Molecular Machinery

Myosin Motor Protein

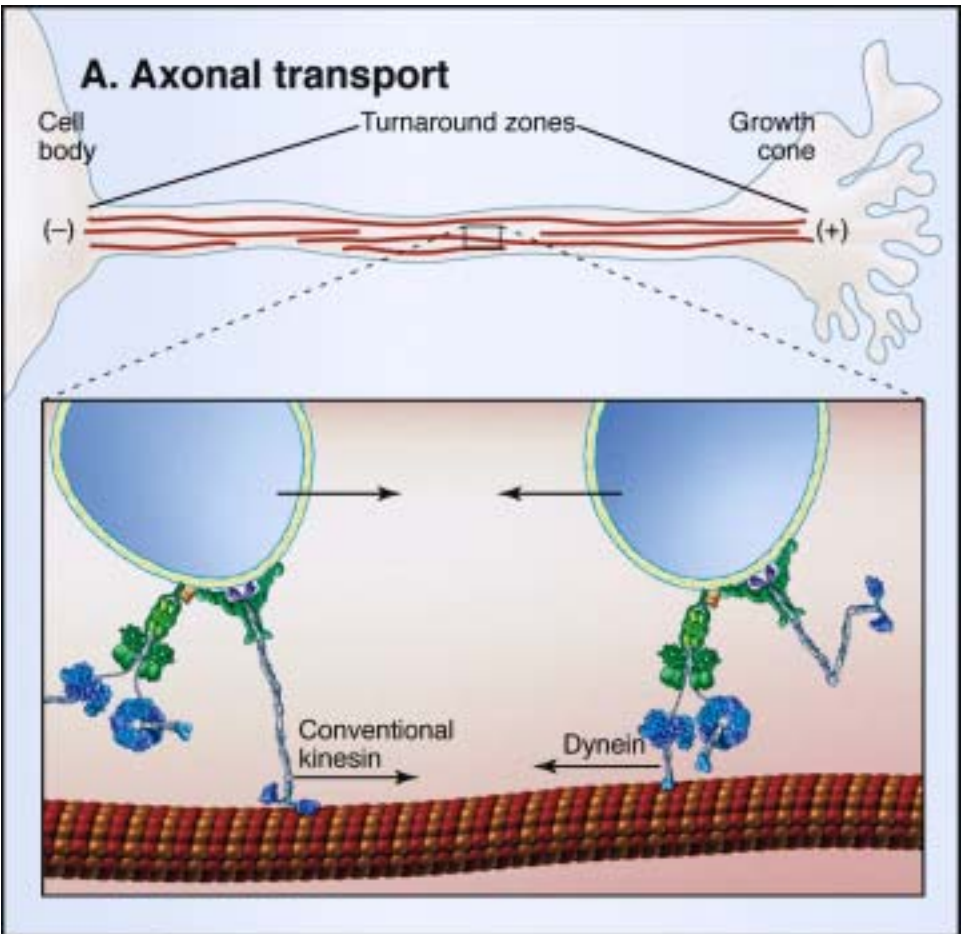
Motor proteins

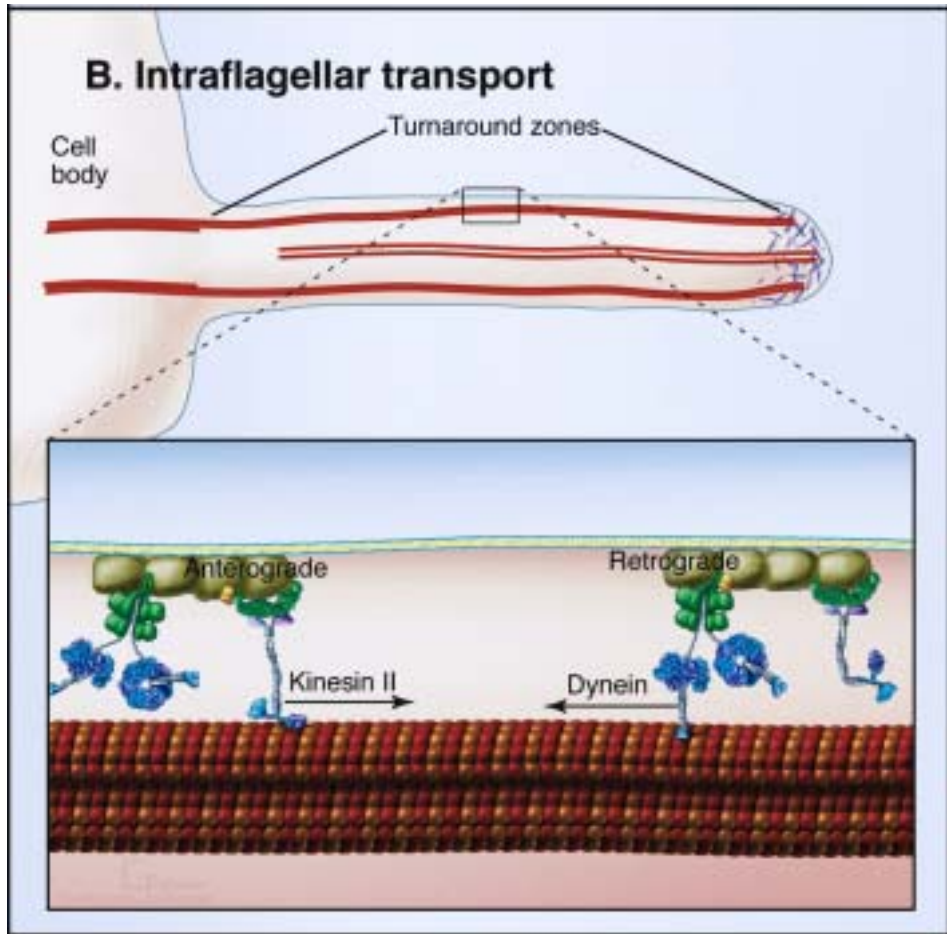
- Motor proteins
  - Derive energy from ATP hydrolysis
  - Bind reversibly to filament tracks
  - Move along filament unidirectionally
- Linear, directed movement
  - Myosin on actin filaments
  - Kinesin and dynein on microtubule filaments
- Circular motion
  - F_0F_1/ATPase

Microtubule motors

- Kinesin and dynein

Vale, 2003 Cell 112, 467-480.

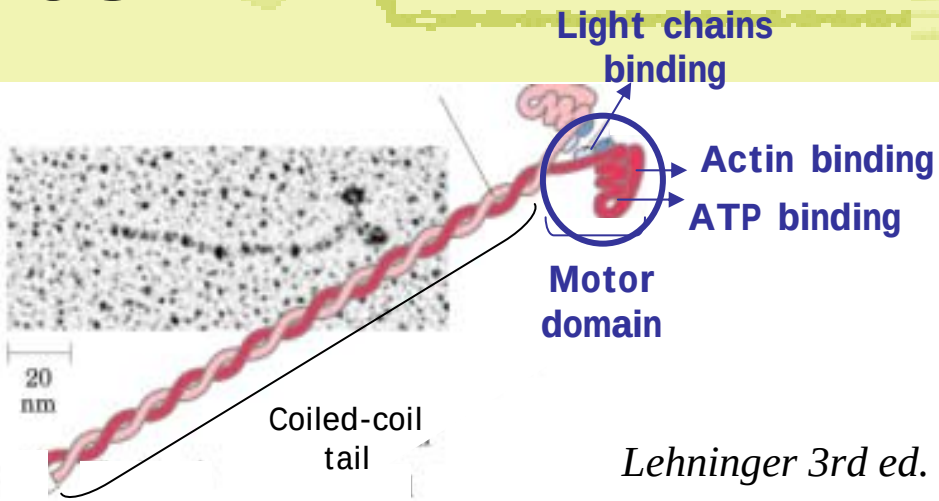




Content

- What is myosin ?
  - Basic structural elements
    - Related motor protein: kinesin (also dynein ?)
- Conventional myosin
  - Myosin II
- Unconventional myosin
  - The “lever-arm” theory and the debate
  - Myosin I
  - Myosin V and Myosin VI
    - Processive motor
    - The “forward” and “reverse” gear
    - Cargo loading and unloading

“Myosin”



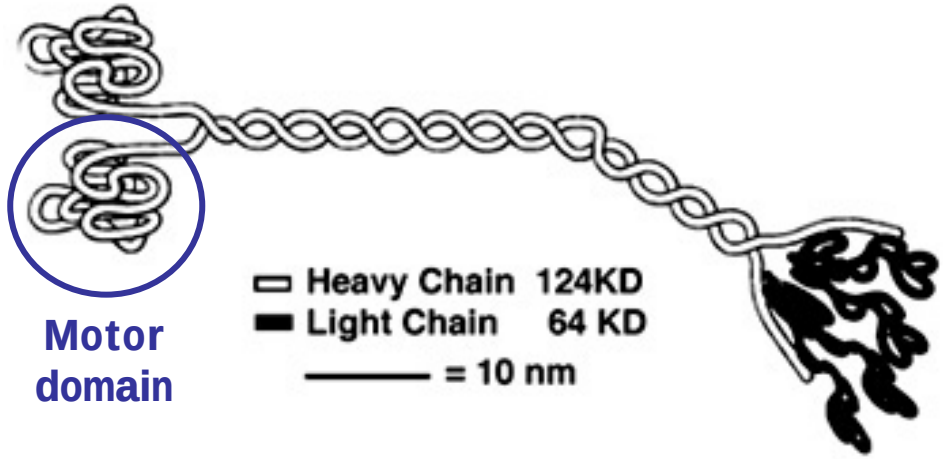
Lehninger 3rd ed.

