Myosin motor proteins and cardiomyopathy – contributions from synchrotron studies

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Overview:

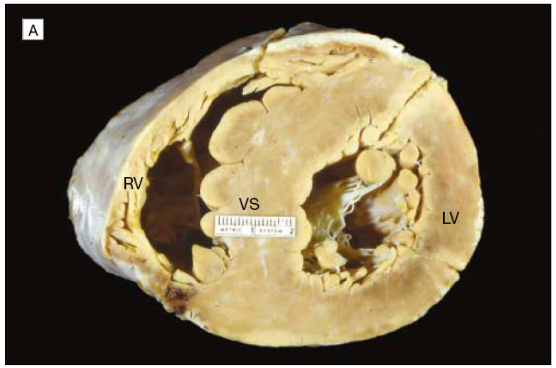
- What is cardiomyopathy
- Cardiomyopathy related mutations
- Muscle fiber diffraction
- What can we learn from fiber diffraction and sarcomeric mutations

  Examples: cardiac myosin binding protein C (cMyBP-C)

  tropomyosin

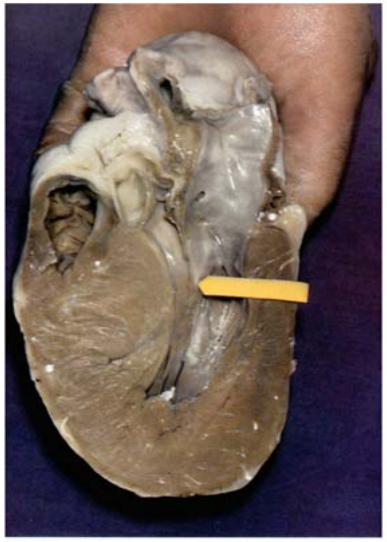
  myosin head domain

Hypertrophic Cardiomyopathy



from Maron, BJ, JAMA 2002

Hypertrophy of LV and Septum



Adapted from G. Farrer-Brown, Farbatlas der Herzkrankheiten, 1980

Typical symptoms:

- asymmetric left ventricular hypertrophy
- cardiac arrhythmias
- fainting
- ventricular fibrillation

→ frequent cause of sudden death, particularly in young athletes

Hypertrophic Cardiomyopathy

THE WALL STREET JOURNAL EUROPE.

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COLUMN ONE

Young Athletes' Deaths Raise Debate in U.S. Over a Hidden Disease

Critics Say Wider Screening, as Done In Italy, Could Detect Heart Defects

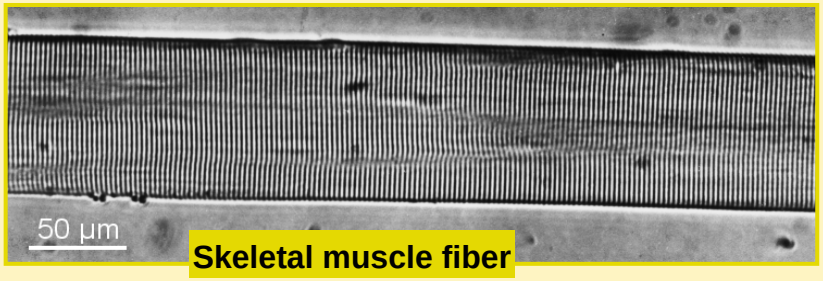
Misreading Shortness of Breath

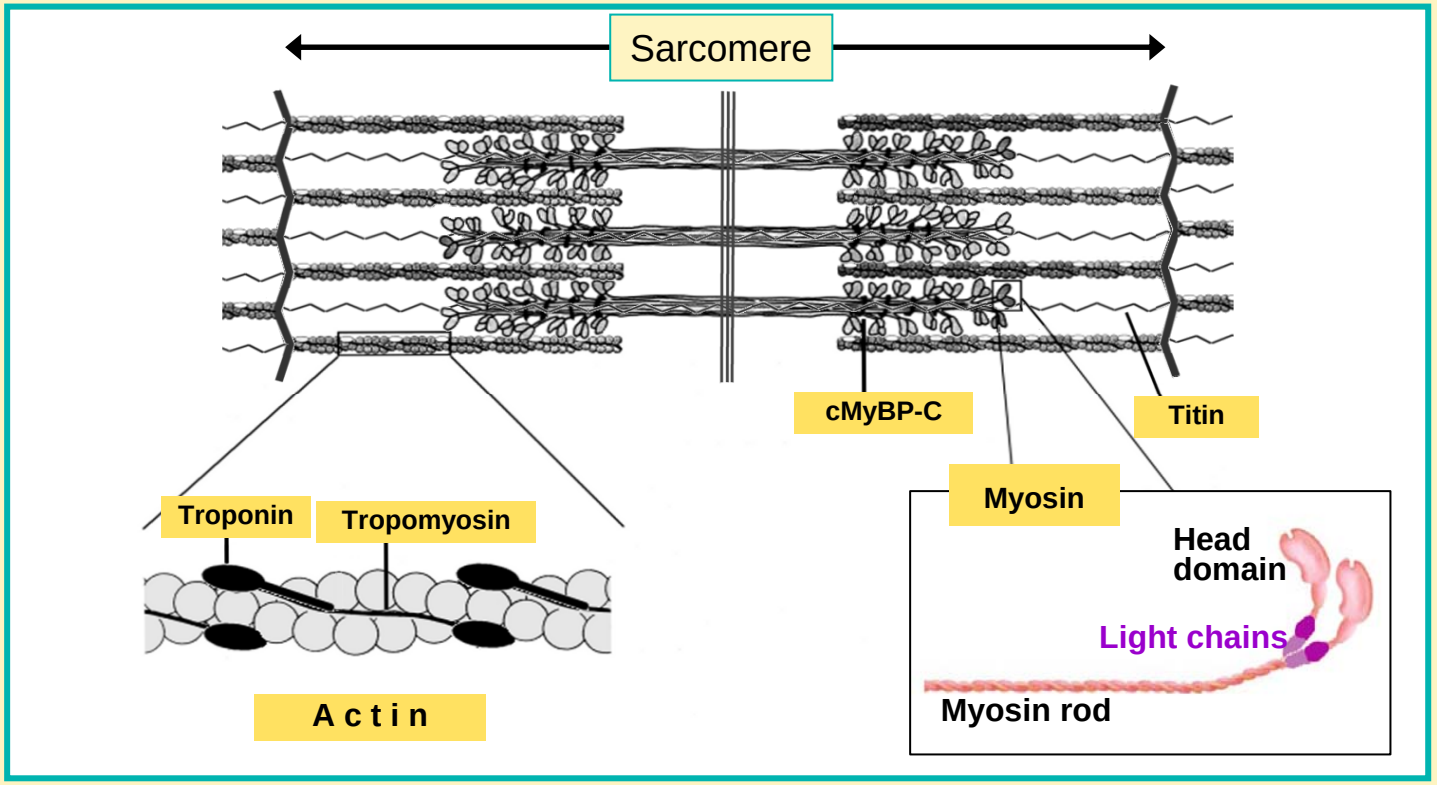
Early this month, Kenny Sirois was jogging beside his identical twin in their hometown of Madawaska, Maine. As the 16-year-olds turned onto their own street, they started sprinting toward home. Kenny never made it.

