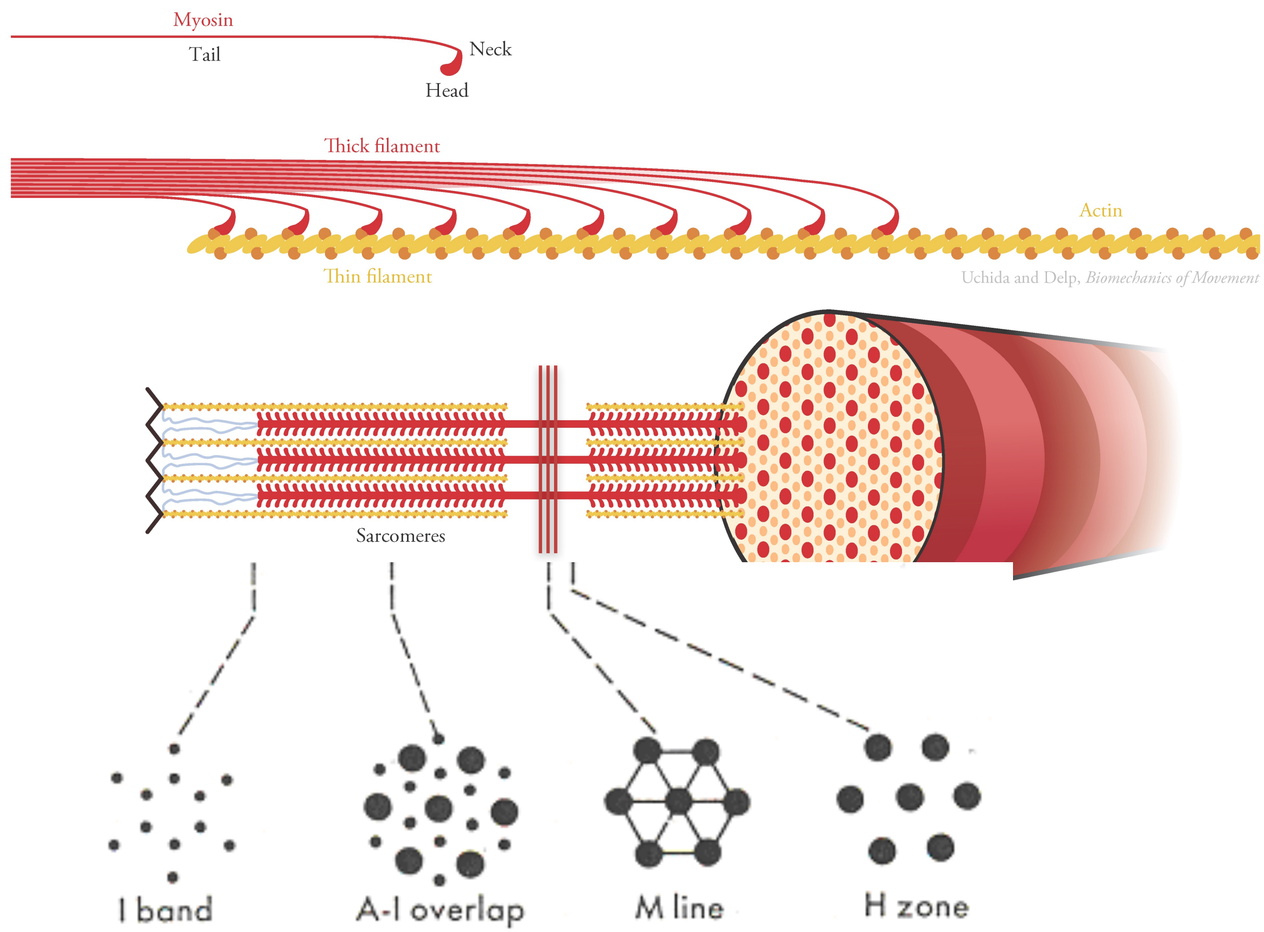


Credit: Slashme
Source: http://en.wikipedia.org/wiki/File:Sarcomere.svg

The Sarcomere

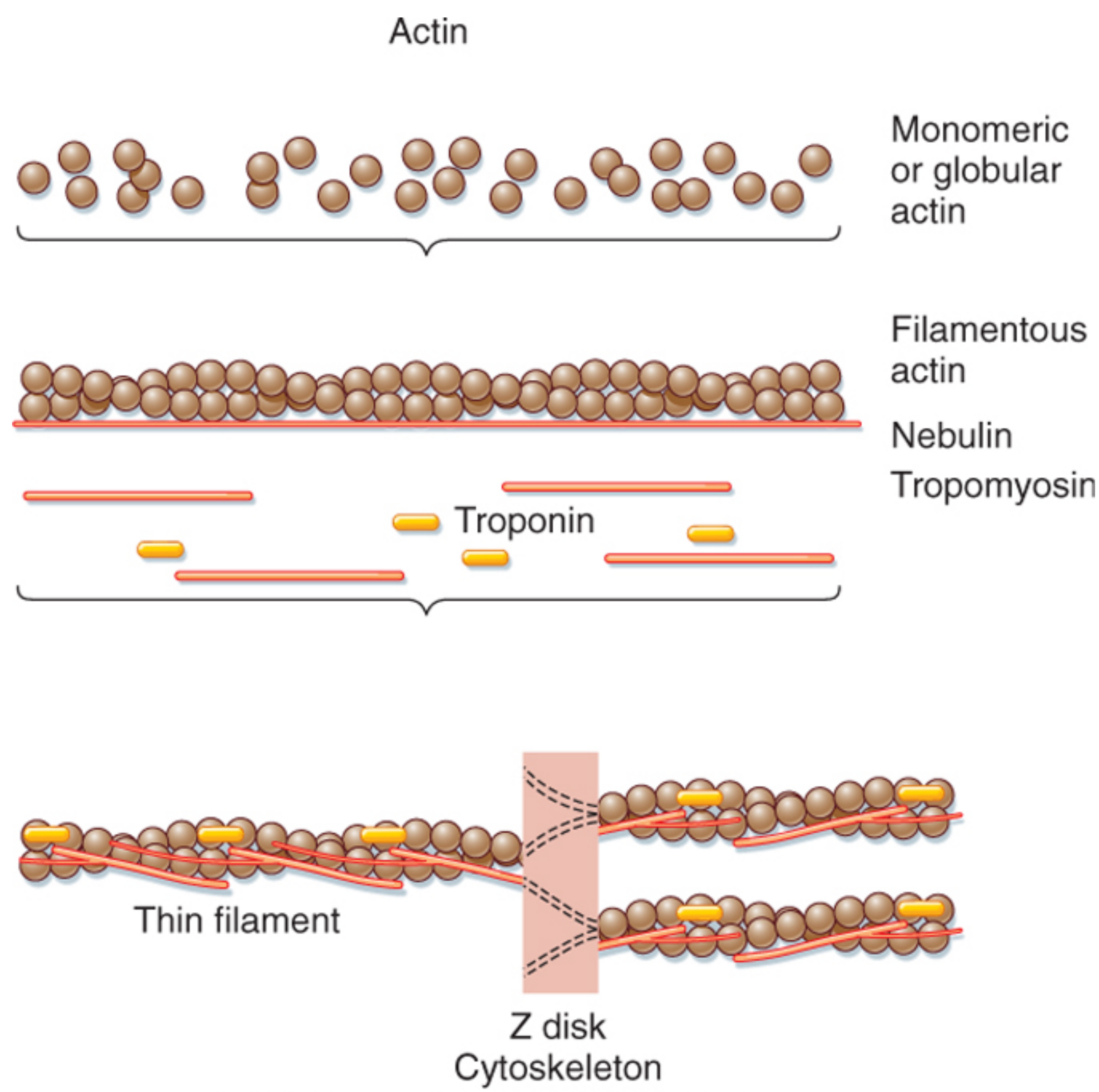
Contractile Filaments
- Thick Myosin Filaments
- Thin Actin Filaments
- Z-line to Z-line
- A and I bands

Titin
- Entropic molecular spring
- Connects Z-line to M-line, allows for force transmission
- Primary cellular contributor to passive stiffness