- Myosin
  - Bind to actin
  - ATPase
  - Generate force and movement along actin filaments

- A superfamily of molecular motors
  - Present in probably all eukaryotic cells
    - A motor/catalytic (head) domain
      - Highly conserved
    - A light chain binding region (the neck region)
      - IQ motif for CaM-like light chain binding
    - A tail region (highly variable)

http://www.proweb.org/kinesin//index.html

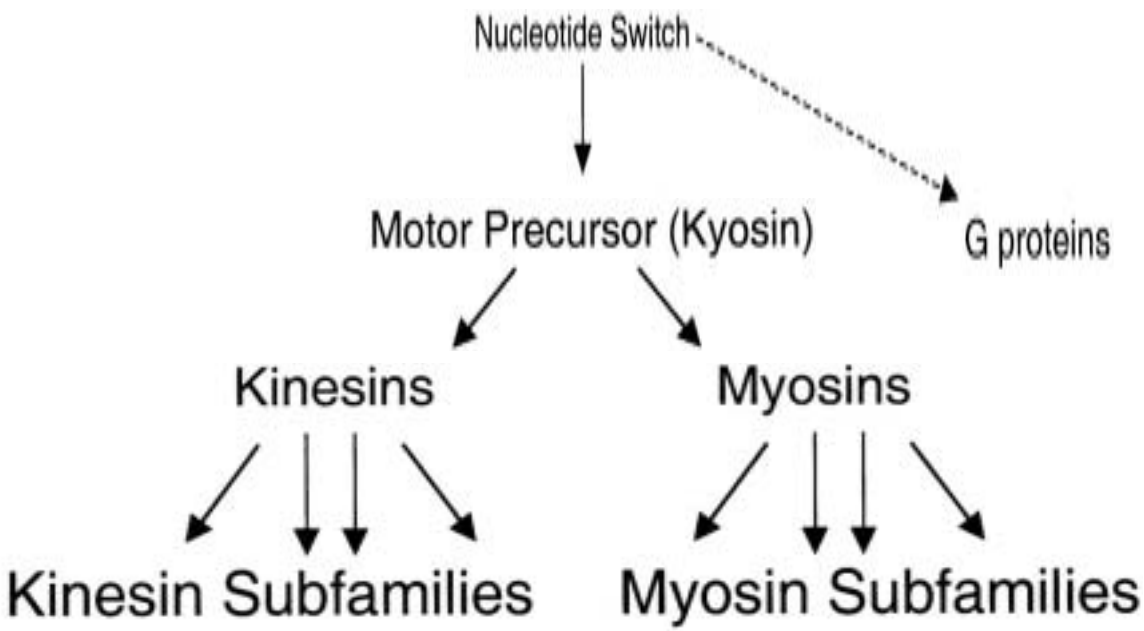
Kinesins



- Kinesin
  - Associate with microtubules
  - Hydrolyze ATP
  - Produce force and movement along microtubules
- A superfamily of molecular motor proteins
  - Found in most if not all eukaryotic cells
    - Mitosis and meiosis
    - Long range vesicle transport

Evolution of Motor Proteins

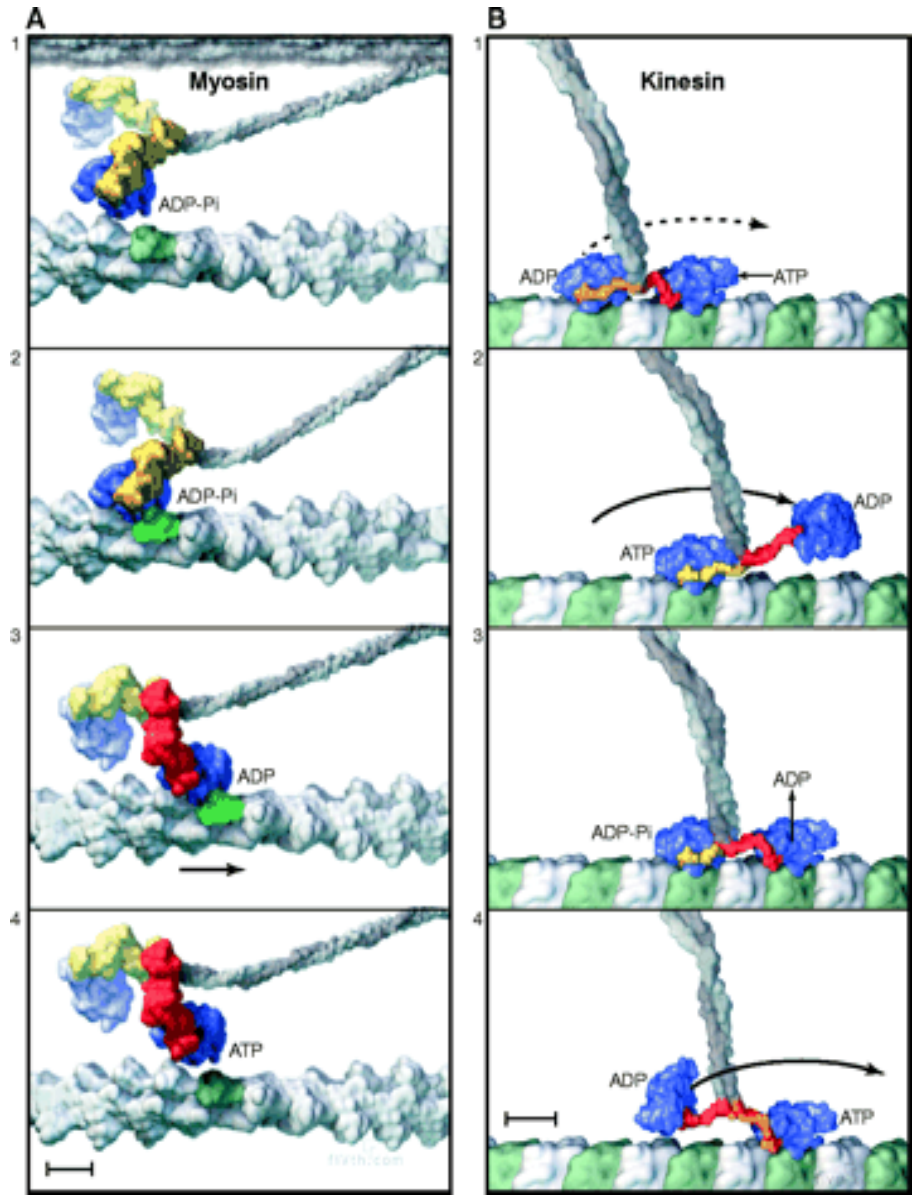
- Structural homology in the motor (catalytic) domain



Vale and Milligan 2000. Science 288, 88.

Are two heads better than one ?

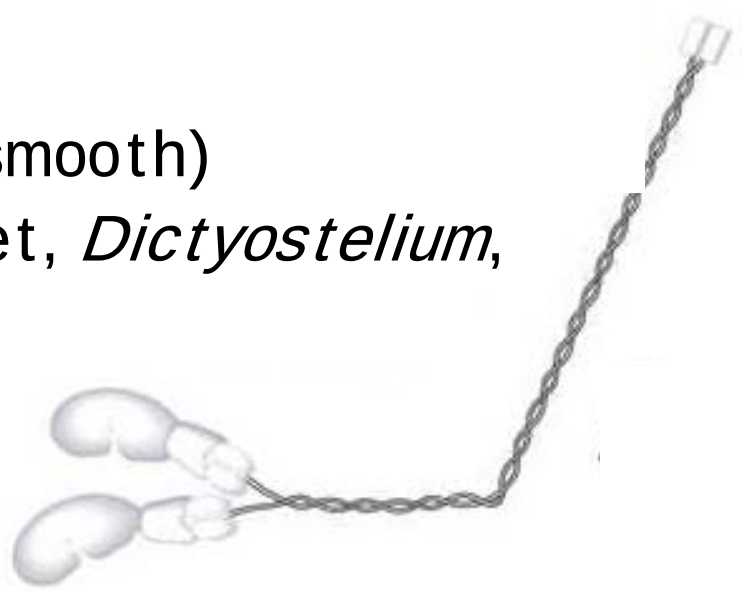
- Myosin vs. Kinesin
  - Myosin:
    - Why two heads?
  - Kinesin:
    - Processive motor
      - Hand-over-hand
      - Stepping stones model

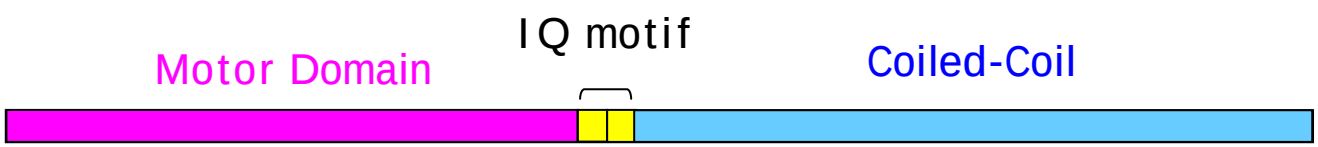


Vale and Milligan 2000. Science 288, 88.