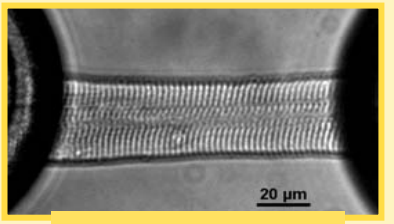
An emergency-room doctor told Vincent and Wendy Sirois their son had died of a hidden heart

Gerald Asamoah
– he suffers from
Hypertrophic Cardiomyopathy

FHC is caused mainly by mutations in sarcomeric proteins

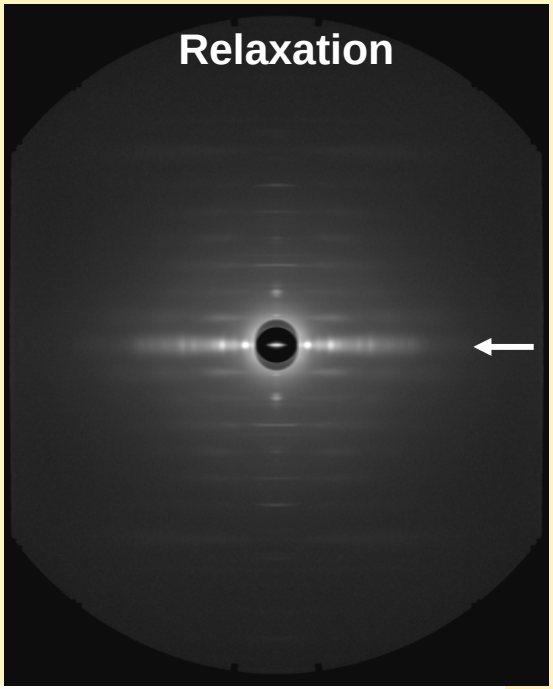




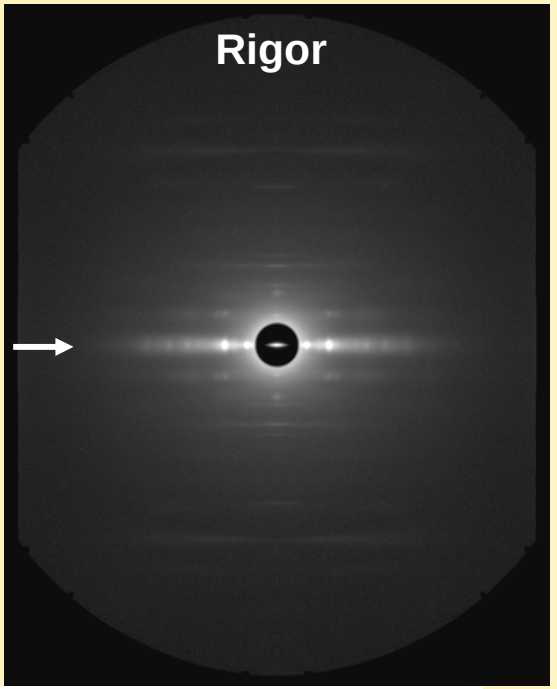


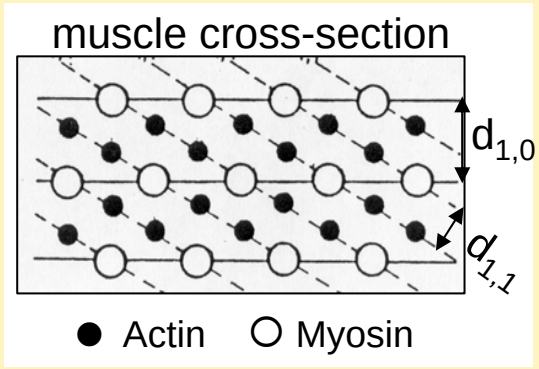
X-ray diffraction of muscle fibers: (1) Equator

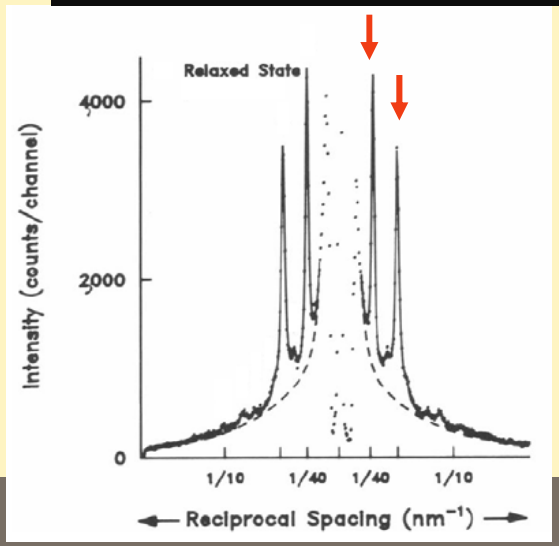
Sarcomere =
highly ordered 3D-assembly of different proteins that interact to produce force / muscle shortening