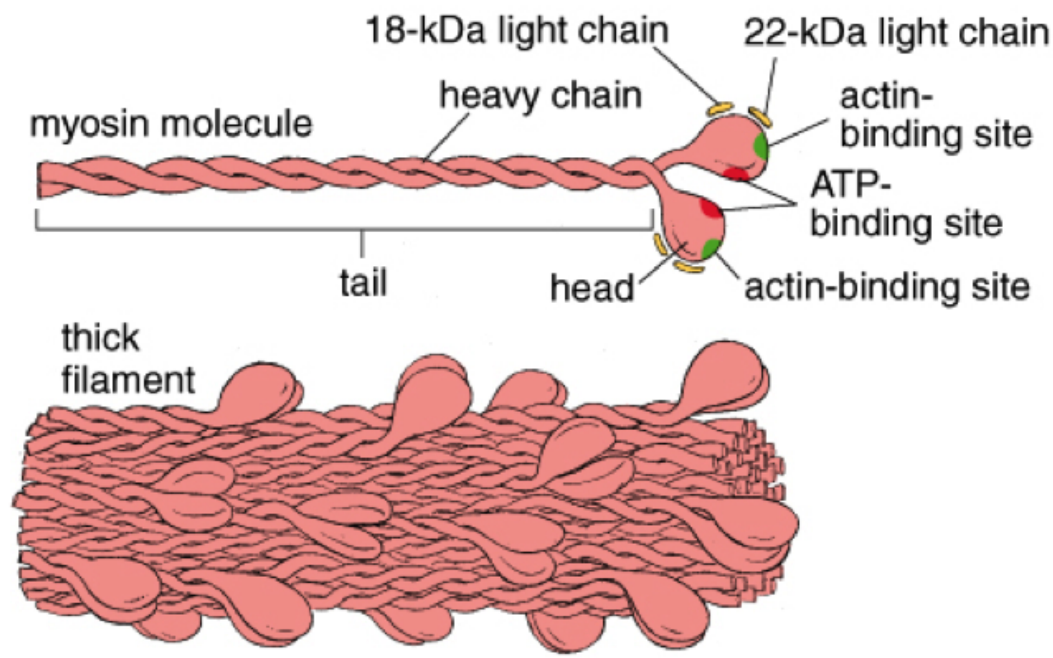
Thin Filament

- F-actin molecule (7 nm diameter)
- Double helix with period ~37 nm
- Nebulin (600–900 kDa) binds ~200 actin monomers
- 1 Tropomyosin ~40 nm long per actin period
- Troponin T, I and C molecules (with a strong affinity for Ca^{2+}) attach to thin filament every 7 G-actin monomers
- Actin filaments anchor at Z-disks



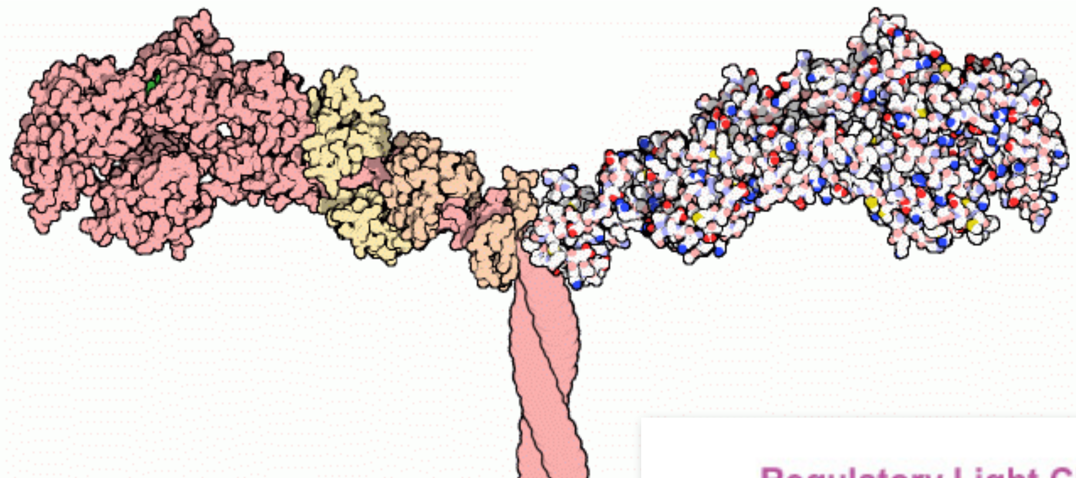
Thick Filament

- Oligamerized myosin molecules
  - Two myosin heavy chains
  - Two myosin light chains per heavy chain
  - One S1-head molecular motor domains per heavy chain
- Heads protrude in pairs at 180º
- ~50 *pairs* of cross-bridges at each end
- 14.3 nm between S1 head pairs
- Thick filament makes a complete twist every 3 pairs

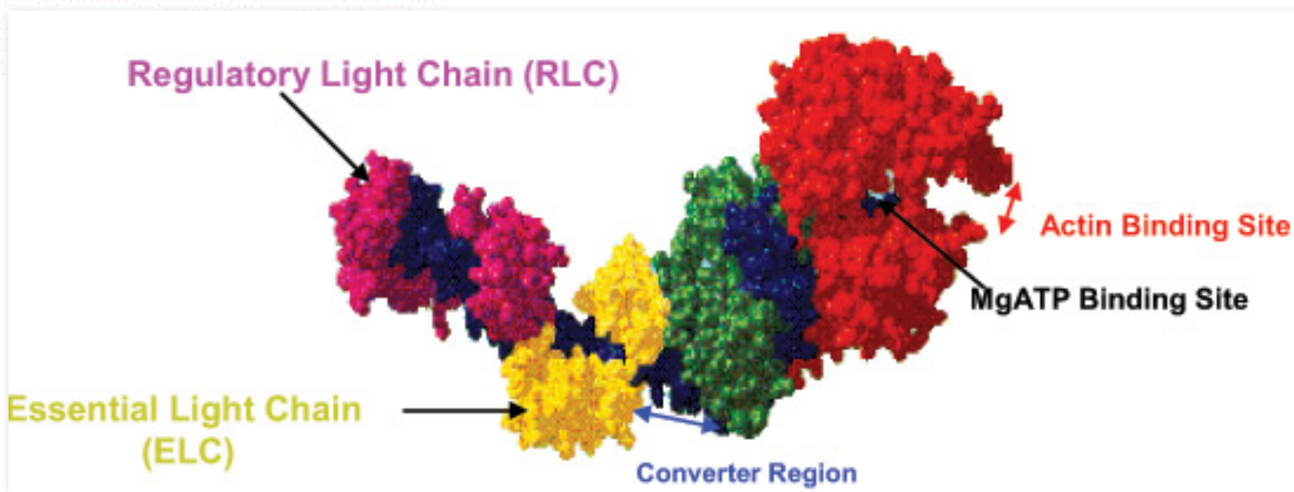


Unfig. 10.2. **Thick filament.**

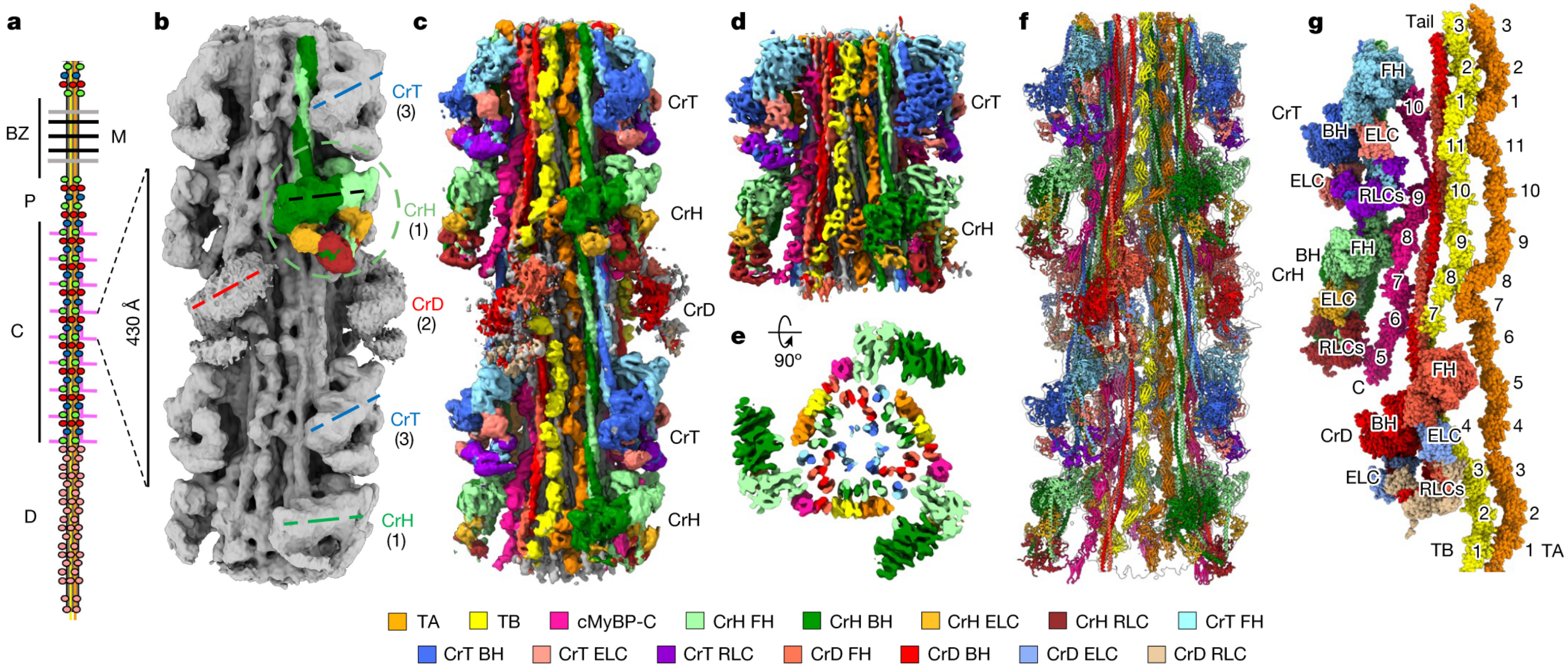
Myosin Light Chains: Regulatory and Essential