Myosin II

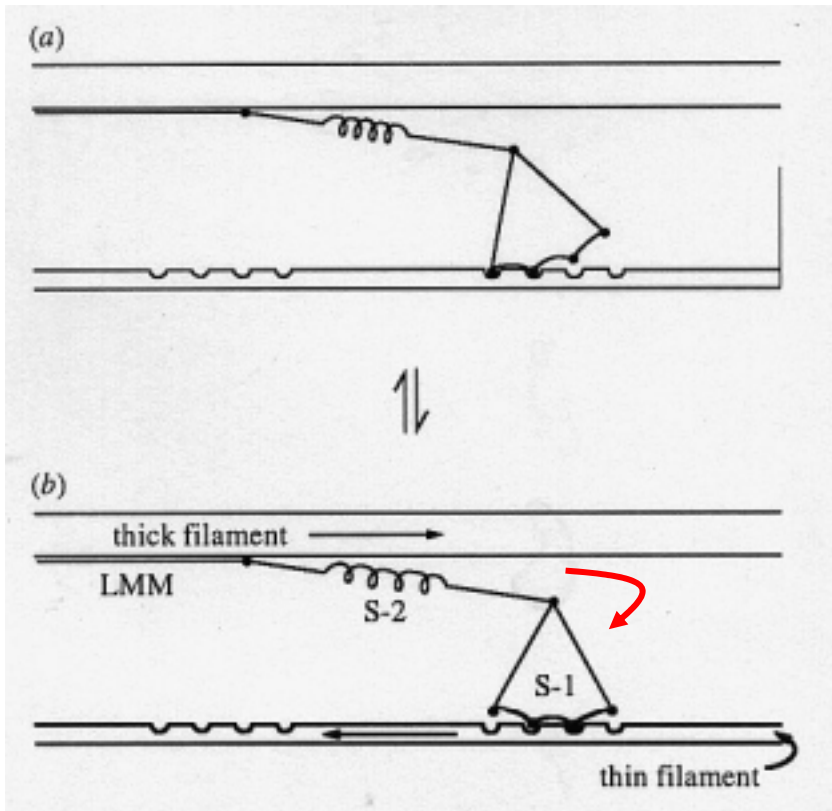
- Conventional myosin
  - Muscle (skeletal/cardiac, smooth)
  - Non-muscle myosin (platelet, *Dictyostelium*, A*canthamoeba*)
- 3 functional domain:
  - A motor domain (head)
  - A neck region (lever arm)
  - A tail region (coiled-coil to form dimmer)





How myosins move ?

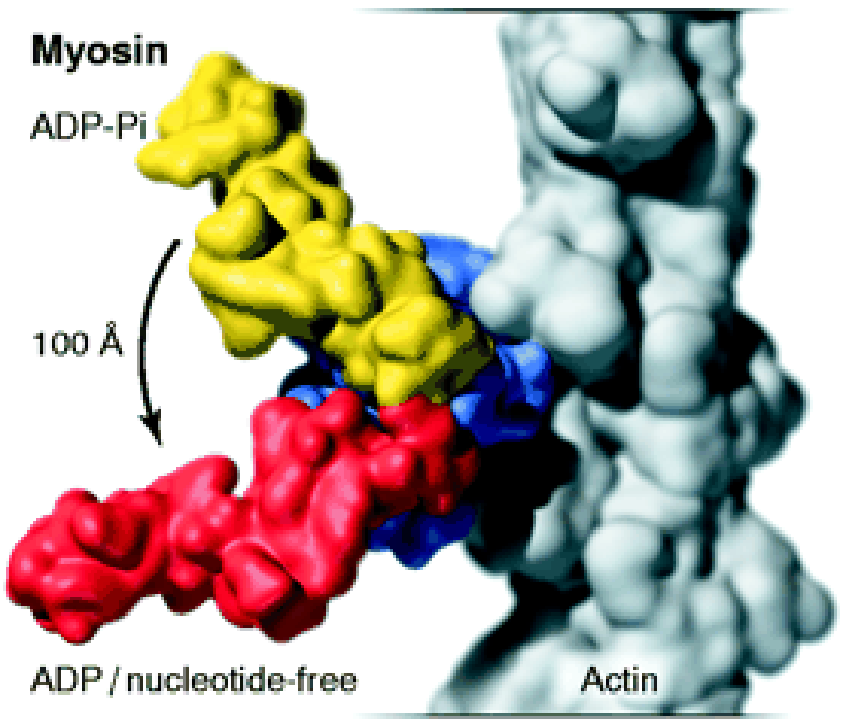
- 1971, A.F. Huxley and Simmons
  - "Tilting crossbridge model"
- 1993, Rayment et al.
  - "Lever-arm model"

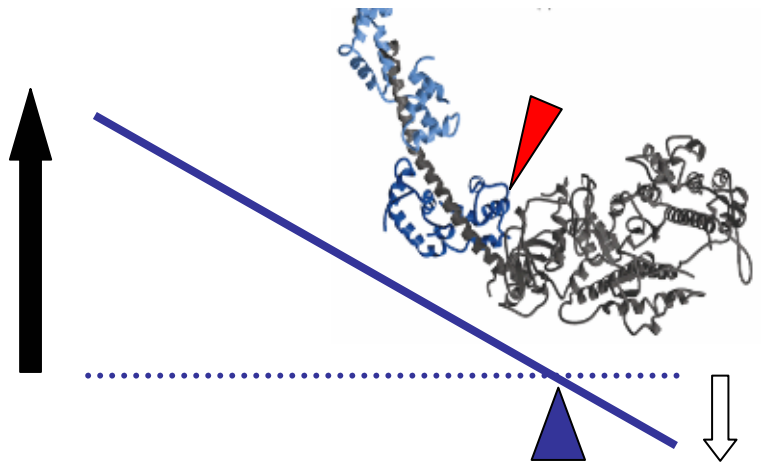


A.F. Huxley 2000. Phil. Trans. R. Soc. Lond. B. 355, 433.

The lever-arm model

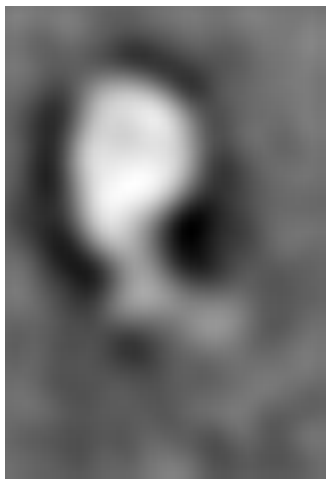
- Displacement $\propto$ lever-arm length





Vale and Milligan 2000. Science 288, 88.

Flexibility within myosin head

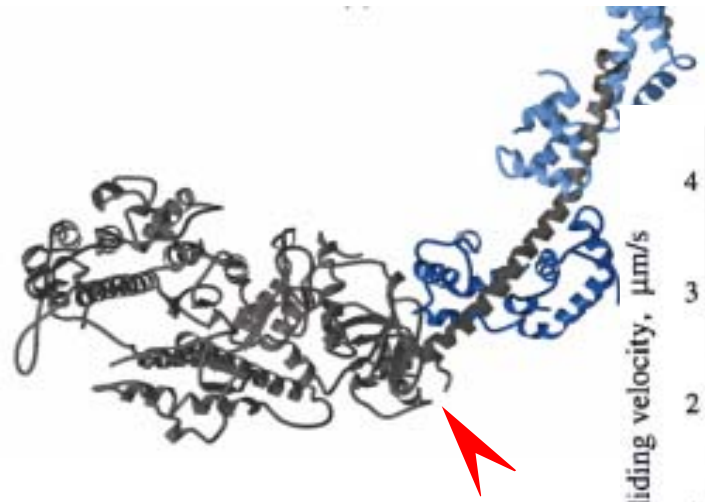


Burgess et al., 1997, J. Cell Biol. 139, 675.
http://www.leeds.ac.uk/chb/muscle/myosinhead.html

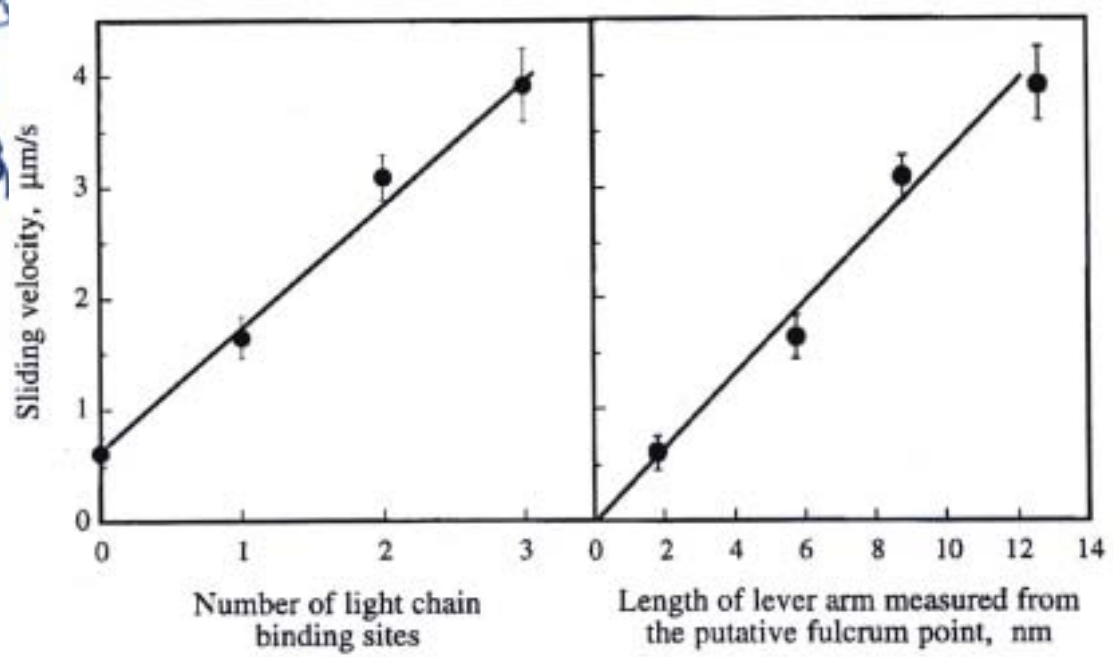
- Revealed by negative stain and single-particle analysis.
- Using digital image processing to combine 2400 images of myosin heads from **EM**.