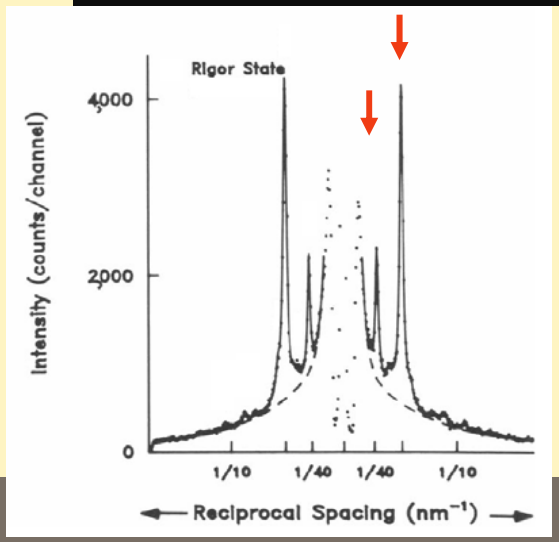


Eq





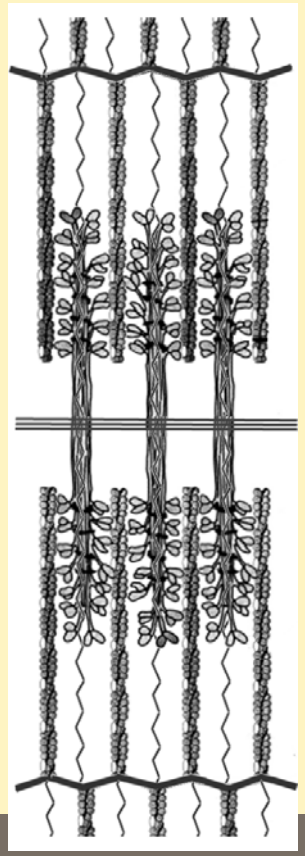


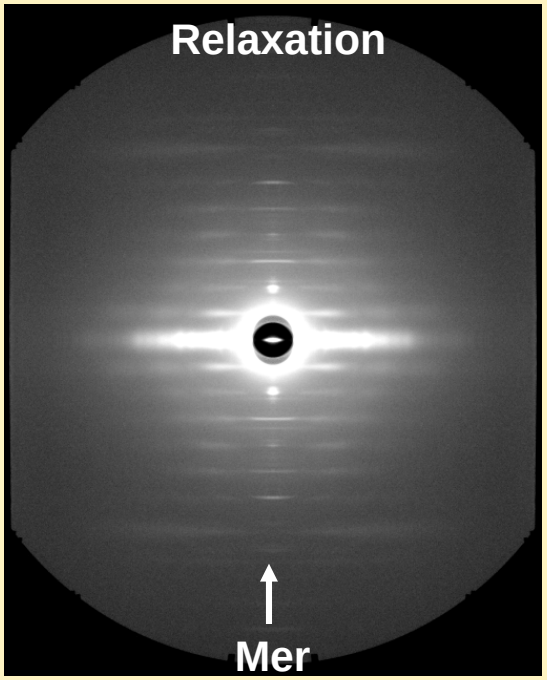


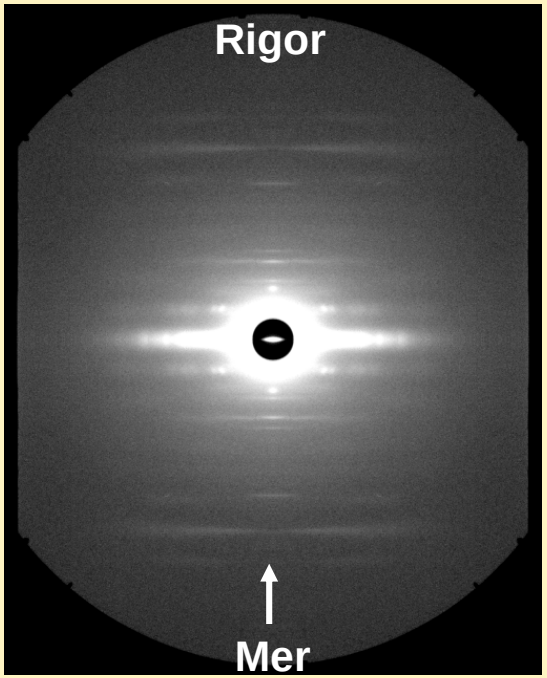
Equatorial profiles

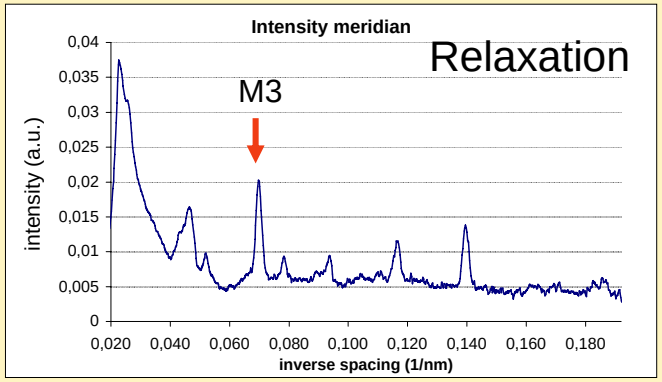
X-ray diffraction of muscle fibers: (1) Meridian and LL‘s

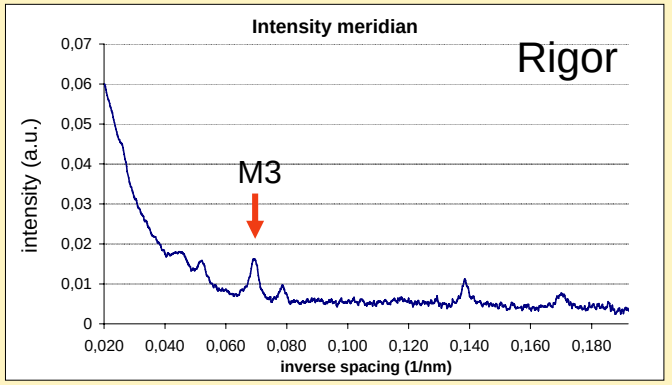
Sarcomere =
highly ordered 3D-assembly of different proteins that interact to produce force / muscle shortening