Heavy chain atoms are pink red on the left-hand side, and light chain atoms are orange and yellow. -
- David S. Goodsell, TSRI

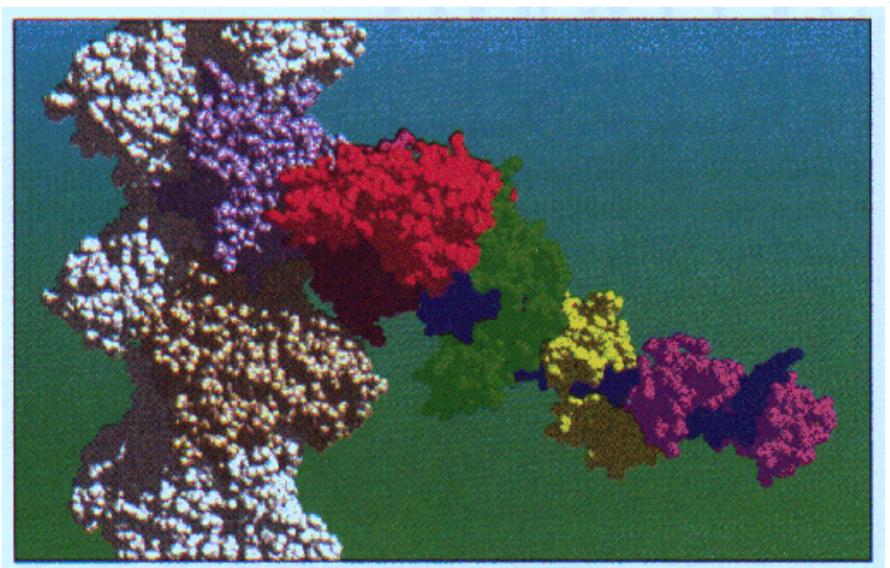


Single-particle 3D reconstruction of the C-zone reveals the organization of titin, cMyBP-C and myosin heads and tails

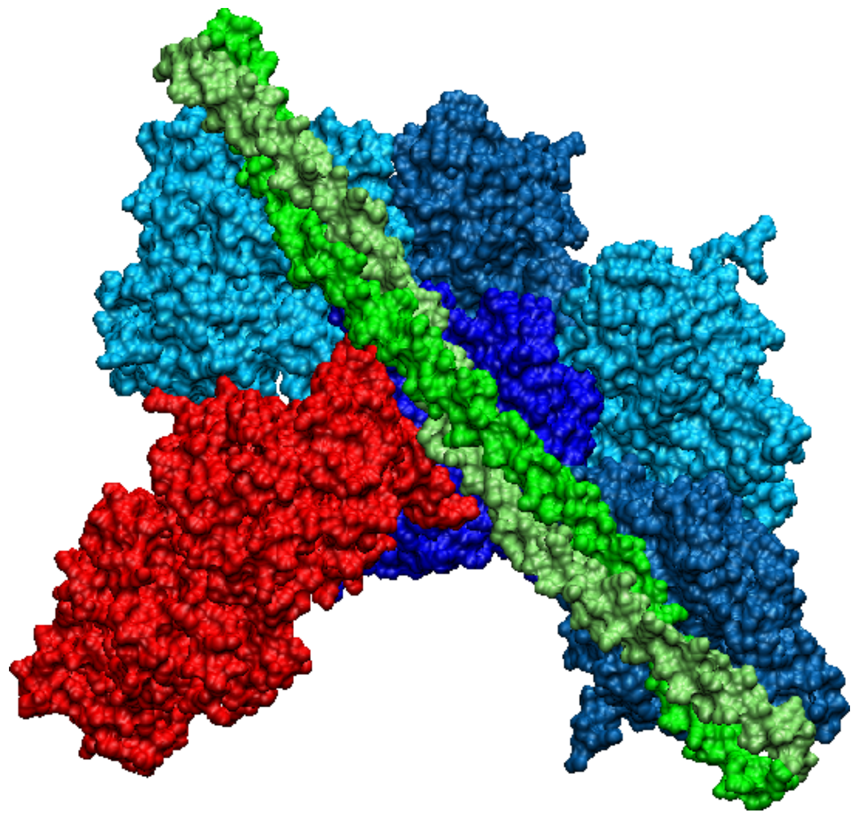


Dutta, D., Nguyen, V., Campbell, K.S. *et al.* Cryo-EM structure of the human cardiac myosin filament. *Nature* **623**, 853–862 (2023). https://doi.org/10.1038/s41586-023-06691-4

Myosin binding to Actin



The actomyosin complex, a molecular motor (1993, Rayment et al.)

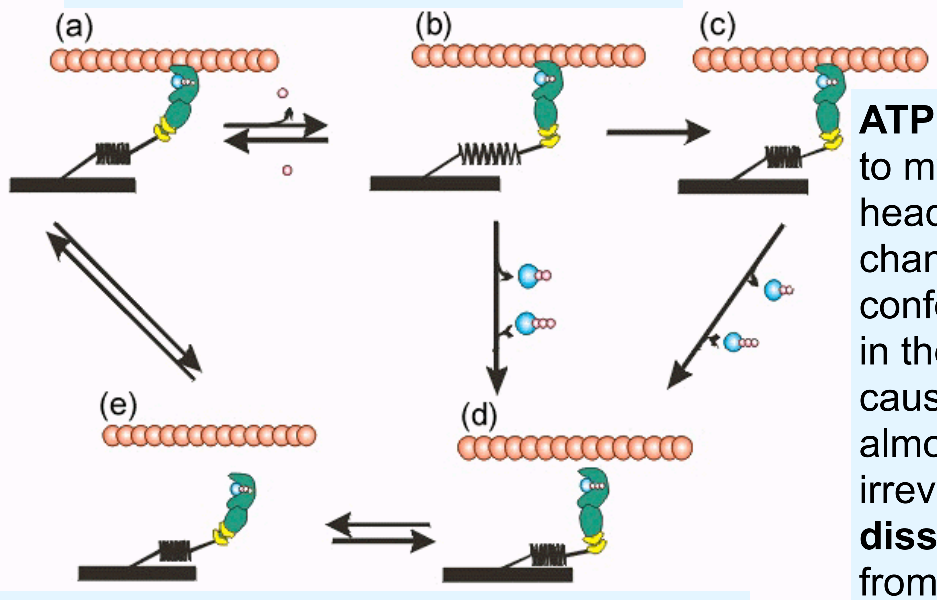


PDB: 4A7F (Behrmann 2012)

Crossbridge Cycle

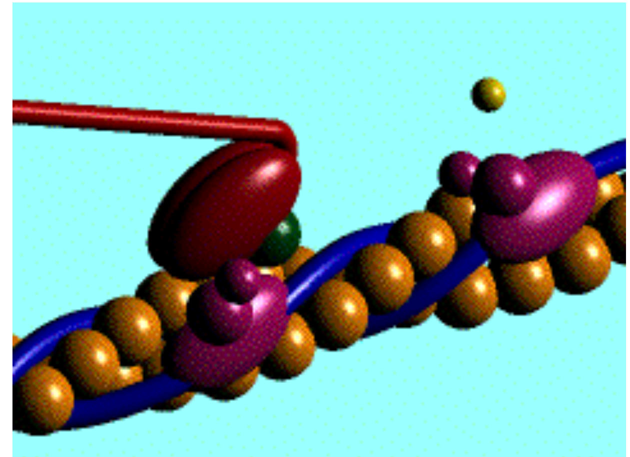
With M bound to A, Pi is released and XB undergoes **powerstroke**

