Molecular motors I

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Talk overview

Part I:

- Overview
- Order of magnitude estimates
- Examples of molecular motors

Part II:

- Theory of motor protein motion
- Models of motion and force generation

Molecular motors

Key property: motion

Motor protein (or protein complex)

Able to generate force and therefore do work

Molecular basis of biological motility

Biological role of molecular motors

Basis of biological motility

- Cell division
- Cell motion
- Transport within cells
- Growth
- Muscle contraction

Outline: part I

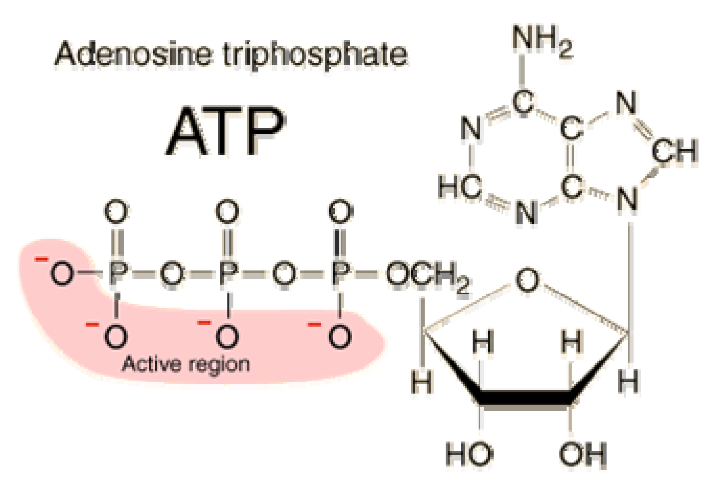
- Energy transduction
- Example: kinesin on microtubule
  - Single-molecule experiments
- Force and energy scales
- Efficiency
- Reynolds number
- Thermal fluctuations
- Heating

Energy transduction

- Motion and work requires energy source
- Energy transduction
  - Conversion of one form of energy (e.g., potential energy of a chemical bond) to another (e.g., motion)
- Typical fuel: ATP hydrolysis

ATP hydrolysis

- **A**denosine **trip**hosphate
- Common fuel in cells
- ATP hydrolysis to ADP+P_i
  - Remove one phosphate (add water)
  - Negative charges move apart