Support for lever-arm theory

- *Dictyostelium* myosin
  - 0 IQ, 1 IQ, 2 IQ (wt), 3 IQ
  - Sliding actin filament *in vitro* motility assay

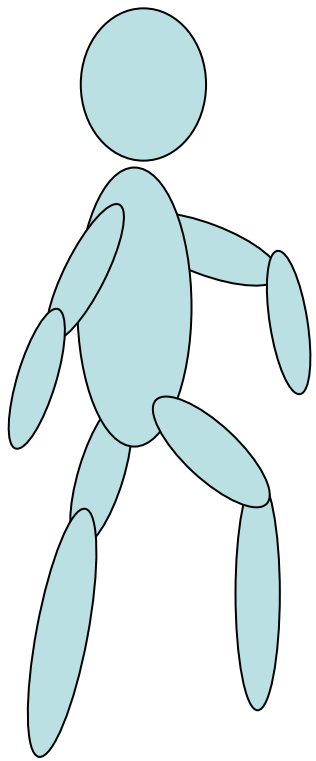


Uyeda et al., 1996. PNAS 93, 4459.



- The neck region acts as a lever arm

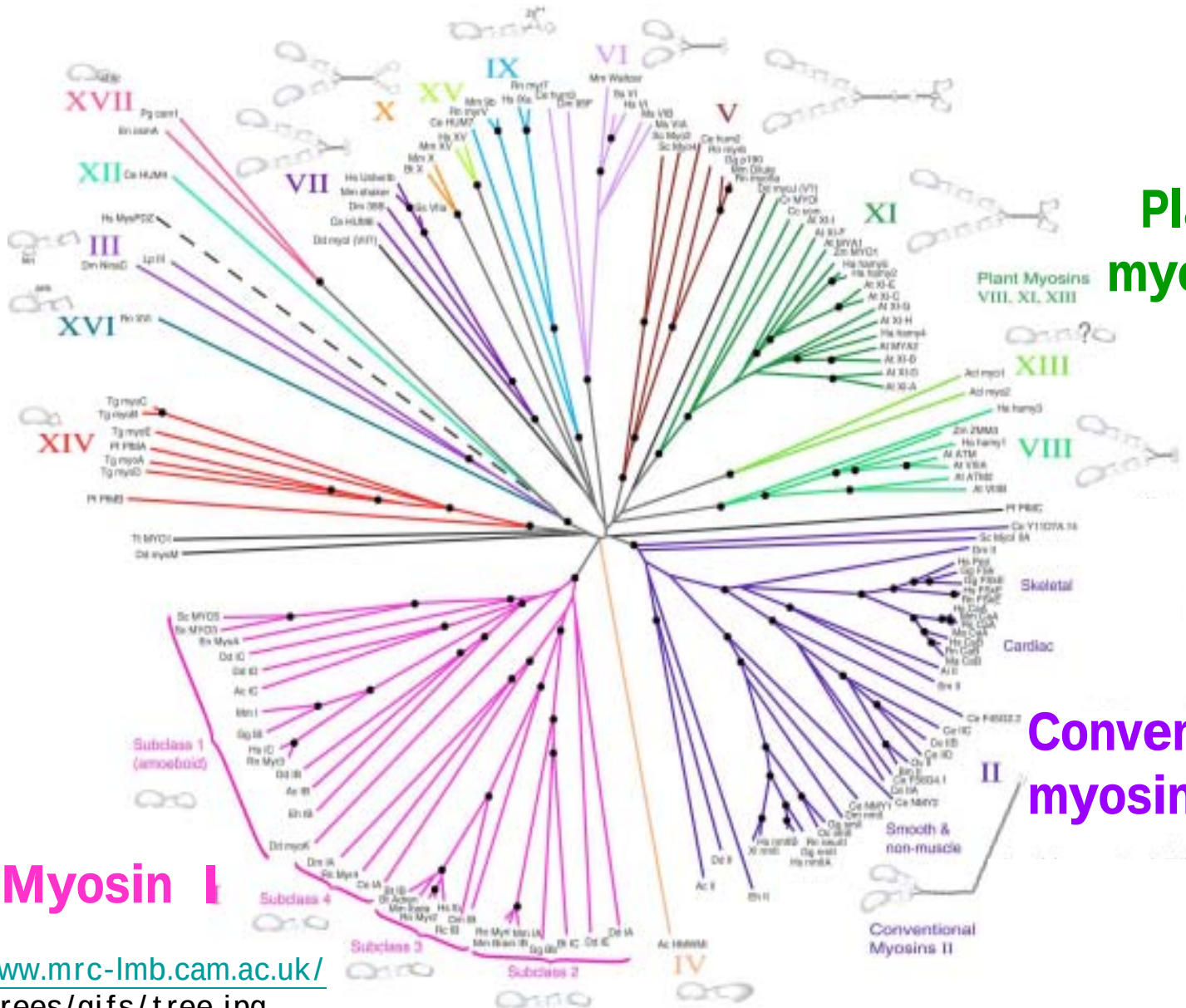
Myosin motors



- Unconventional

- Conventional

Phylogenetic tree of myosins



Plant myosins

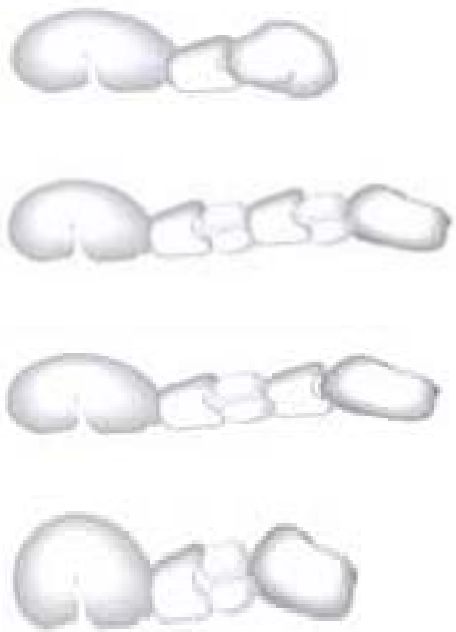
Conventional myosin II

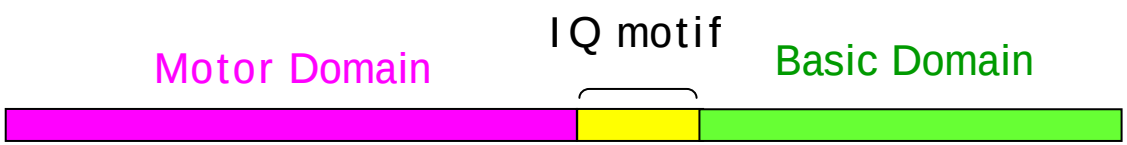
Myosin I

http://www.mrc-lmb.cam.ac.uk/myosin/trees/gifs/tree.jpg

Myosin I

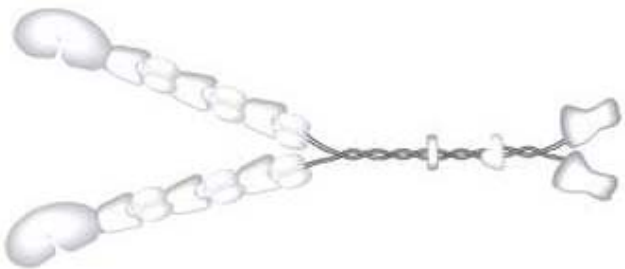
- Discovered by Thomas D. Pollard in 1973
  - From Amoeba
  - Based on ATPase assay
    - K-EDTA ATPase activity
    - Actin-activated ATPase activity
- 1st unconventional myosin
  - Monomer (only one head)

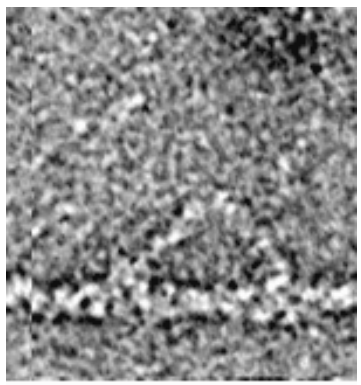




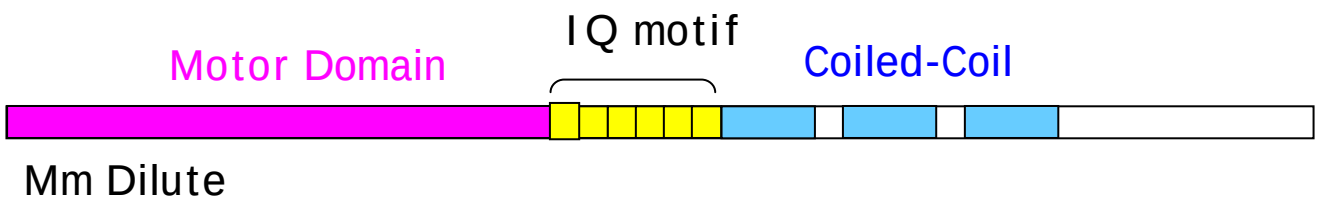
Myosin V

- Mouse: *dilute*
  - Coat color loss
    - Melanosome transport
- Yeast: Myo2p
  - Vacuolar inheritance
- A processive motor