ADP and Pi stay bound until **M.ADP.Pi binds to A**. If an actin site is within reach of the myosin head, it will bind rapidly & reversibly

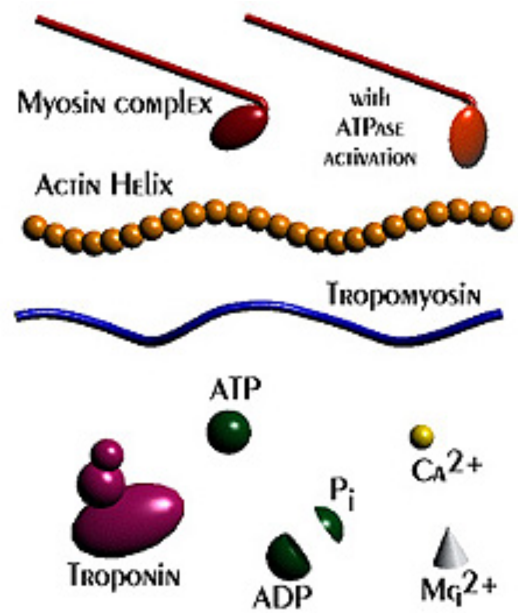


ATP binding to myosin head (**b or c**) changes conformation in the head causing rapid, almost irreversible **dissociation** from actin (**d**).

After detachment, **ATP is** rapidly (10 ms) **hydrolyzed** but ADP and Pi remain bound. Hydrolysis is reversible (Keq = 10), i.e. free energy of ATP remains in the M.ADP.Pi complex. Hydrolysis causes **conformational change repriming t**he power stroke.



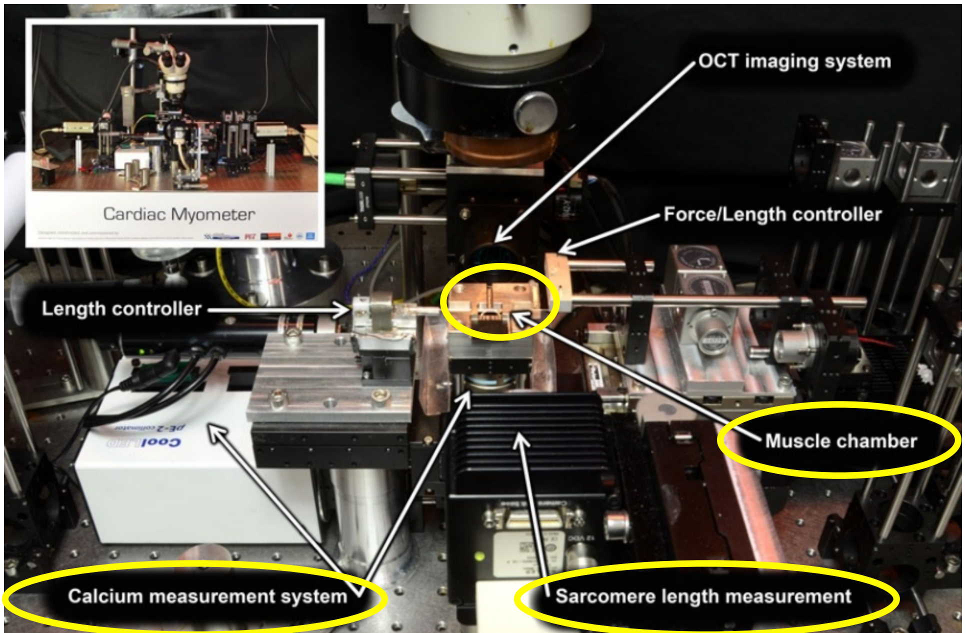
ATP/ADP, Blue/Pink
Pi, Pink
Stefan Weiss & Mike Geeves
Actin monomers
Myosin head



Primary Determinants of Force Production in Cardiac Muscle

- Intracellular calcium concentration
- Sarcomere length (SL)
- Rate of change in SL (velocity)

Cardiac Muscle Testing



Tissue chamber and preparation

- isolated right ventricular trabeculae
- 4000x200x90 μm

Force transducers

- cantilever - laser interferometry
- piezoresistive 1 *V/mN,* capacitive 0.1 *V/mN*