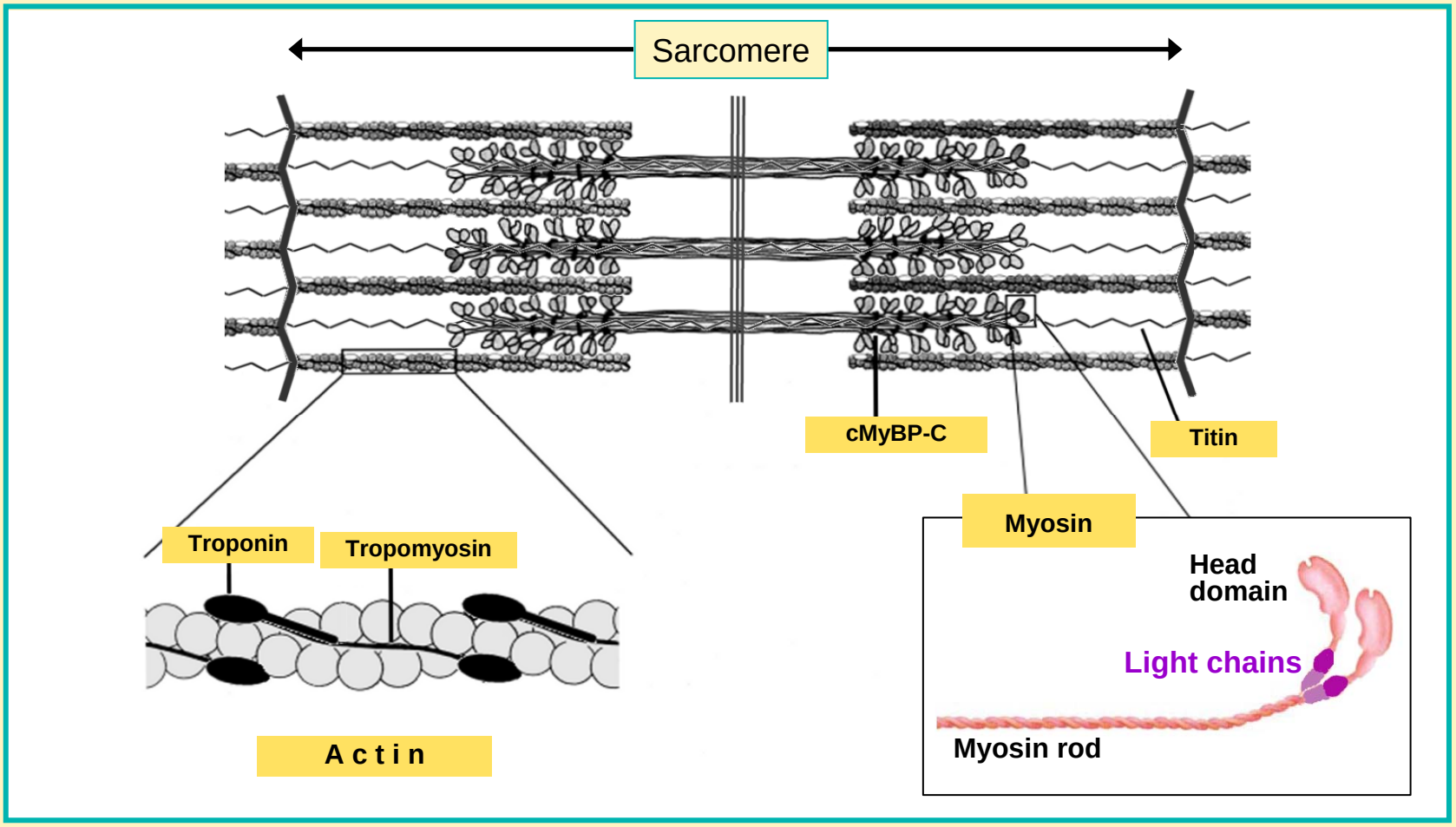






Meridional profiles

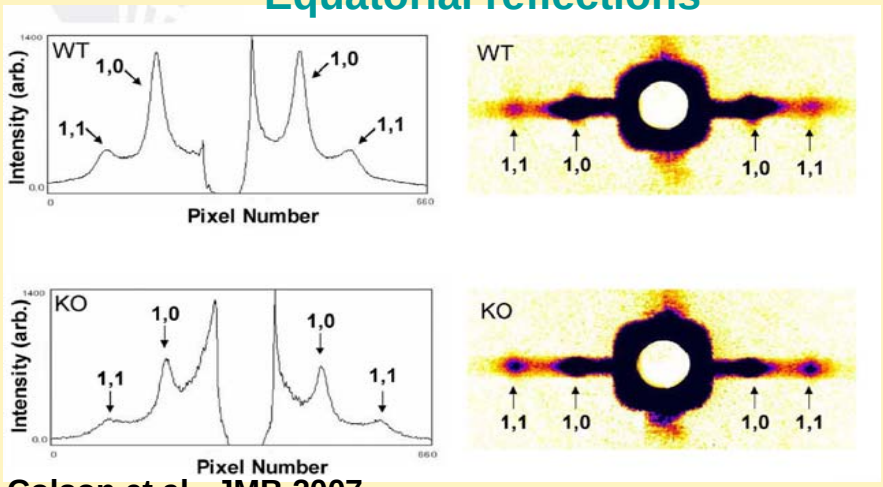
Effects of cardiomyopathy mutations on diffraction patterns?



Mutations in cMyBP-C

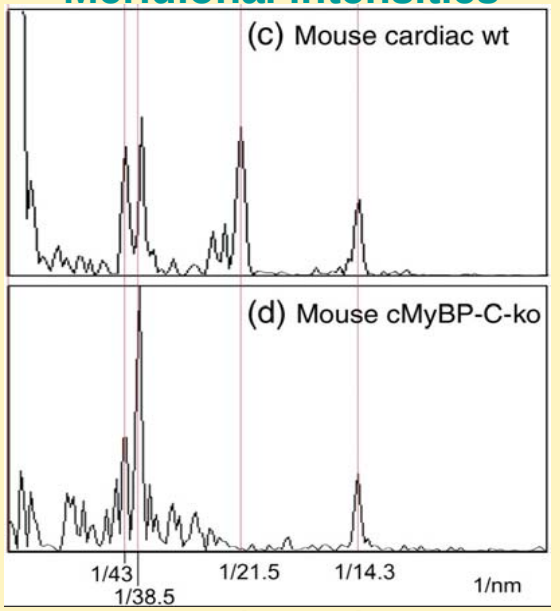
- cMYBP-C mutations are frequent and often cause premature degradation of the faulty protein

Equatorial reflections



Colson et.al., JMB 2007

Meridional intensities

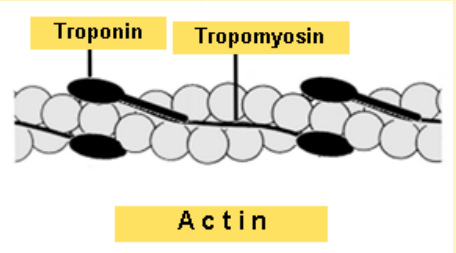


Luther et al., JMB 2008

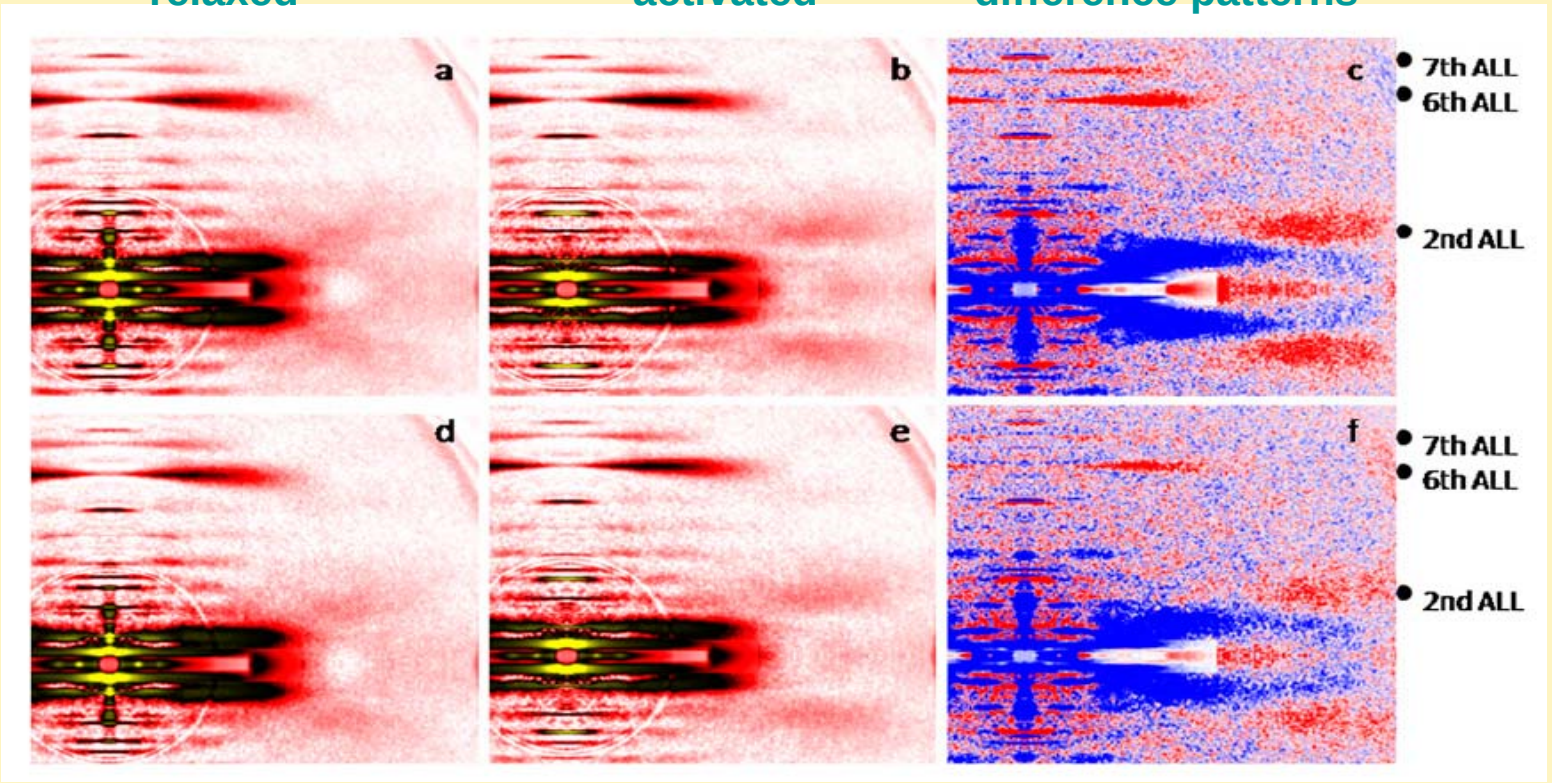
Heart muscle from cMyBP-C k.o. mice:

- Increase in intensity ratio of the two innermost equatorial reflections ($I_{1,1}/I_{1,0}$)
  → myosin heads move away from thick filament backbone
- Fourier-transforms obtained from electron micrographs show massive decrease of forbidden reflections like at 21.5nm → myosin assumes more helical order