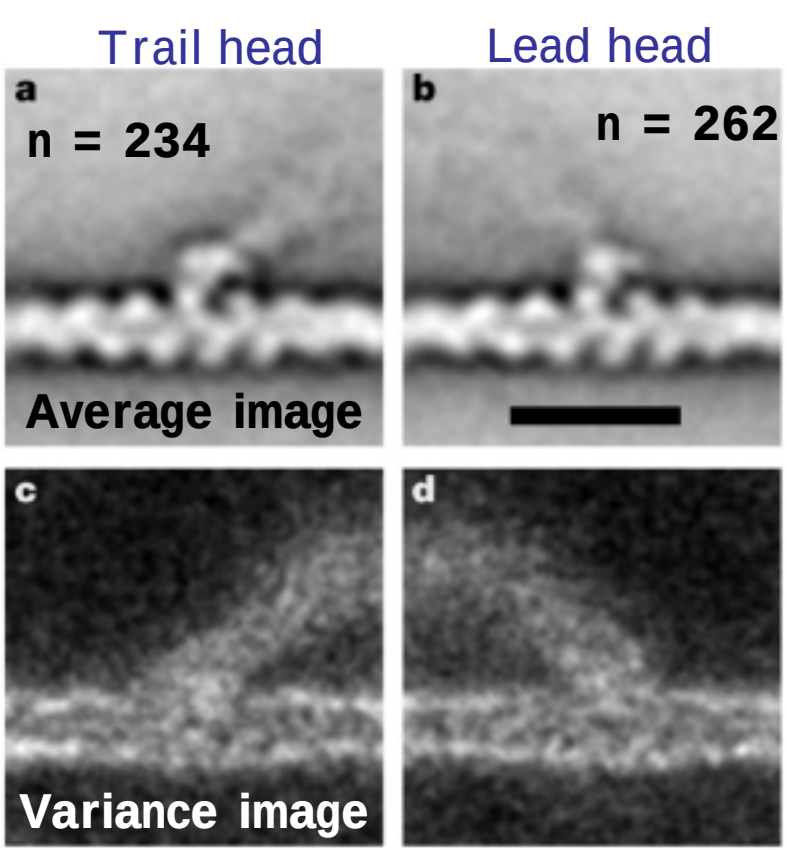


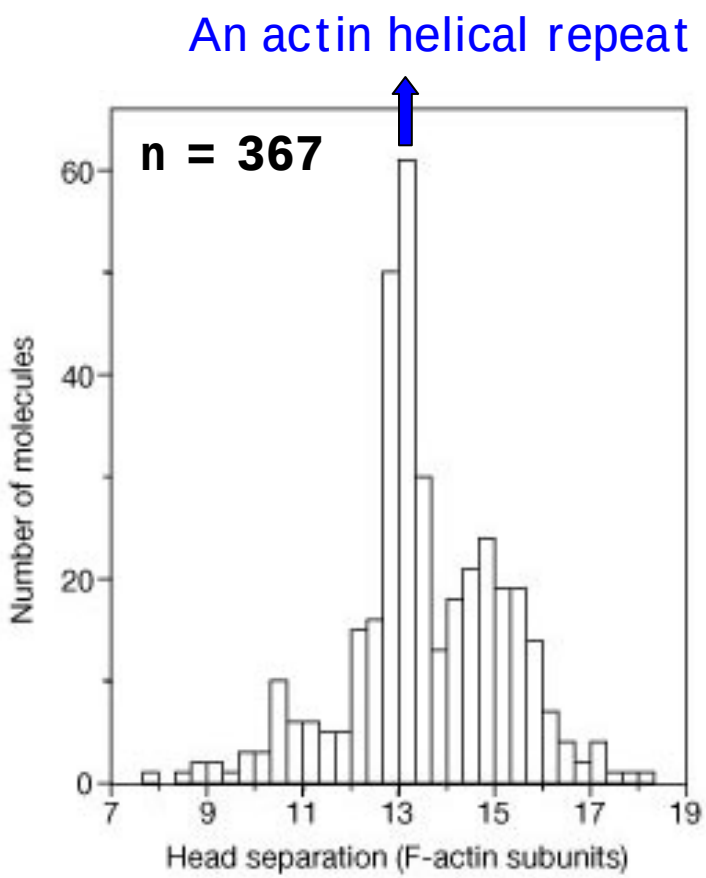
*Walker et al., 2000. Nature, **405**, 804.*



Myosin V

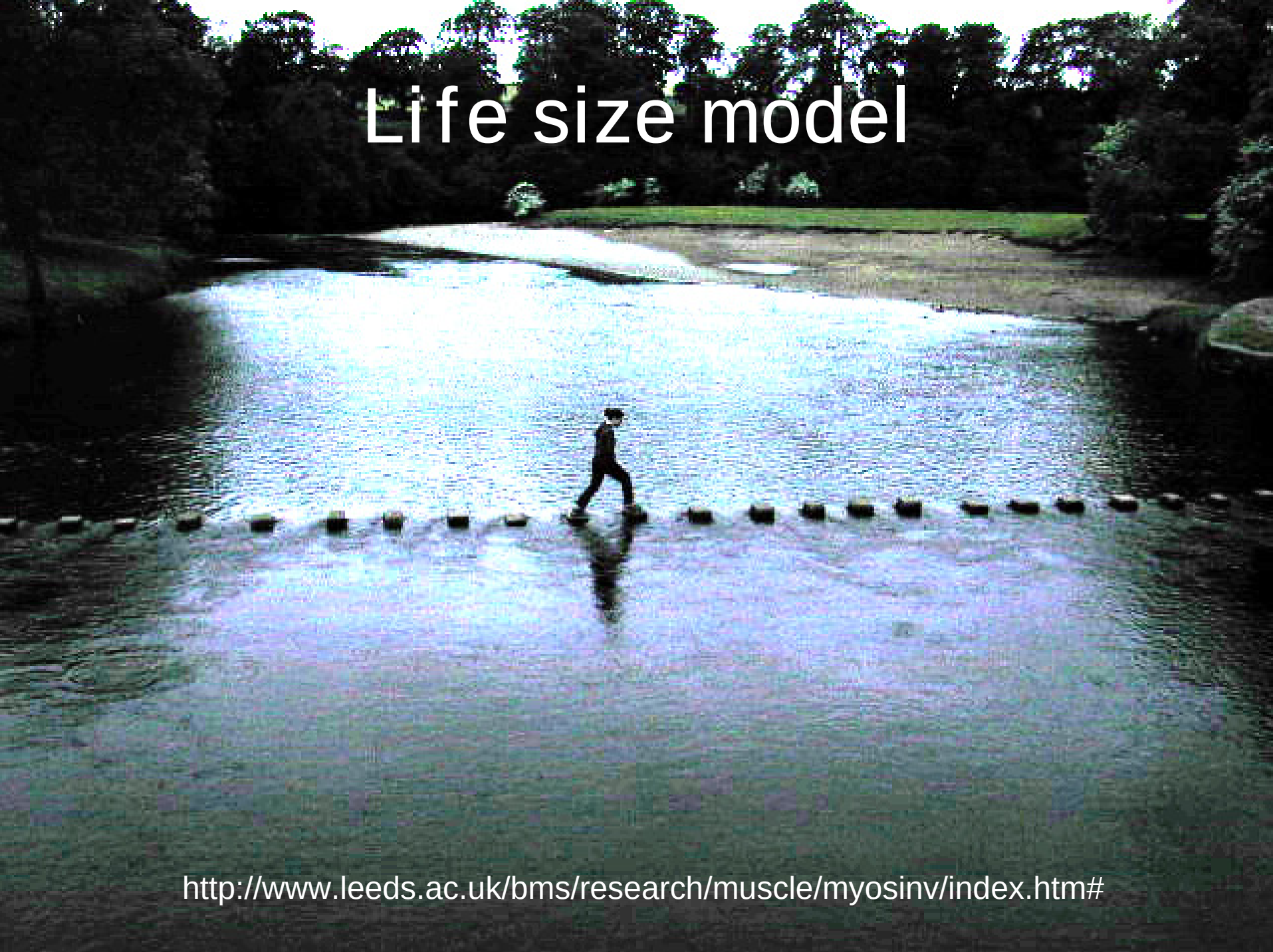
- A processive motor with a large step
  - 36 nm vs. 5 nm for mysoin II





*Walker et al., 2000. Nature, **405**, 804.*

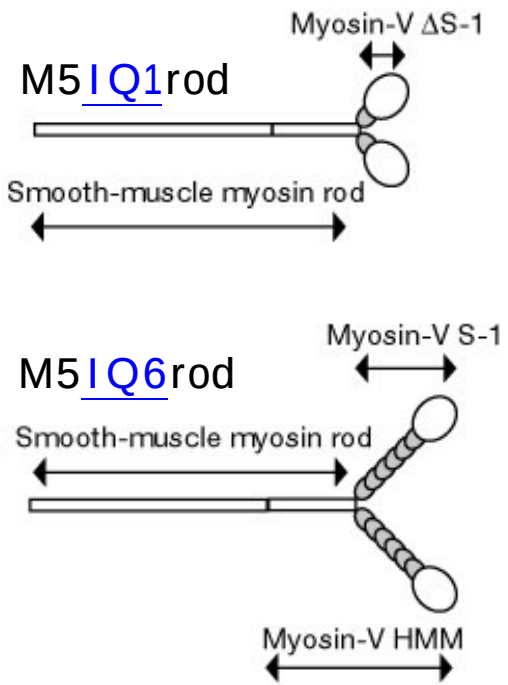
Life size model

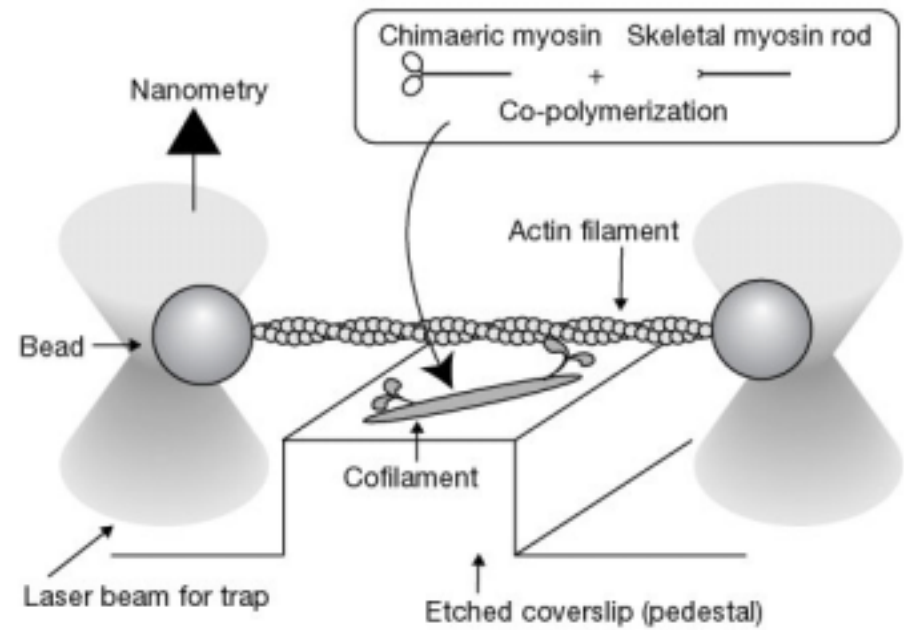


http://www.leeds.ac.uk/bms/research/muscle/myosinv/index.htm#

Test the lever-arm model (I)