Motor

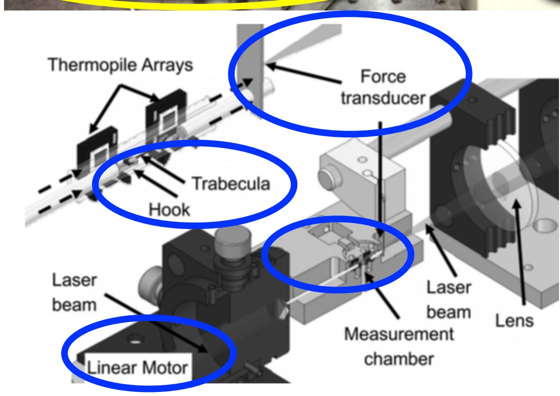
- linear servomotor

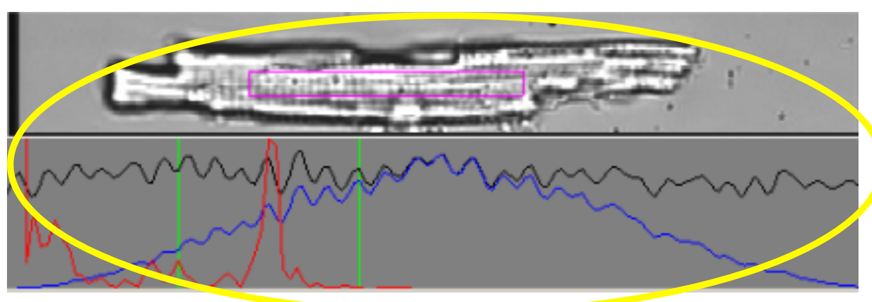
Sarcomere length

- laser diffraction or Fourier imaging

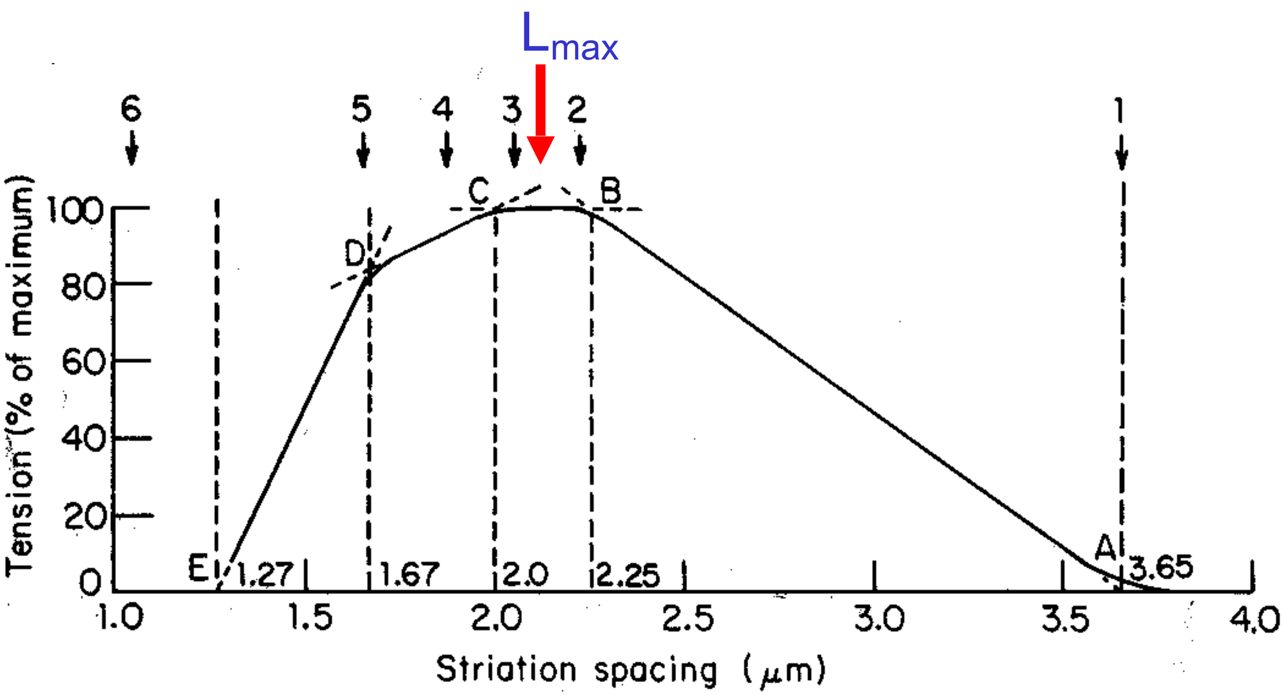
Calcium

- ratiometric fluorescence imaging



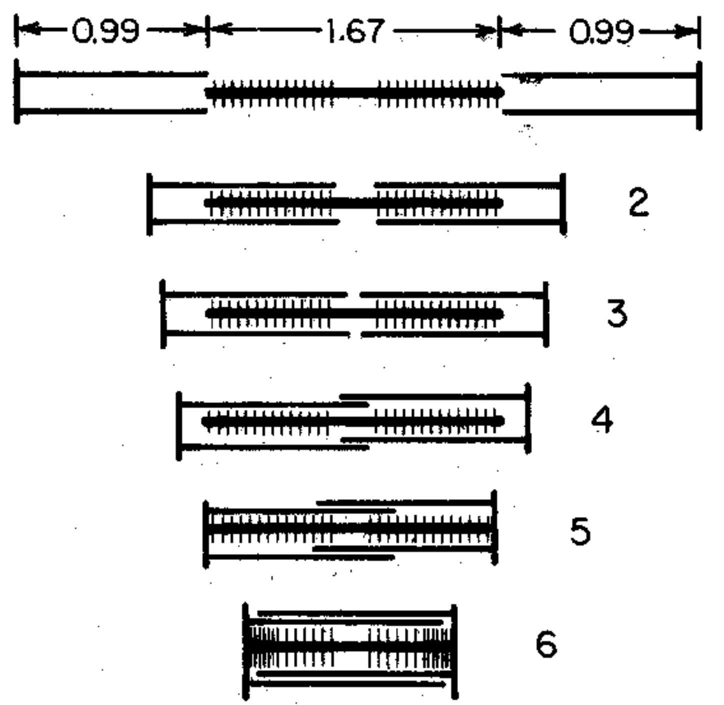


Skeletal Muscle Isometric Tension: Sliding Filament Theory

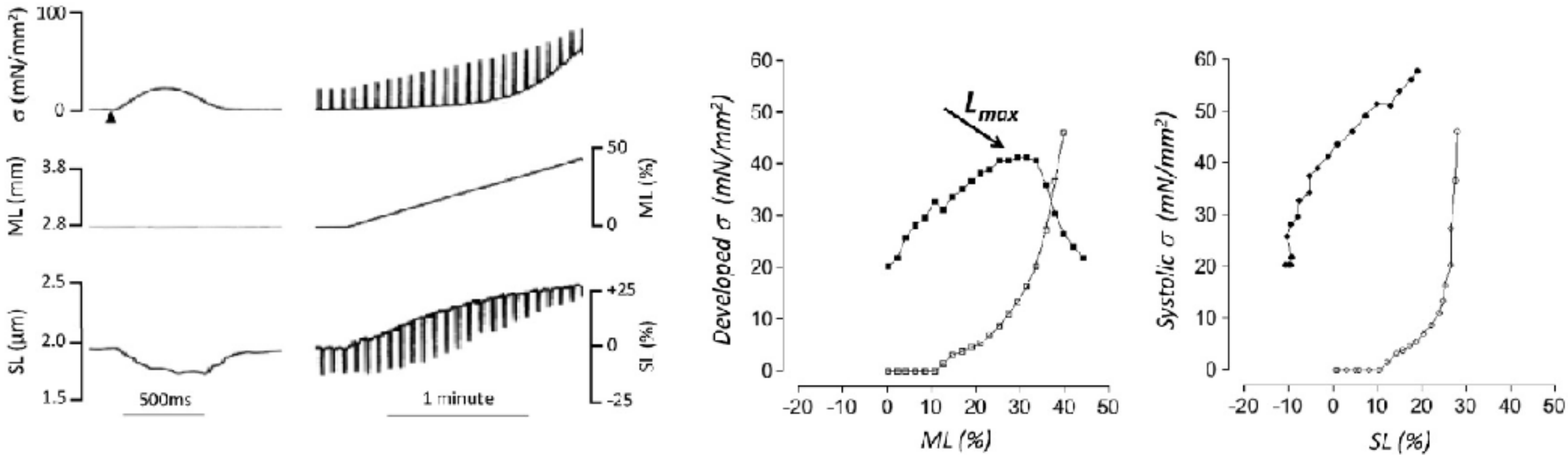


Developed tension versus length for a single isometric fiber of frog semitendinosus muscle