Myopathy mutation in tropomyosin



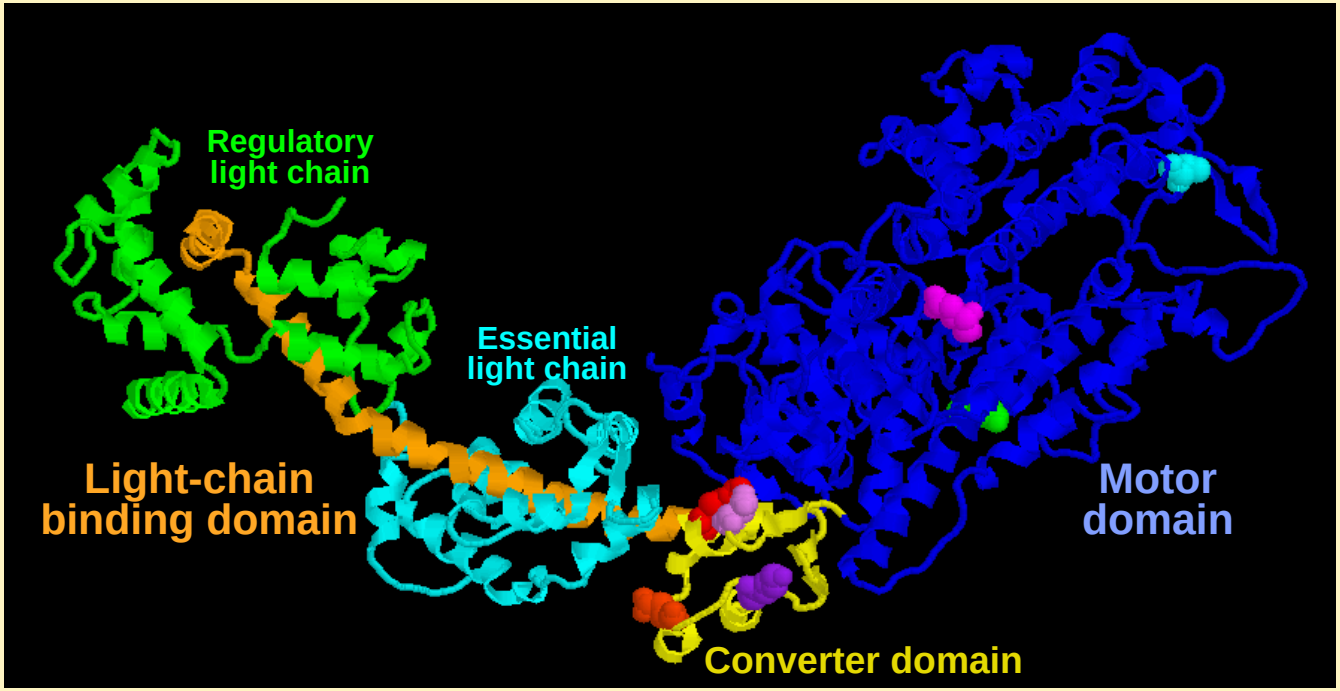
relaxed activated difference patterns



Ochala et al., PNAS 2010

- Point mutation in tropomyosin (R133W) causes charge change
  → movement of tropomyosin over thin filament is impaired as seen from actin layer line intensity changes.

Some FHC-mutations in the myosin head domain

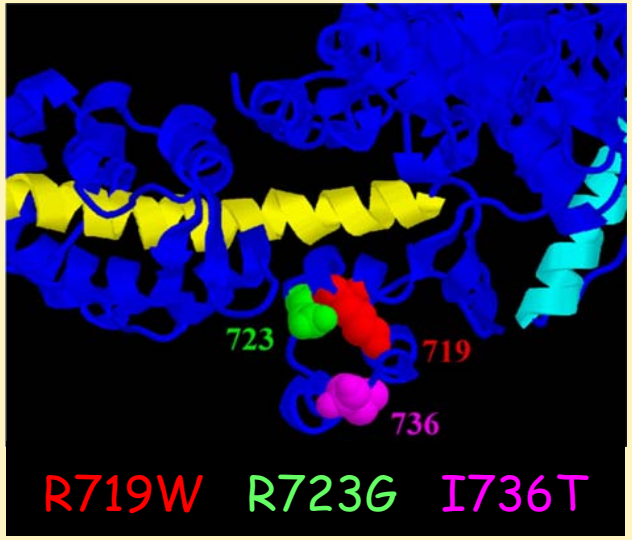


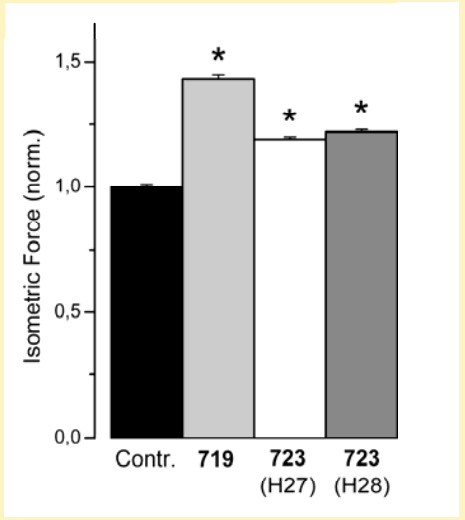
Adapted from Rayment et al., Science 261, 1993

Converter domain: forms an anchoring socket for the long alpha helix of the „lever arm“ (light chain binding domain)

Functional effects of converter domain mutations R723G and R719W?

Functional effects of mutations in the myosin converter domain



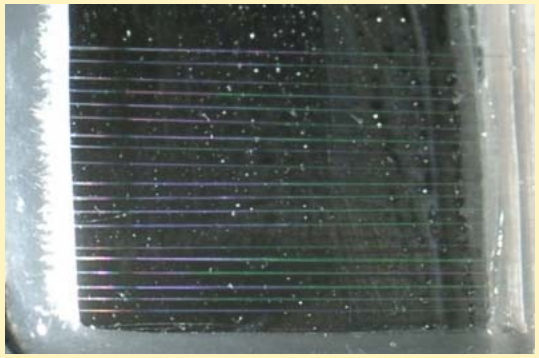


Biomechanical studies:

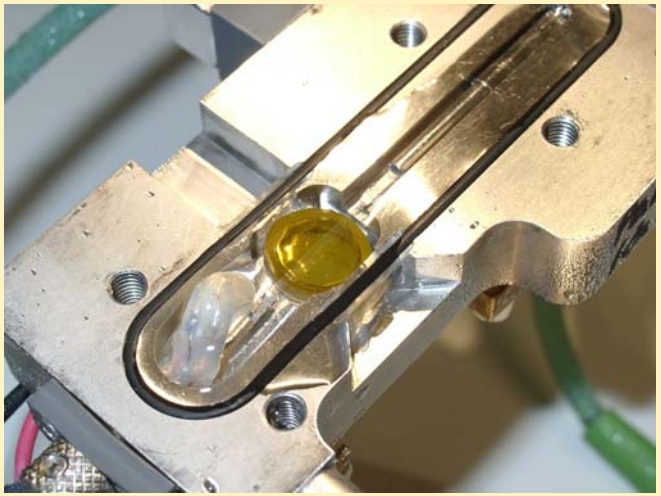
→ Mutations R719W and R723G cause increase in isometric force generation

→ what is the molecular basis for that?