- Measure displacement of single-molecule
  - Myosin V: 6 light chains
  - Short vs. long lever arm

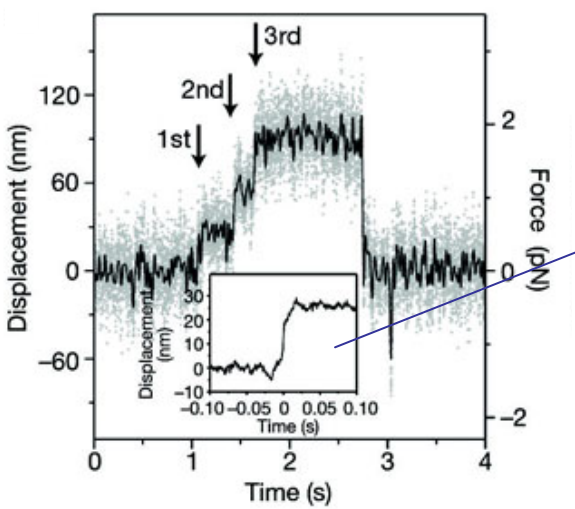




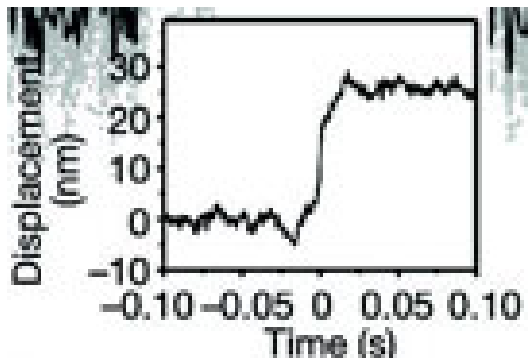
Tanaka et al., 2002. Nature 415, 192.

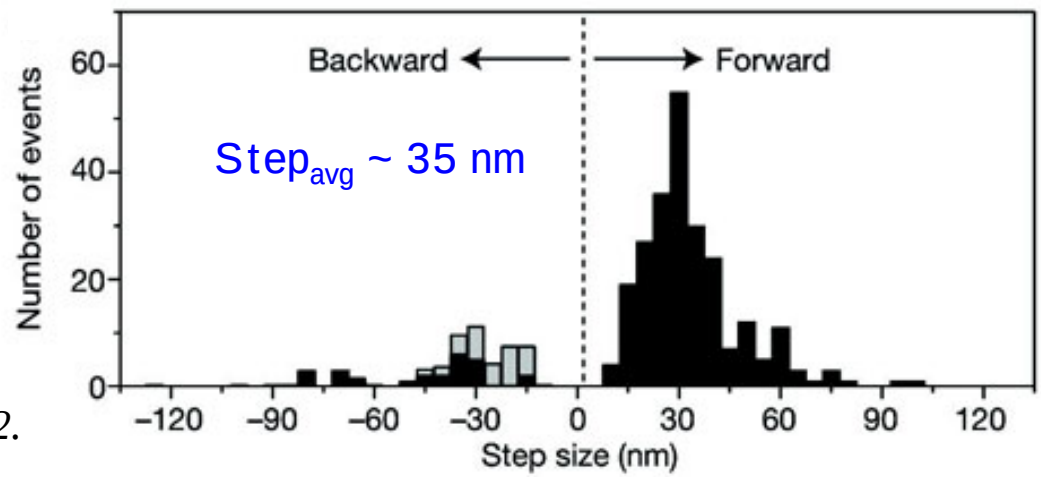
Test the lever-arm model (II)

- Displacement by single M5IQ1rod



Gray dot: raw data
Black line: low-pass filtered



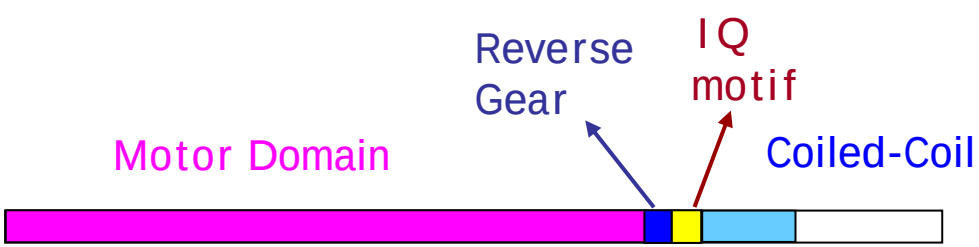


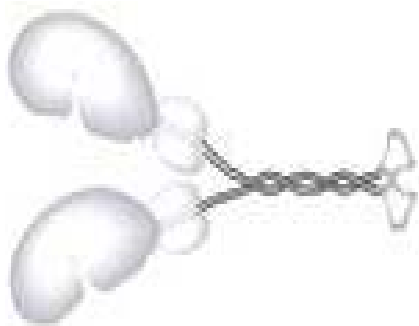
Tanaka et al., 2002. Nature 415, 192.

- The long neck is not essential for the large step.

Myosin VI

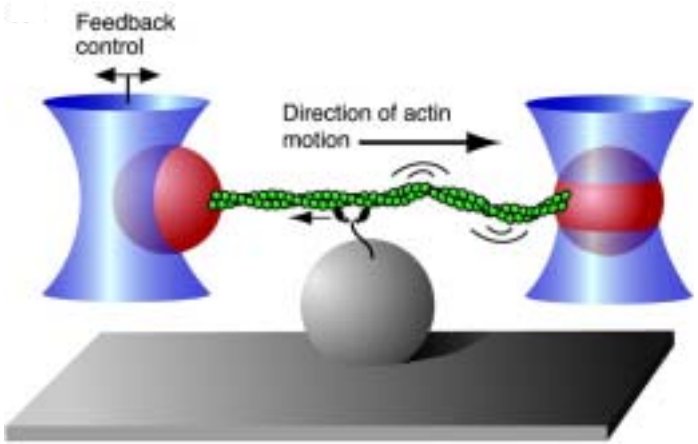
- Mouse, *Drosophila, C. elegans*
  - Hearing, cell migration, embryo development and spermatogenesis
- Reverse motor
  - Move towards the minus end of actin filament
  - Myosin IX
- Processive

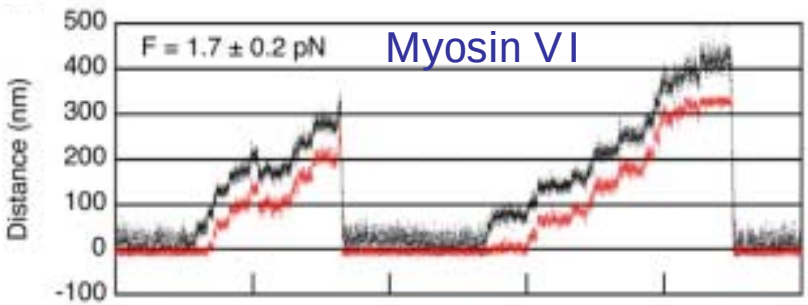




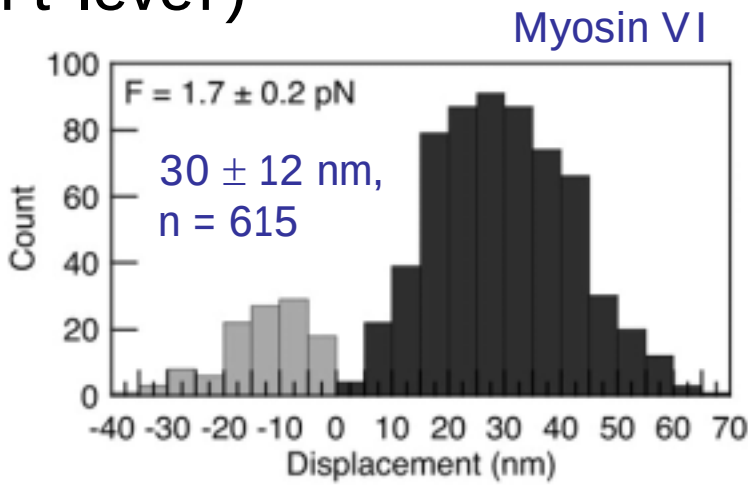
Myosin VI step size ?