Sliding filament theory

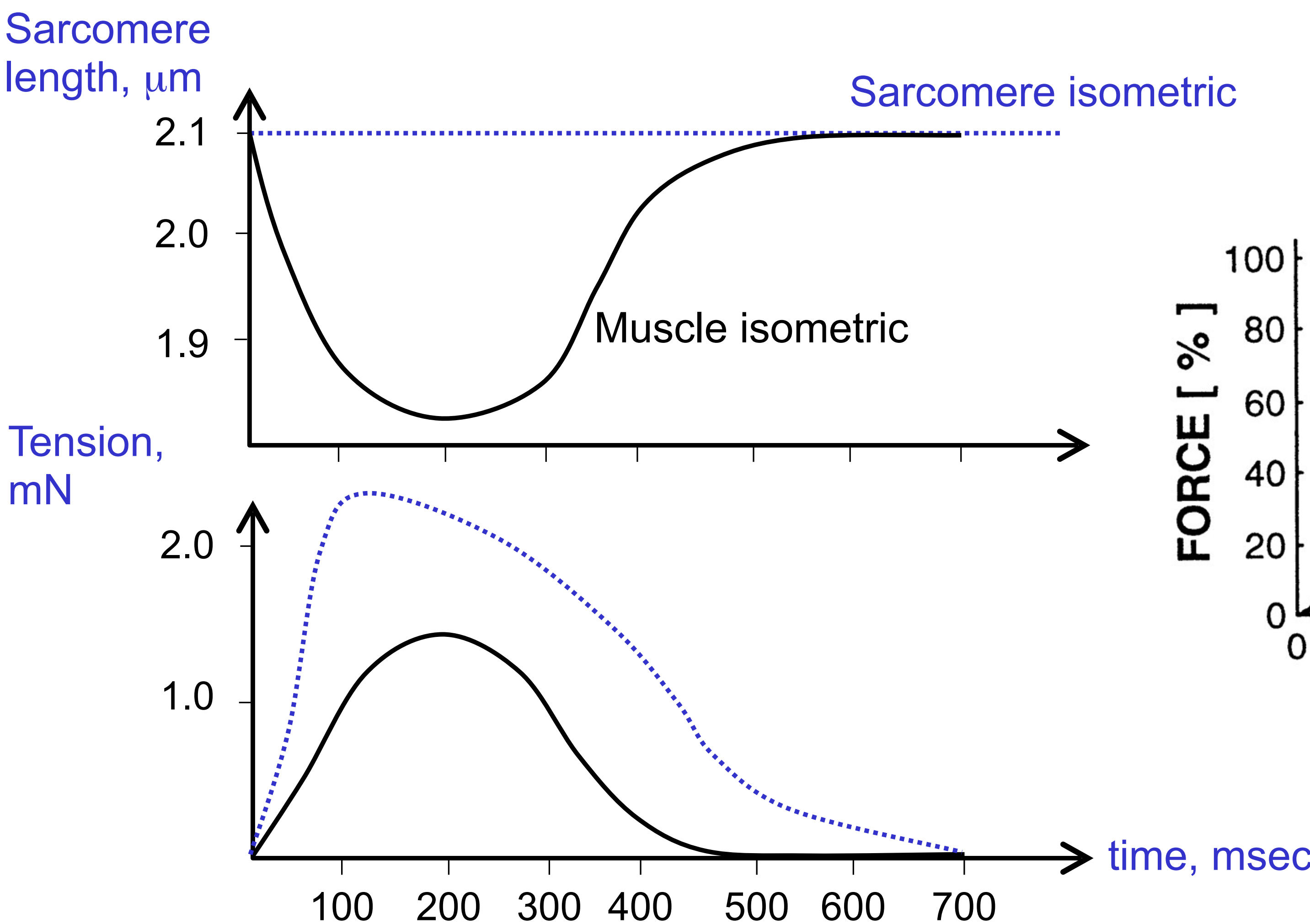


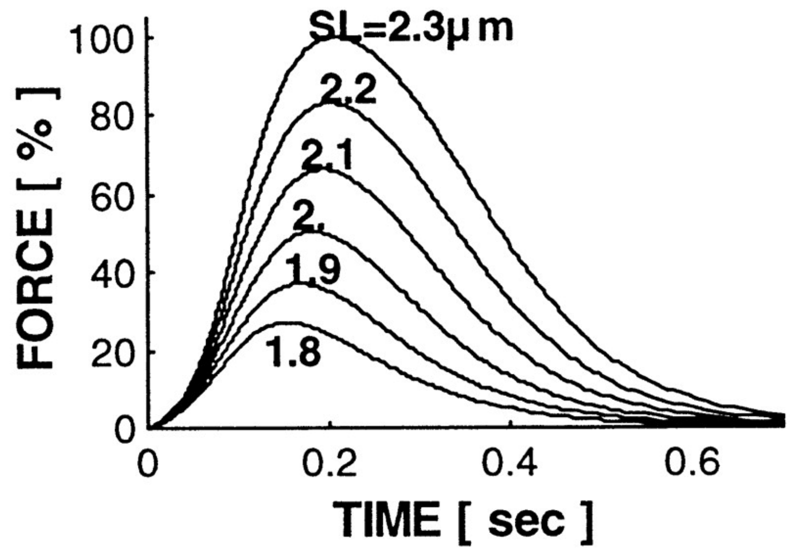
Cardiac Muscle Isometric Twitch Testing



At constant muscle length, muscle preparation shortens in the middle at the expense of lengthening at the damaged ends. de Tombe and ter Keurs (2016) *Journal of Molecular and Cellular Cardiology* 91: 148–150

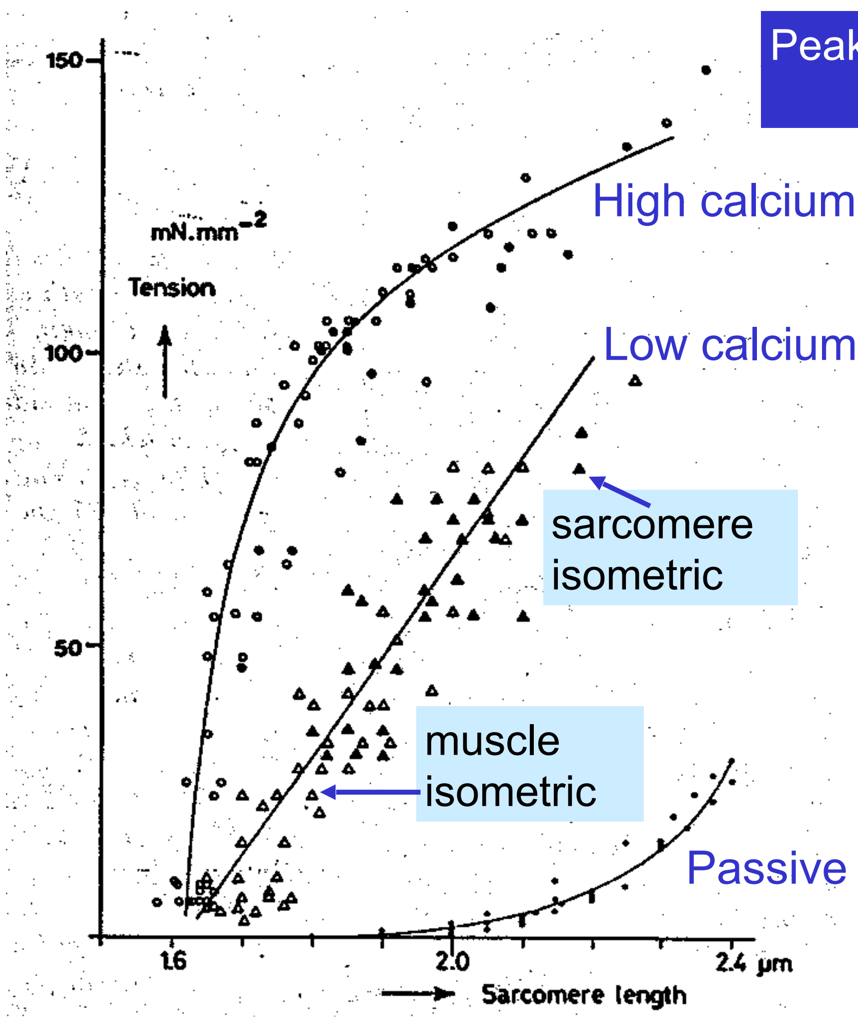
Cardiac Muscle Isometric Twitch Testing

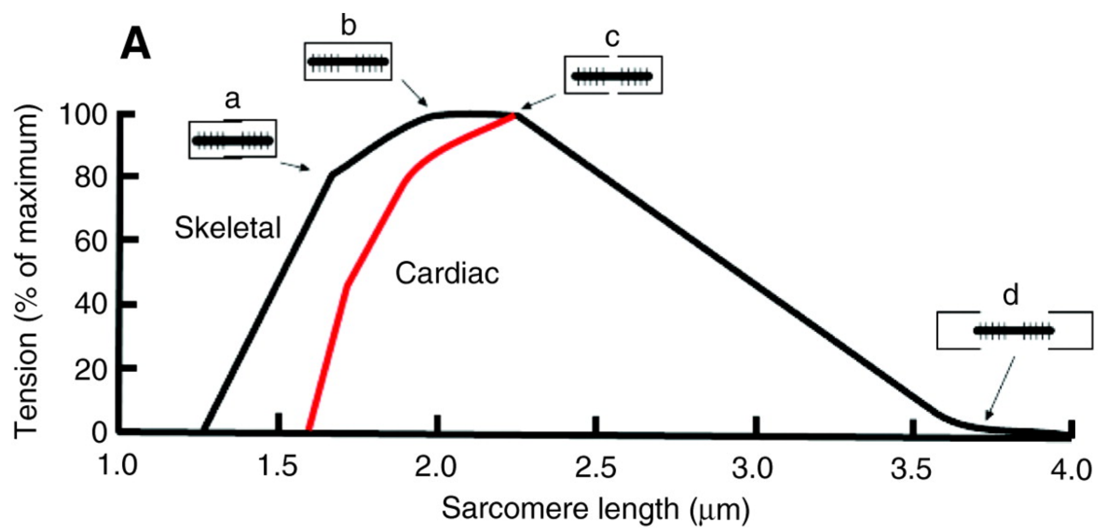




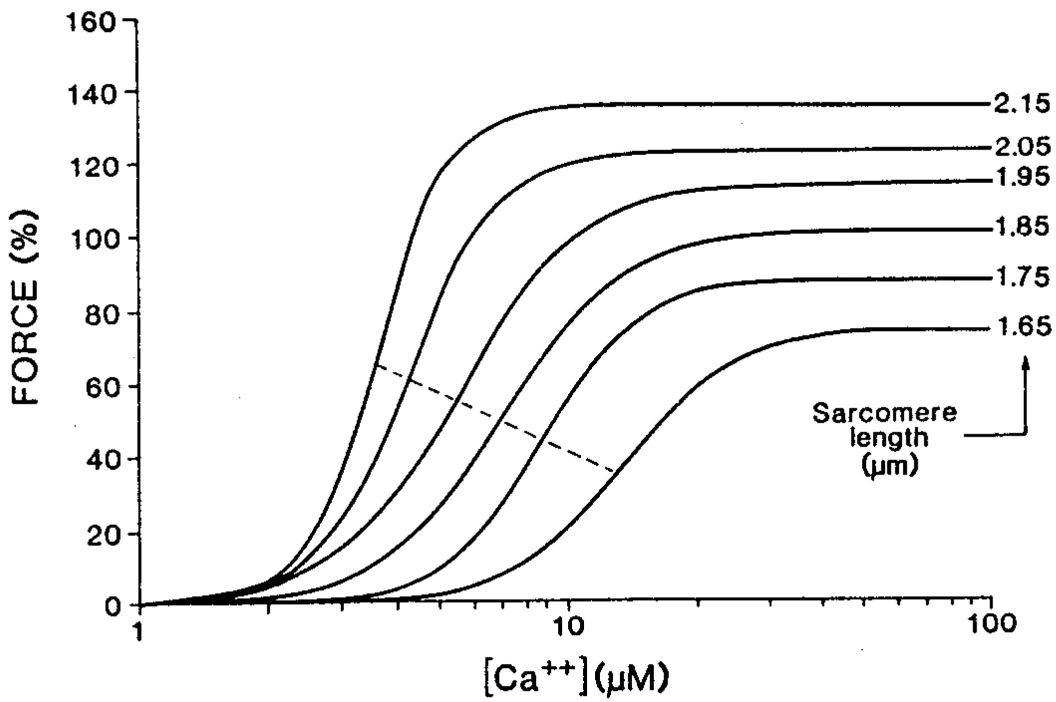
Cardiac Muscle Isometric Length-Peak Tension Curve