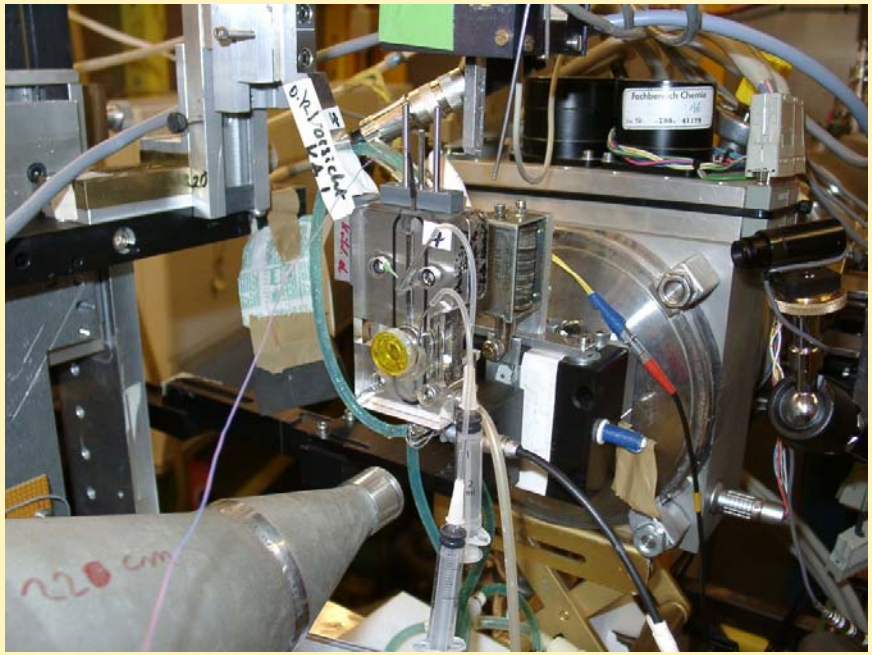
Experimental approach



Isolated single muscle fibers

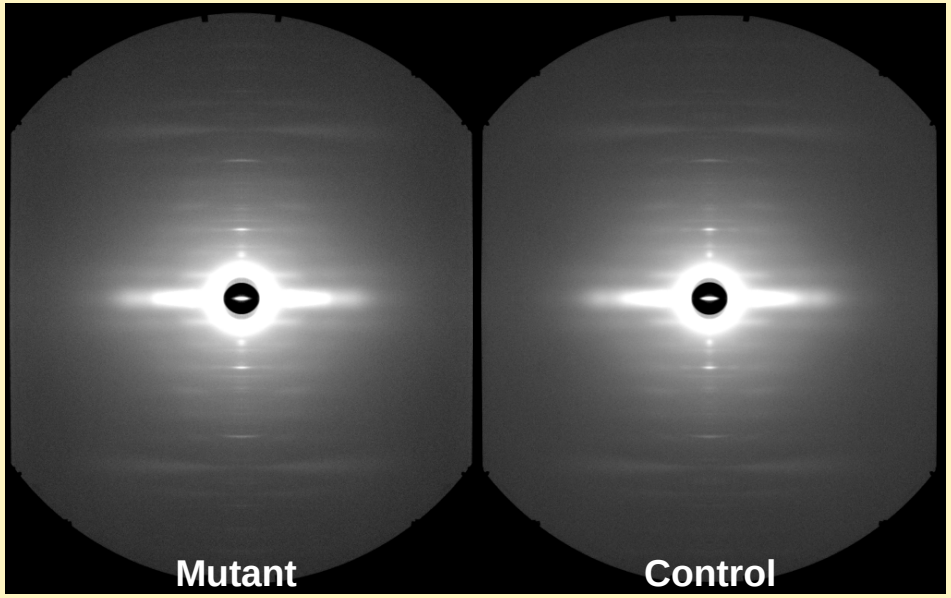


Single muscle fibers mounted into experimental chamber

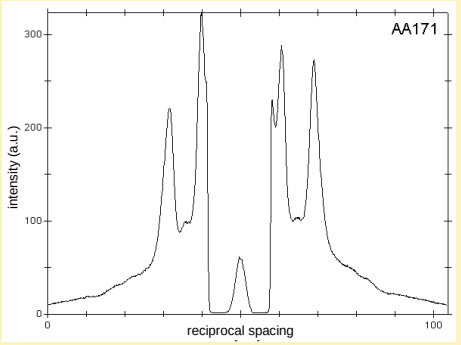


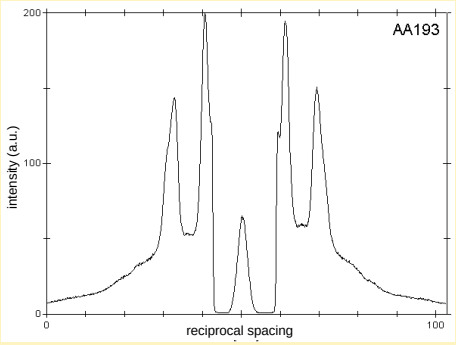
Experimental chamber mounted on beamline A2

Do mutations R723G or R719W affect lattice spacing and/or myosin helical repeat?



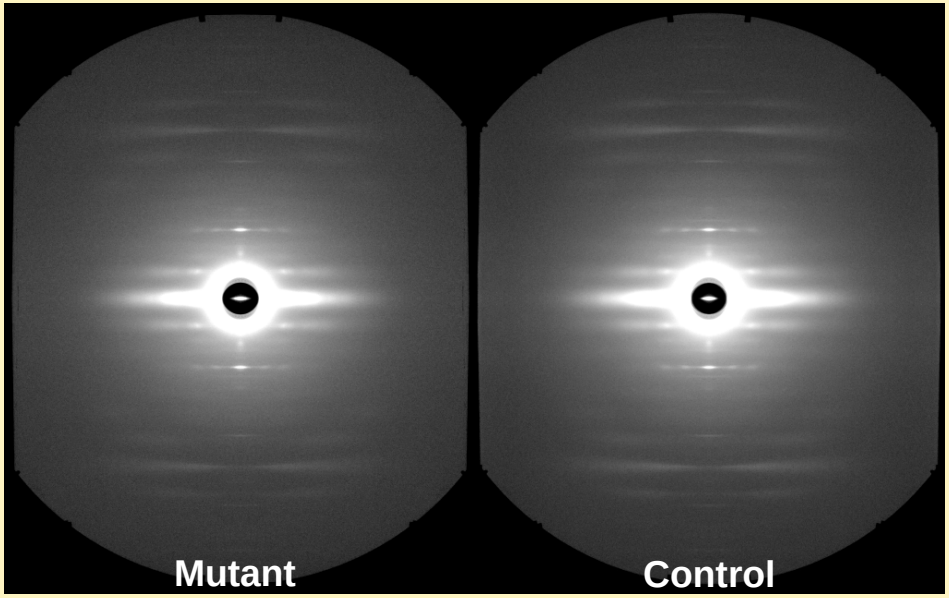
2D-patterns from soleus muscle fibers of patient and control recorded under **relaxing conditions**



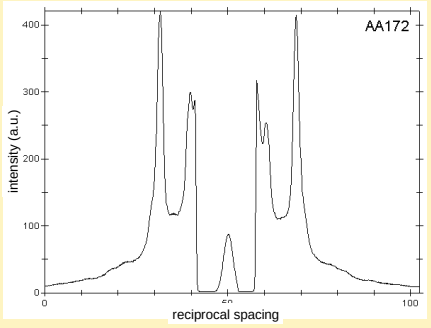


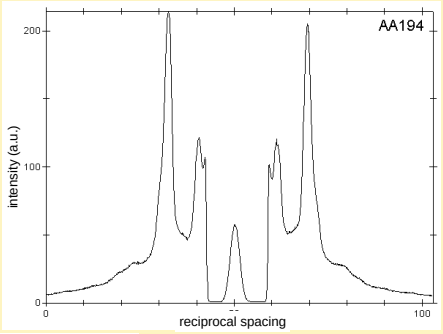
Equatorial profiles

Do mutations R723G or R719W affect lattice spacing and/or myosin helical repeat?