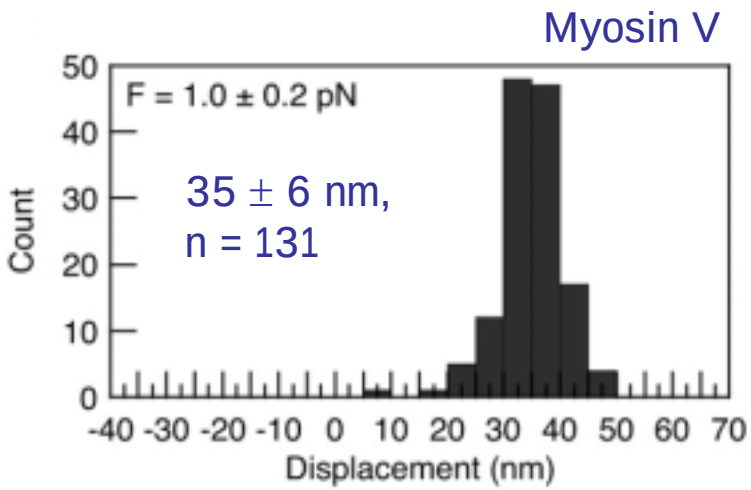
- Myosin V: 6 light chains (long lever)
- Myosin VI: 1 light chain (short lever)





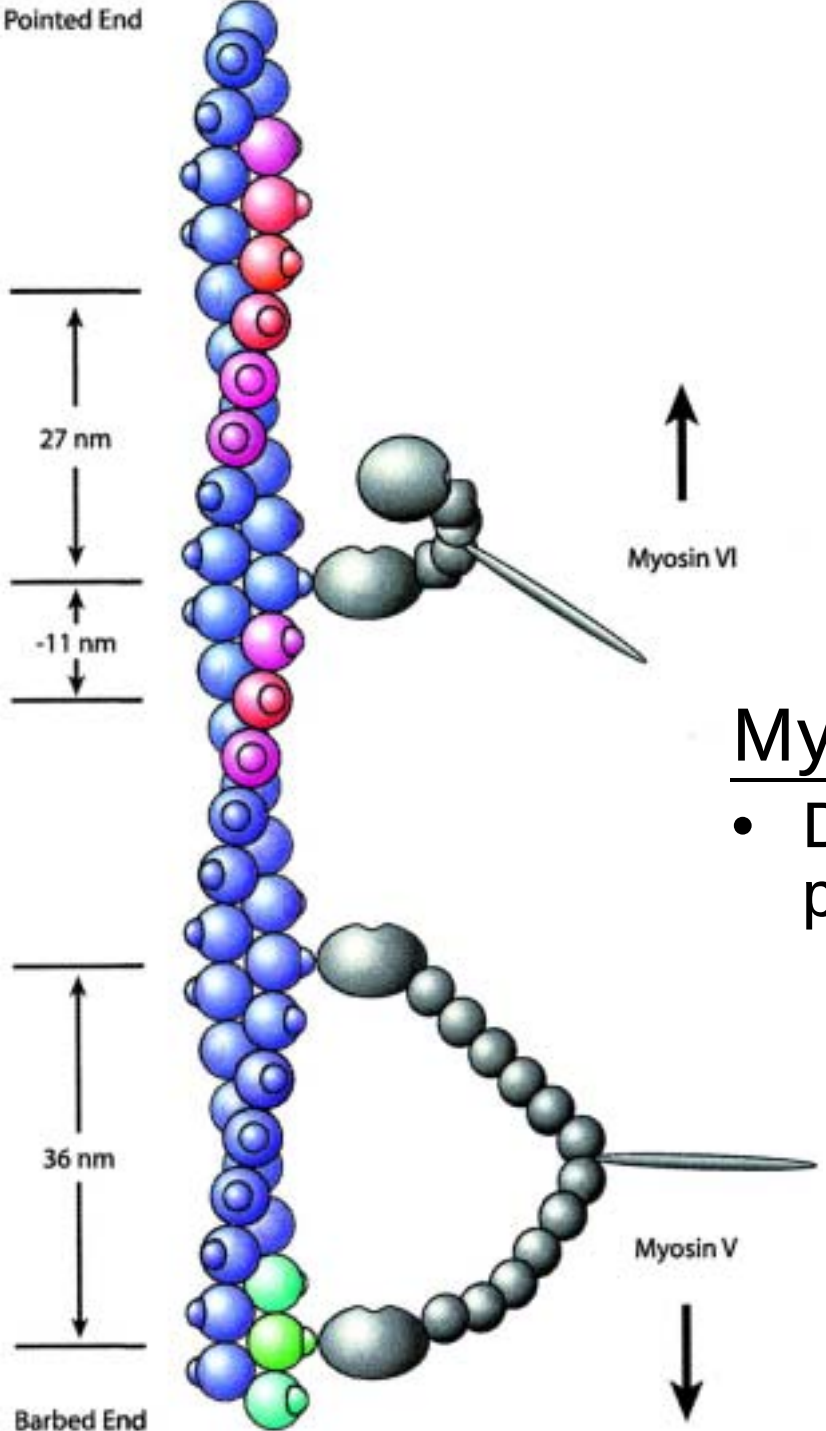
Rock et al., 2001. PNAS 98, 13655.





- Myosin VI takes unexpected large step.

Stepping model

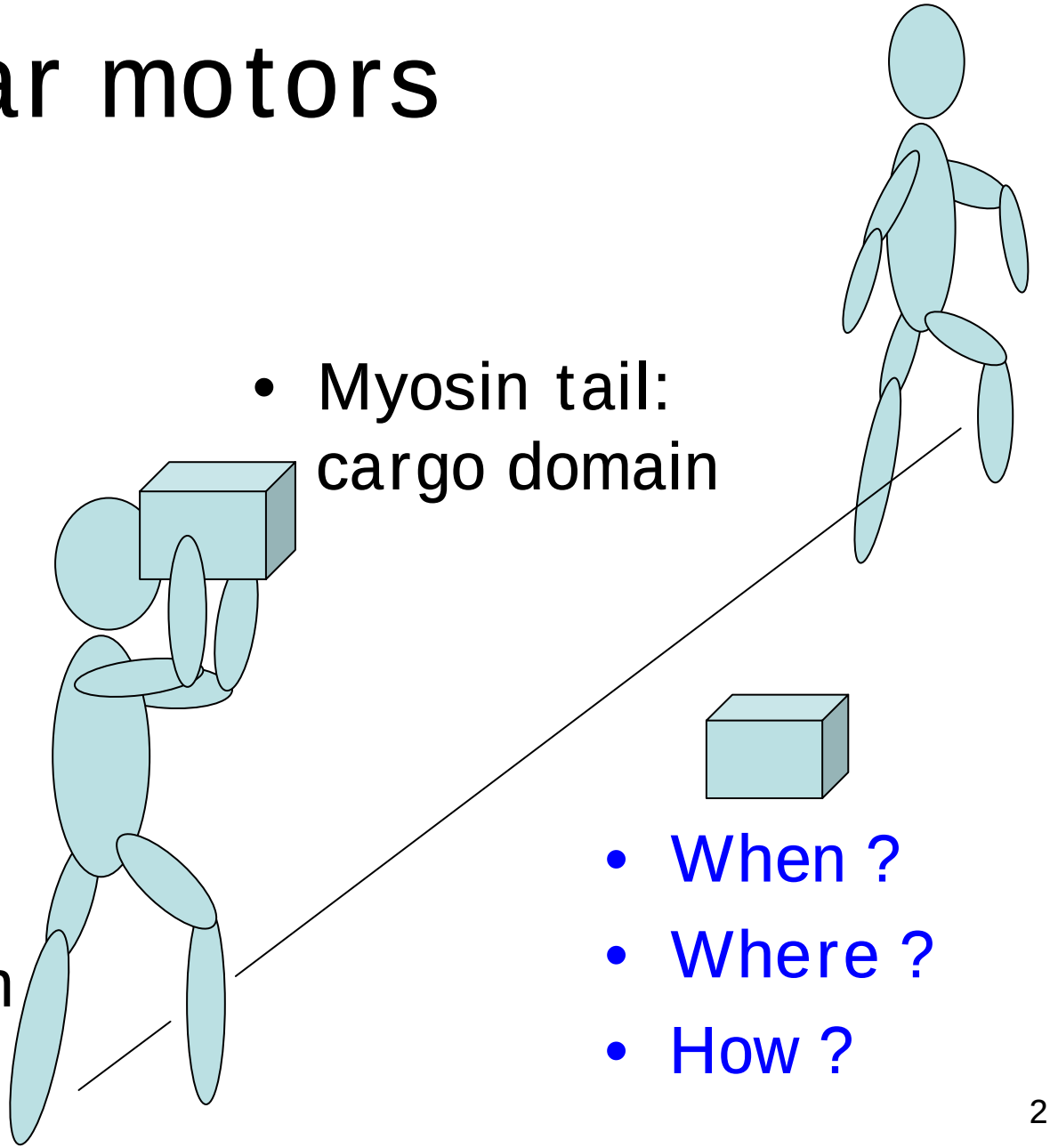


Myosin VI step size

- Determined by the actin filament periodicity ?
  - A left-handed rotation of the lever arm from EM.
  - An actin helical repeat in two steps
    - 36 nm ~ (11 + 27) nm

Rock et al., 2001. PNAS 98, 13655.