Peak developed isometric twitch tension (total-passive)





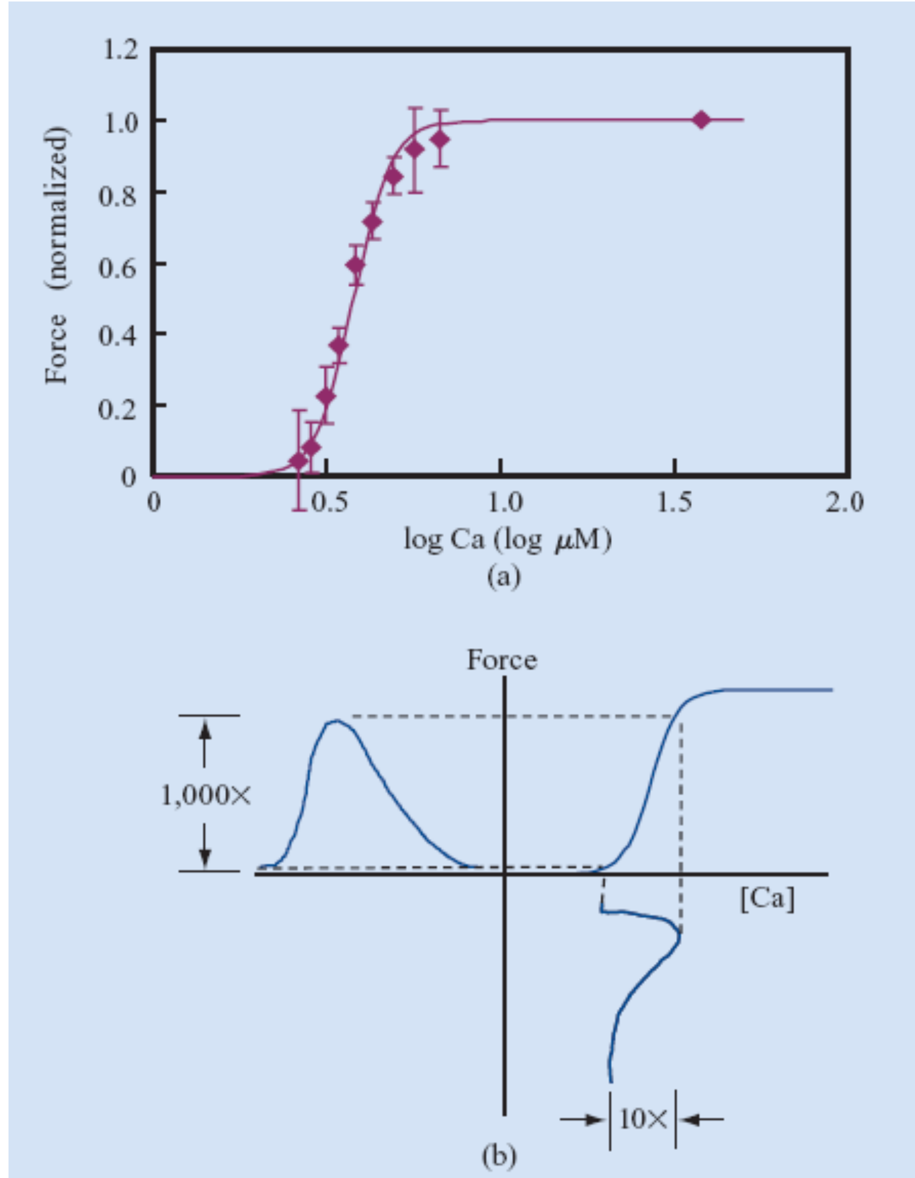
Length-Dependent Activation



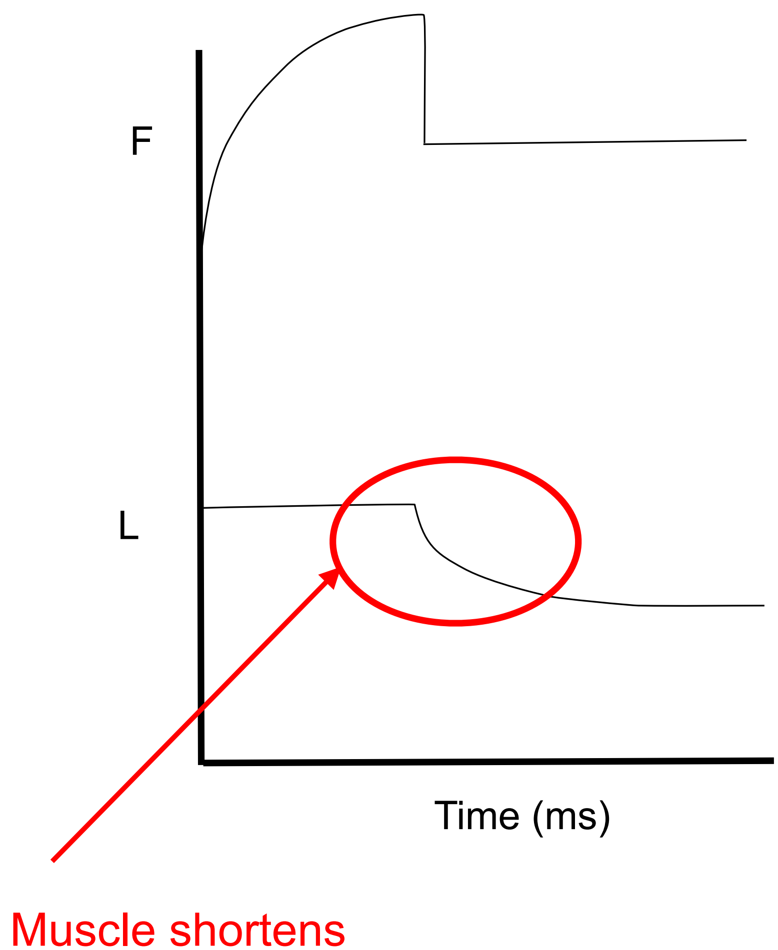
Isometric peak twitch tension in cardiac muscle continues to rise at sarcomere lengths >2 μm due to sarcomere-length dependent increase in myofilament calcium sensitivity

Cooperative Myofilament Activation

- Developed force is a steep nonlinear function of [Ca].
- Experimental data from rat cardiac muscle.
- Small change in [Ca] produces a larger relative change in force
- Twitch forces rises slower than the calcium transient and falls faster



Isotonic (constant force) Test



Here, the isotonic force is ~ 50% of the isometric max. Now if, the isotonic force is only about 20% of the isometric value. Which is true?

(A) The shortening velocity is lower
(B) The shortening velocity is higher
(C) The shortening velocity is the same in but the total shortening is less
(D) The shortening velocity is the same in but the total shortening is more
(E) The shortening velocity and the total amount of shortening are the same

Hill's Force-Velocity Relation

Dimensionless forms:

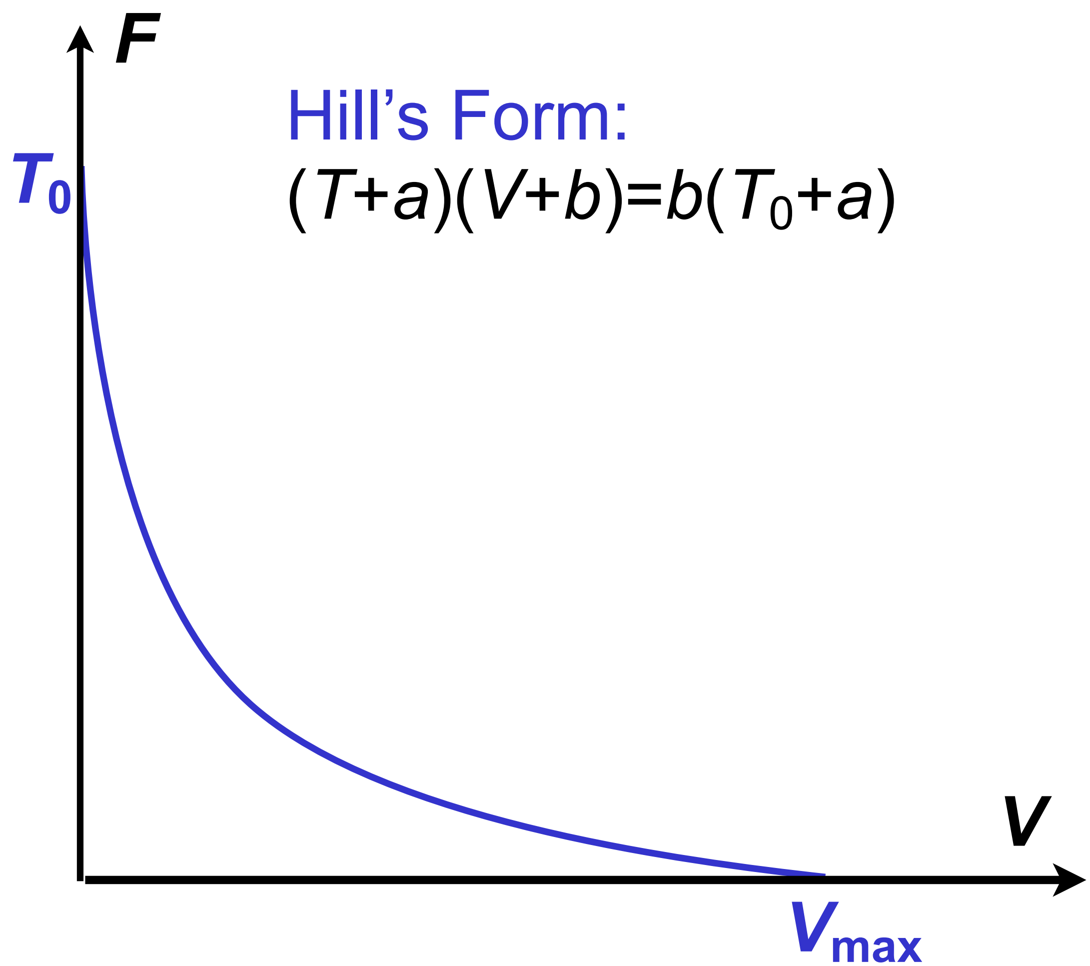
$$\frac{V}{V_{max}} = \frac{1 - T/T_0}{1 + c\left(T/T_0\right)}$$

$$\frac{T}{T_0} = \frac{1 - V/V_{max}}{1 + c\left(V/V_{max}\right)}$$

- -a, -b = asymptotes
- T_0 = Isometric tension
- V_{max}= unloaded shortening velocity
- c = T_0/a (ranges from 1.2-4.0)

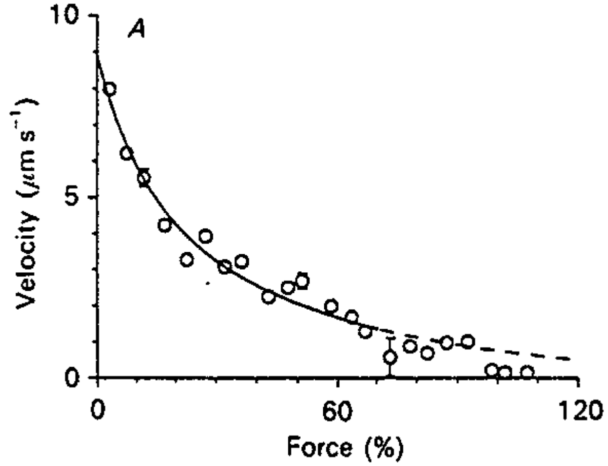
Hill's Form:

$(T+a)(V+b)=b(T_0+a)$

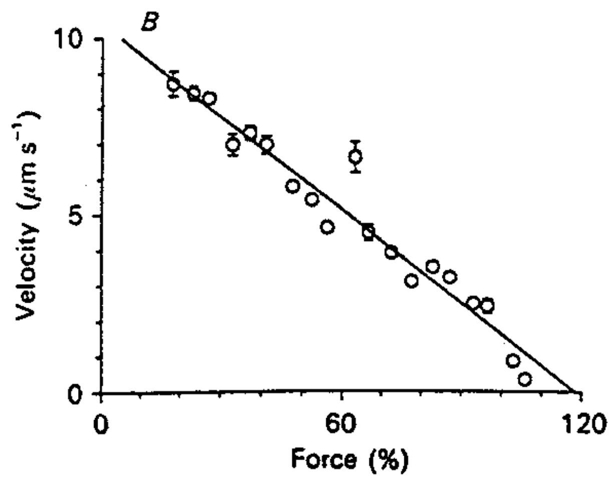


Isotonic Force-Velocity Relation

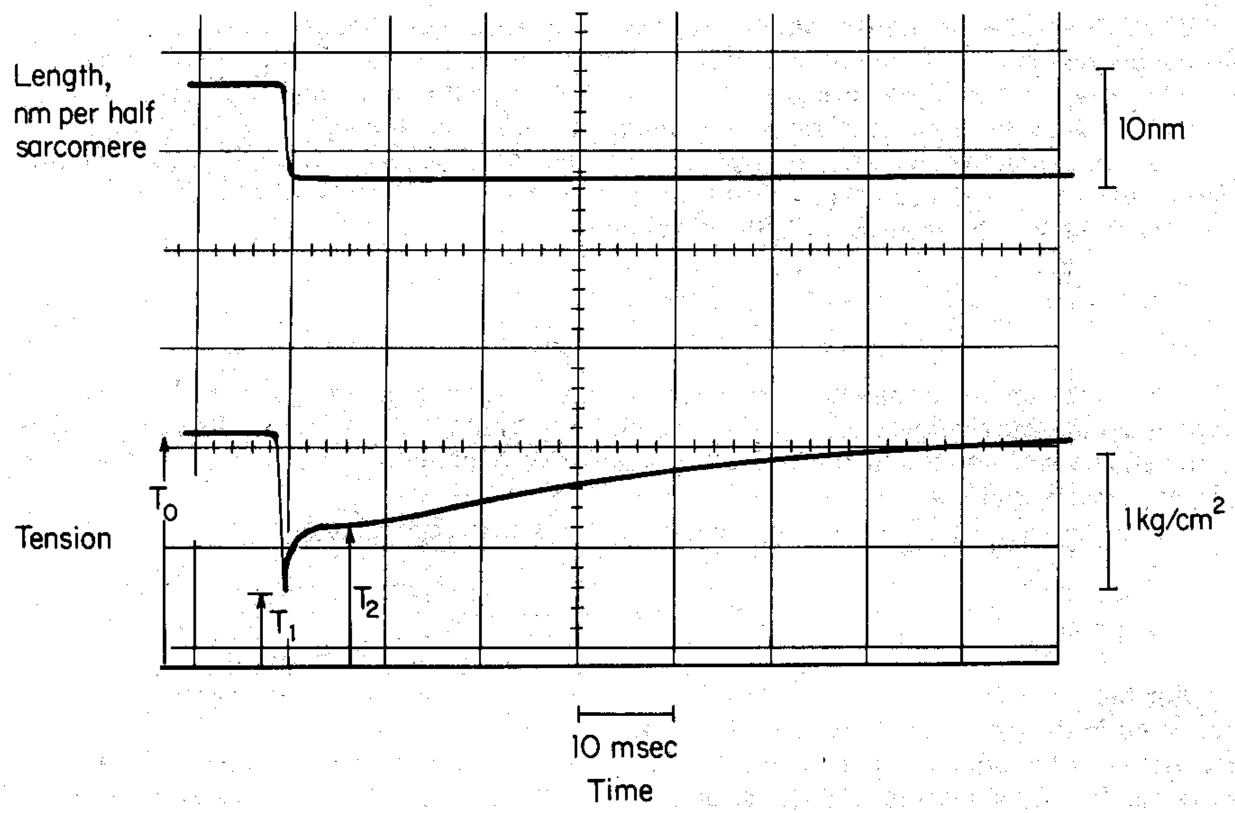
Isovelocity release experiment conducted during a twitch



Cardiac muscle force-velocity relation corrected for viscous forces of passive cardiac muscle which reduce shortening velocity



Transient Responses



Tetanized single frog muscle fiber at 0ºC during a 1% shortening step lasting 1 ms

Cardiac Muscle: Summary

- *Cardiac muscle fibers* (cells) are short and rod-shaped but are connected by *intercalated disks* and collagen matrix into a spiral-wound *laminar fibrous architecture*
- The *cardiac sarcomere* is similar to the skeletal muscle sarcomere
- Cardiac muscle has a very slow twitch but it can not be tetanized because the *cardiac action potential* has a refractory period
- *Calcium* is the intracellular trigger for cardiac muscle contraction
- *Cardiac muscle testing* is much *more difficult* than skeletal muscle: *laser diffraction* has been used in trabeculae
- Cardiac muscle has relatively *high resting stiffness* due to more collagen and shorter titin isoforms.
- The *cardiac muscle isometric length-tension curve* has no real descending limb