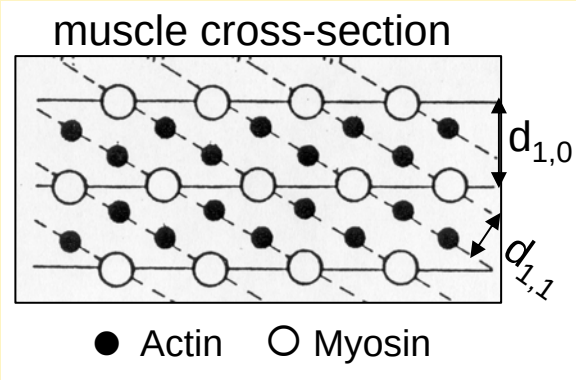
2D-patterns from soleus muscle fibers of patient and control recorded under **rigor conditions**

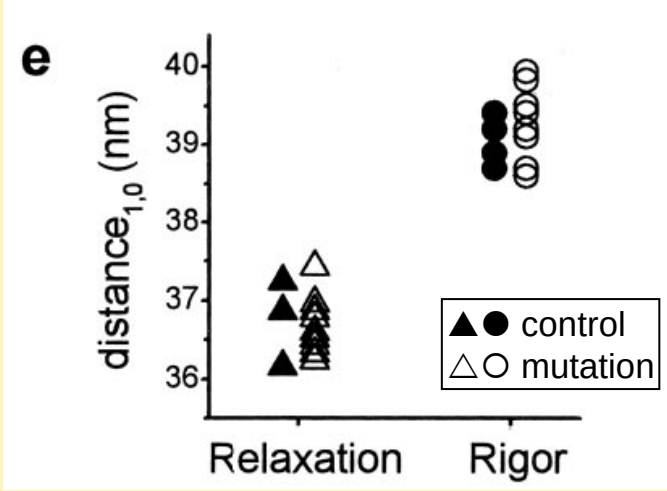




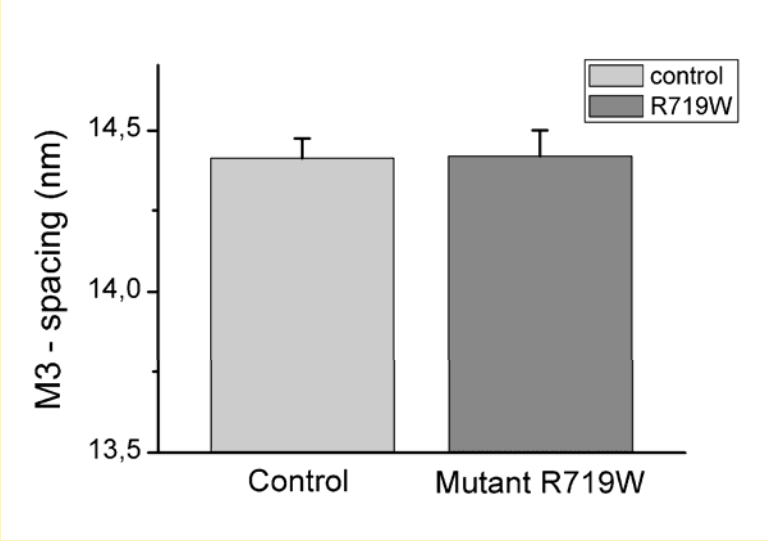
Equatorial profiles

Myosin head domain mutations do not affect myofilament architecture





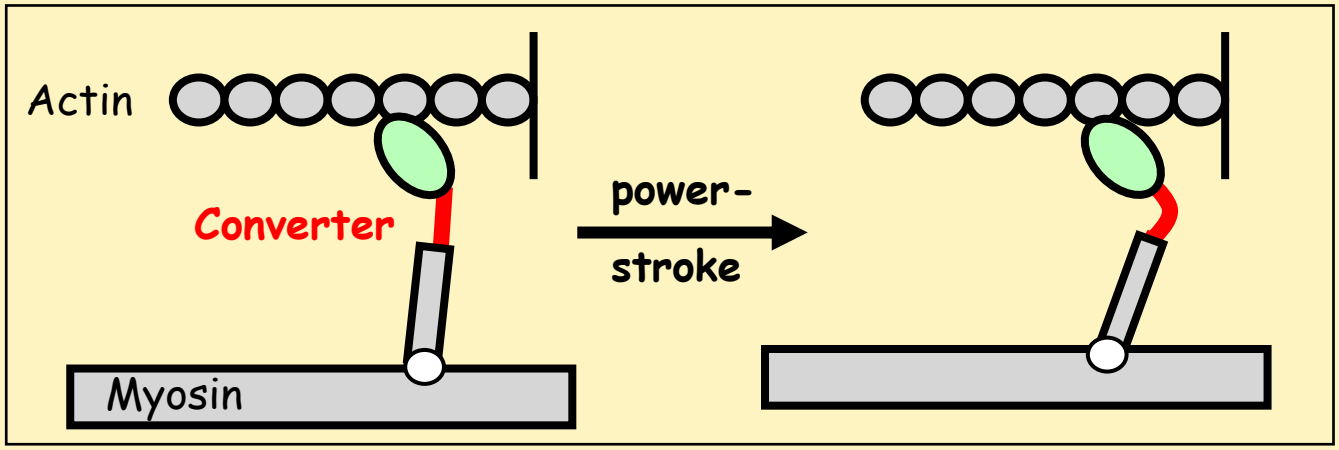
Distance of the 1,0 plane ($d_{1,0}$), calculated from the two innermost equatorial reflections.



Spacing of the M3 meridional reflection (ca. 14.5nm) in rigor. M3 corresponds to the repeat distance between the crowns of myosin heads along the thick filaments.

→ Mutation R719W neither affects lattice spacing nor packing of the myosin filaments.

Mutations R719W and R723G in the myosin converter domain cause higher resistance to elastic distortion



Force generation at the molecular level:

- conformational changes during the power stroke cause elastic distortion of myosin head / converter domain.

Explanation for higher force with mutations?

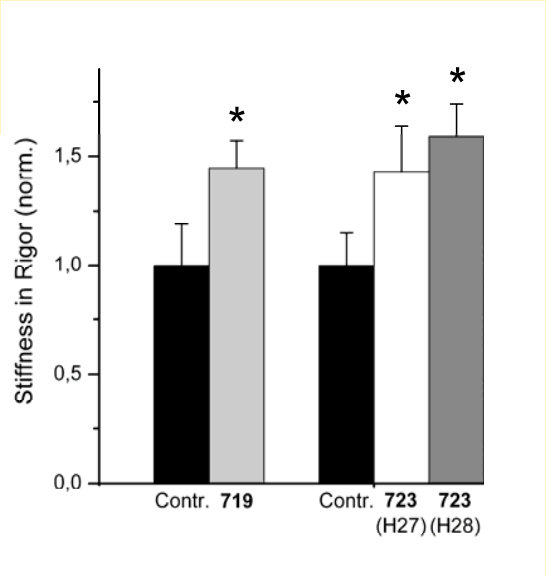
- higher resistance to elastic distortion of converter will therefore result in higher force of myosin head and muscle fiber

Evidence?