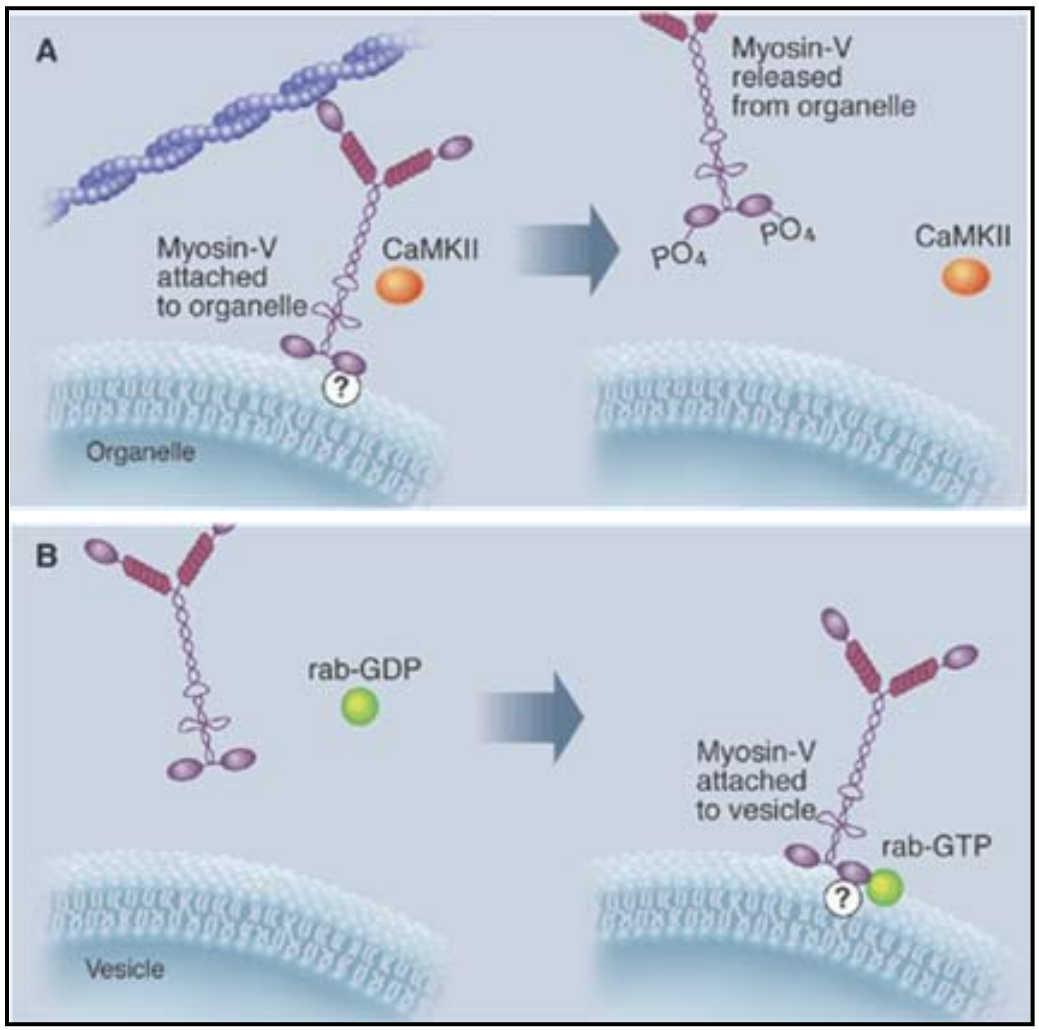
Molecular motors



- Myosin tail: cargo domain
- Myosin head: motor domain
- When ?
- Where ?
- How ?

A switch to release the motor

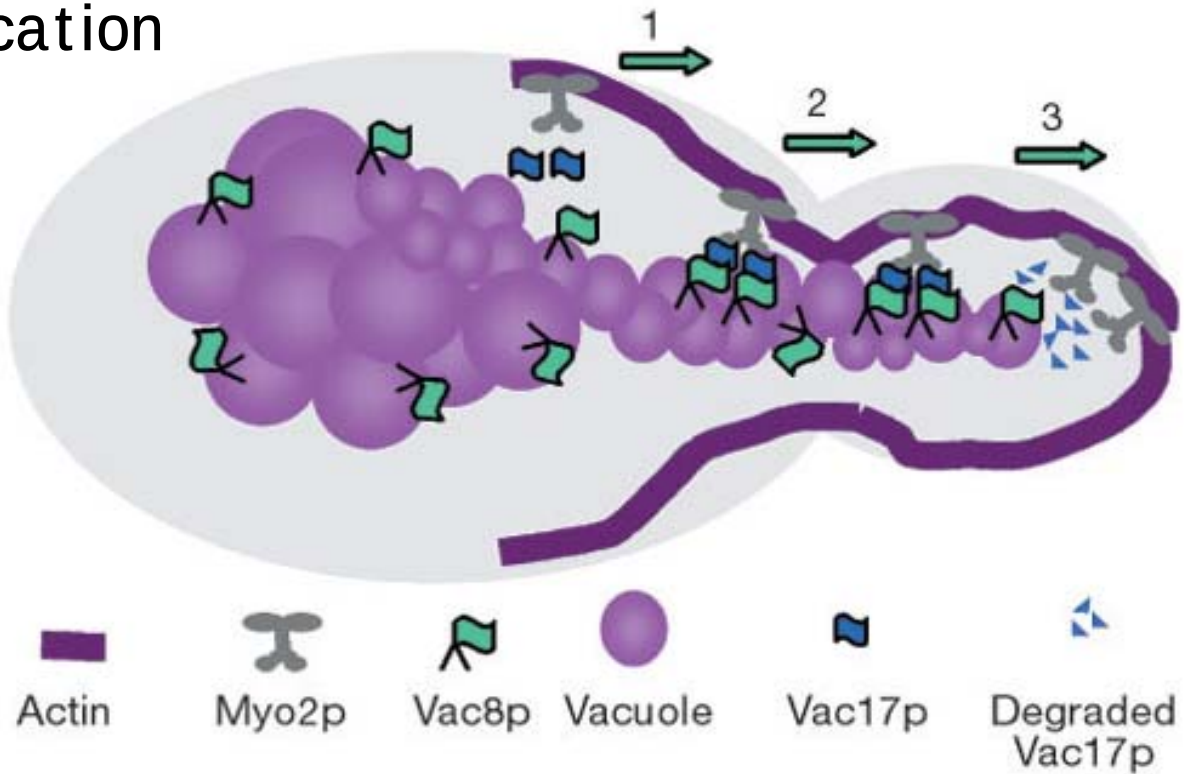
- Regulating traffic



Cheney and Rodriguez 2001 Science 293, 1263.

Cargo-specific receptor

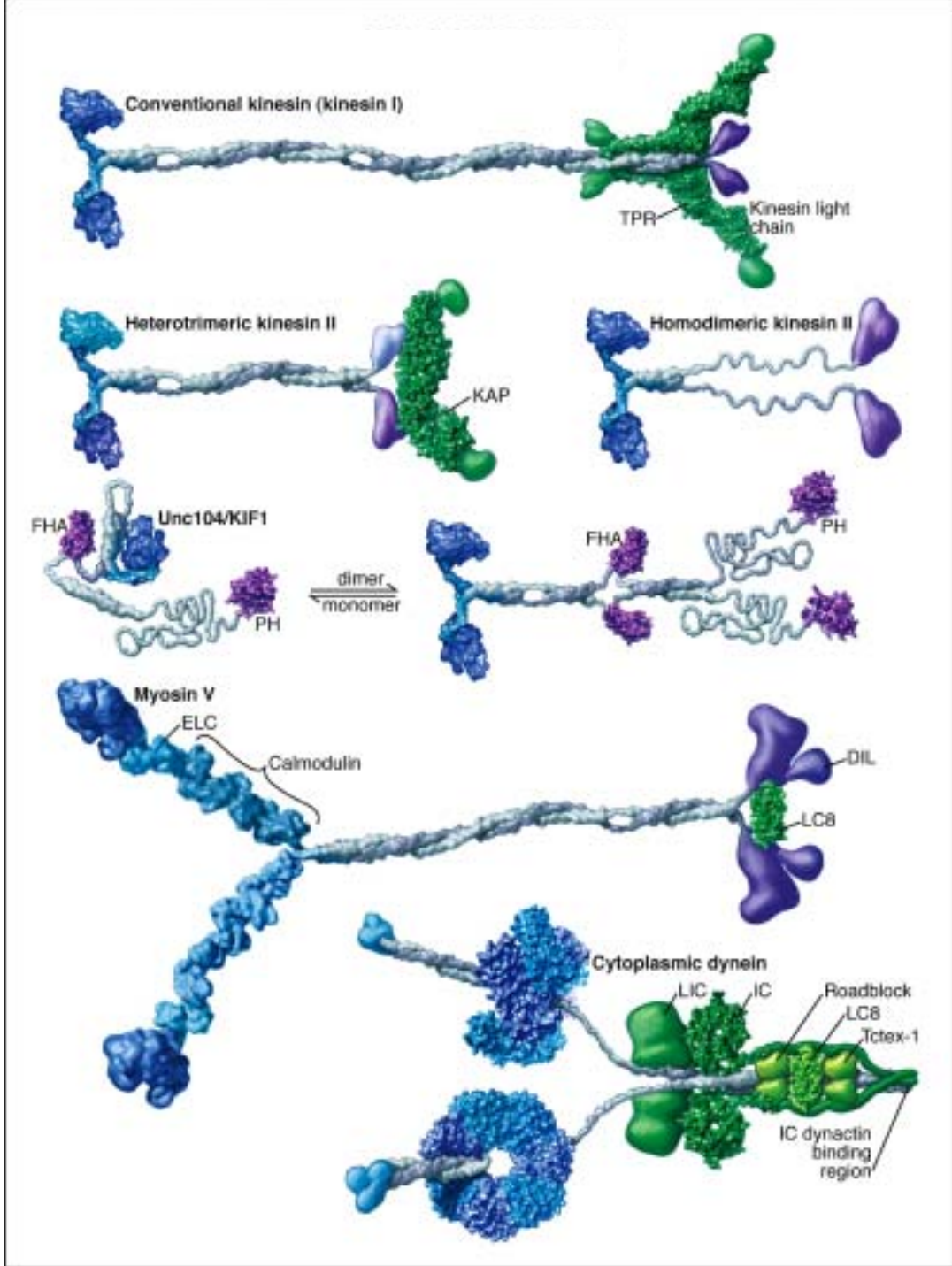
- Regulated cargo binding
- Regulated linker degradation
  - Timing
  - Location



Tang et al., 2003 Nature 422, 87.

Motor Toolbox

- Head domain
  - Conserved motor
- Neck domain
- Tail domain
  - Accessory



Vale, 2003 Cell 112, 467-480.

References

- The Myosin Homepage
  - http://www.mrc-lmb.cam.ac.uk/myosin/myosin.html
- The Kinesin Homepage
  - http://www.proweb.org/kinesin//index.html
- Burgess et al., 1997. J. Cell Biol. 139:675-681.
- Rock et al., 2001, PNAS, 98, 13655-13659.
- Uyeda et al., 1996. PNAS, 93, 4459-4464.
- Vale and Milligan. 2000. Science, 288, 88-95.
- Vale, 2003. Cell, 112, 467.
- Walker et al., 2000. Nature, 405, 804.