Mutations R719W and R723G in the myosin converter domain cause higher resistance to elastic distortion

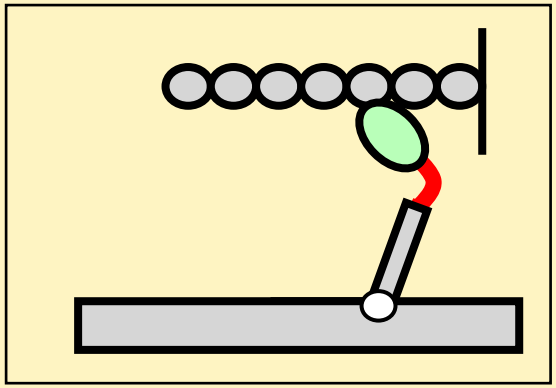
Evidence comes from measurements of fiber stiffness (resistance to elastic distortion)

→ stiffness of individual mutated myosin heads is increased (2-3fold)

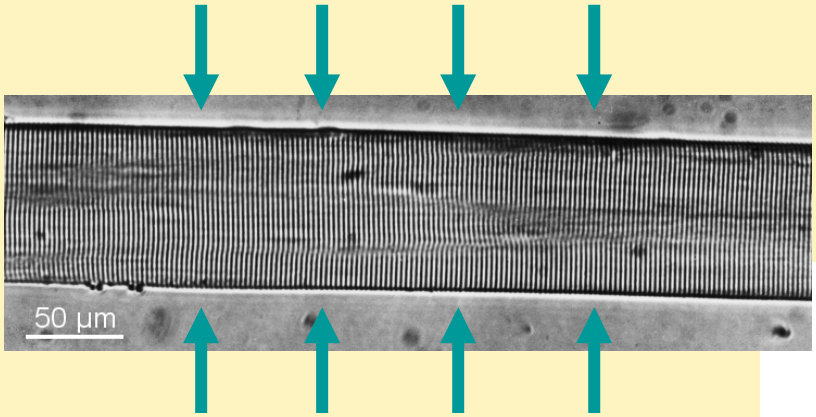


Can we test this conclusion?

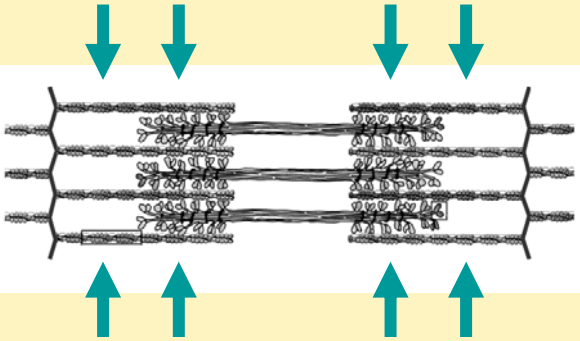
- Do the mutations also affect the compliance of the myosin heads in **radial** direction?

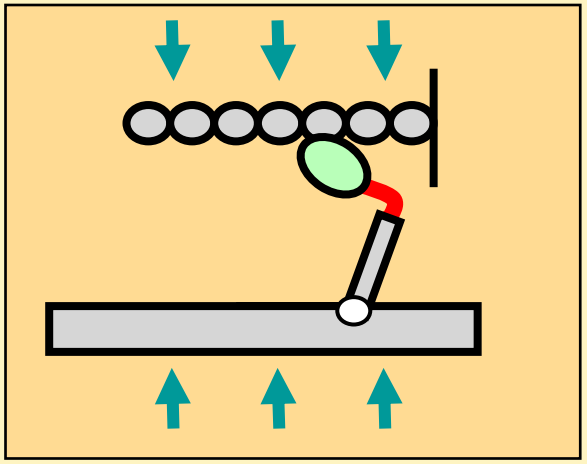


Approach to measure resistance to radial distortion of the myosin heads



Osmotic pressure

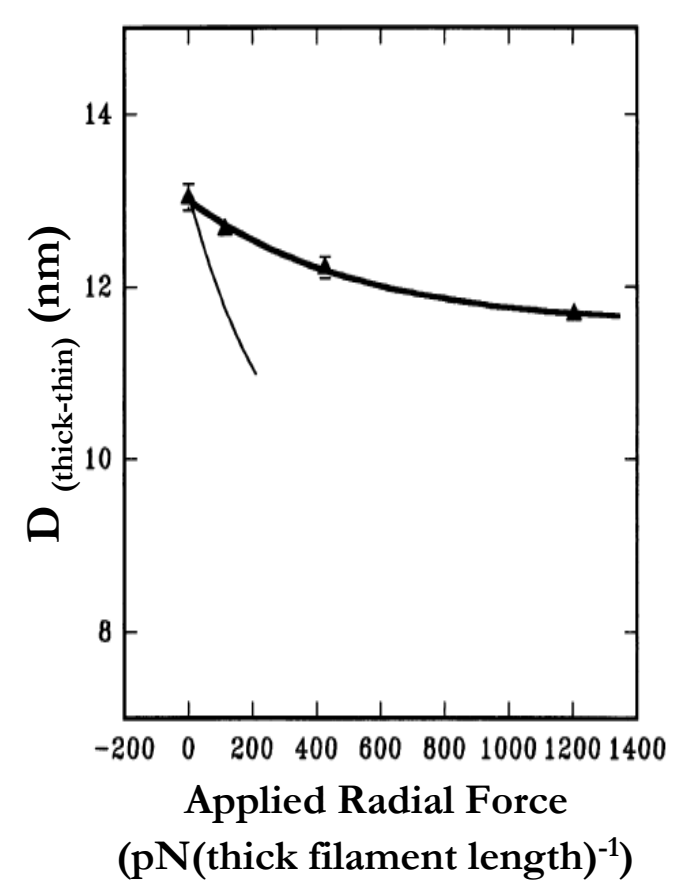




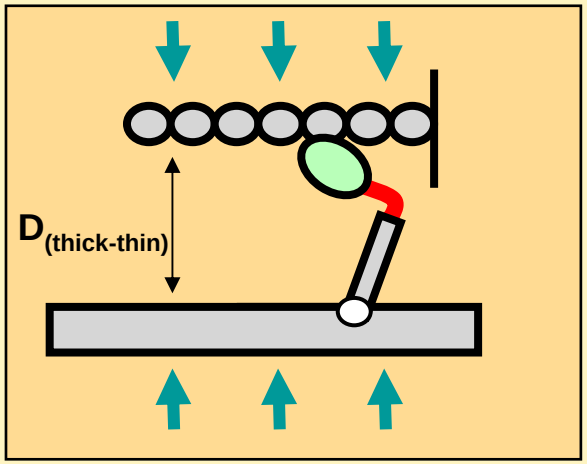
Test:

- By osmotic compression using high MW dextran solution → radial force is applied
- Lattice spacing is determined precisely by fiber diffraction

Approach to measure resistance to radial distortion of the myosin heads



Radial compression in rigor.
Thin line indicates compression without attached myosin heads.



Spacings of equatorial reflections allow to calculate the distance between actin and myosin filament surface ($D_{(thick-thin)}$)

→ From $D_{(thick-thin)}$ vs. radial force we can derive the radial stiffness of myosin heads.

Summary:

Synchrotron radiation provides **important, unique contributions** for functional characterization of cardiomyopathy related mutations

and

studies on disease related mutations provide valuable insight into the function of the respective protein

e.g., assessment of the function of the myosin head domain at molecular level (biomechanical measurements, X-ray diffraction, protein crystallography)

to understand

→ pathomechanisms of the disease

→ molecular basis of protein compliance

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