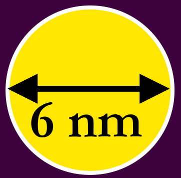


http://hyperphysics.phy-astr.gsu.edu/hbase/biology/atp.html

Problem #1
Protein transport in neurons

Spine to fingertip distance: 1 m

Typical protein size: 3 nm

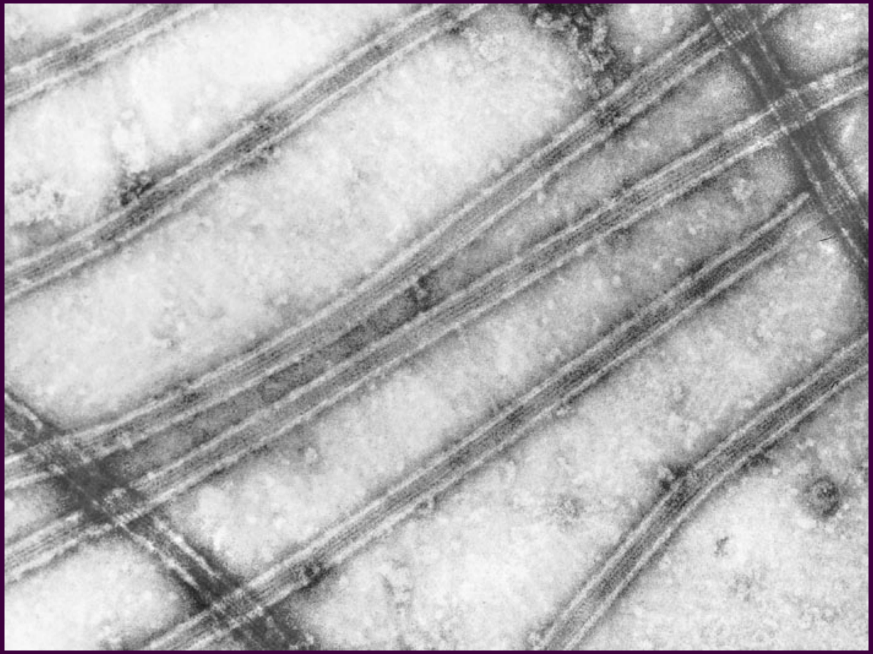


Solution

Specialized intracellular transport between center and edge of cell

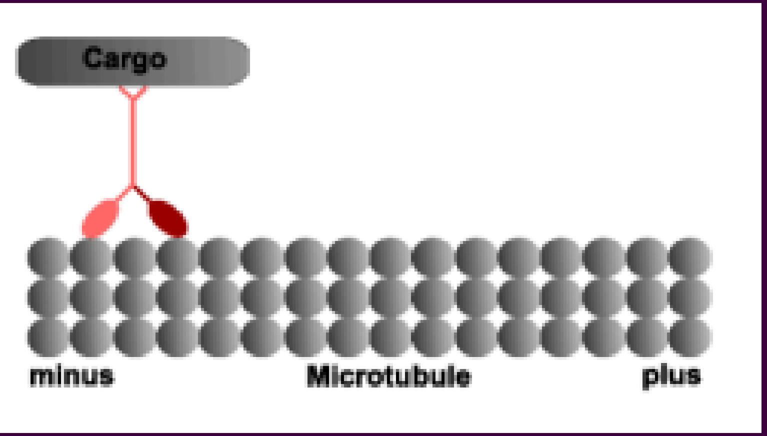
Highways: microtubules

20-nm filaments

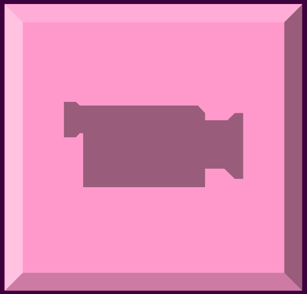


Transporter: kinesin

"Walks" on microtubules

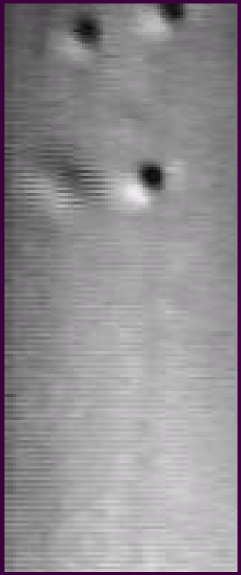


Kinesin *in vivo*



Michael Welte, http://www.bio.brandeis.edu/weltelab/movies/live.mov

Kinesin *in vitro*

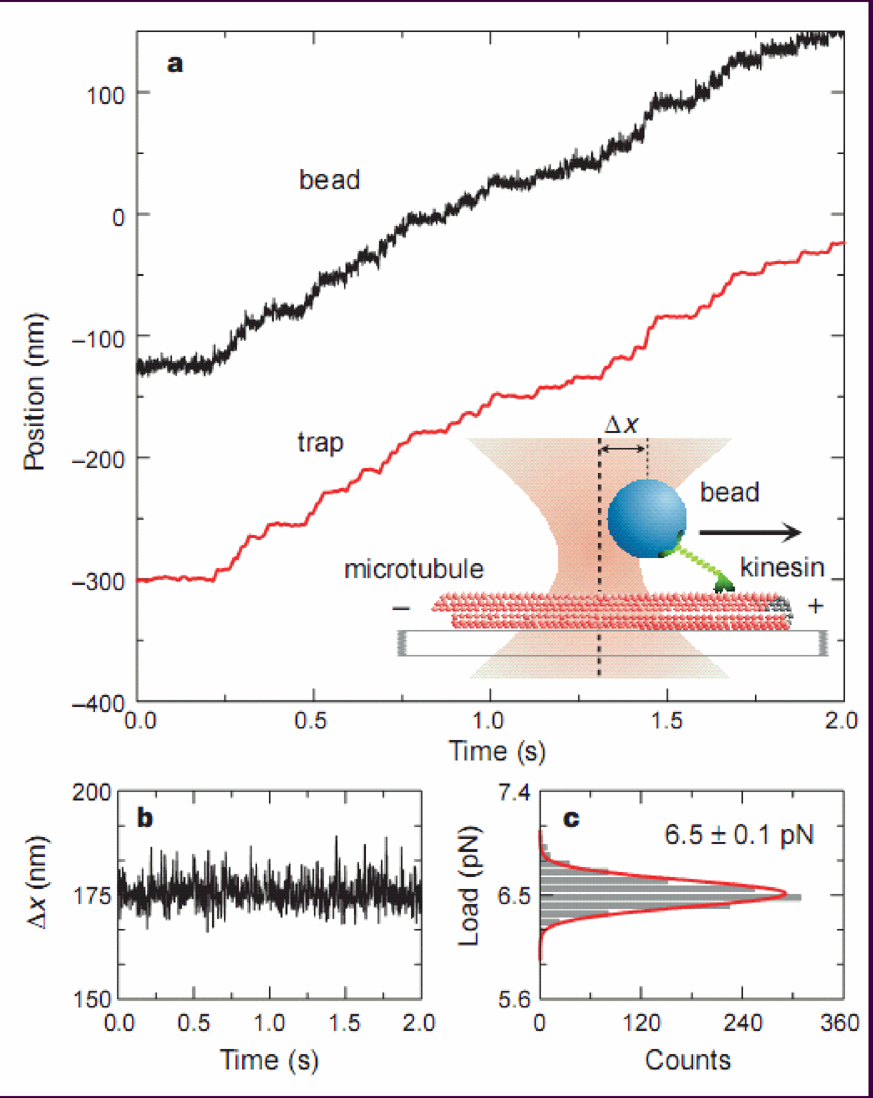


Single kinesin attached to bead

Jeff Gelles, http://www.bio.brandeis.edu/~gelles/kamppnp/index.html

Single-molecule experiments

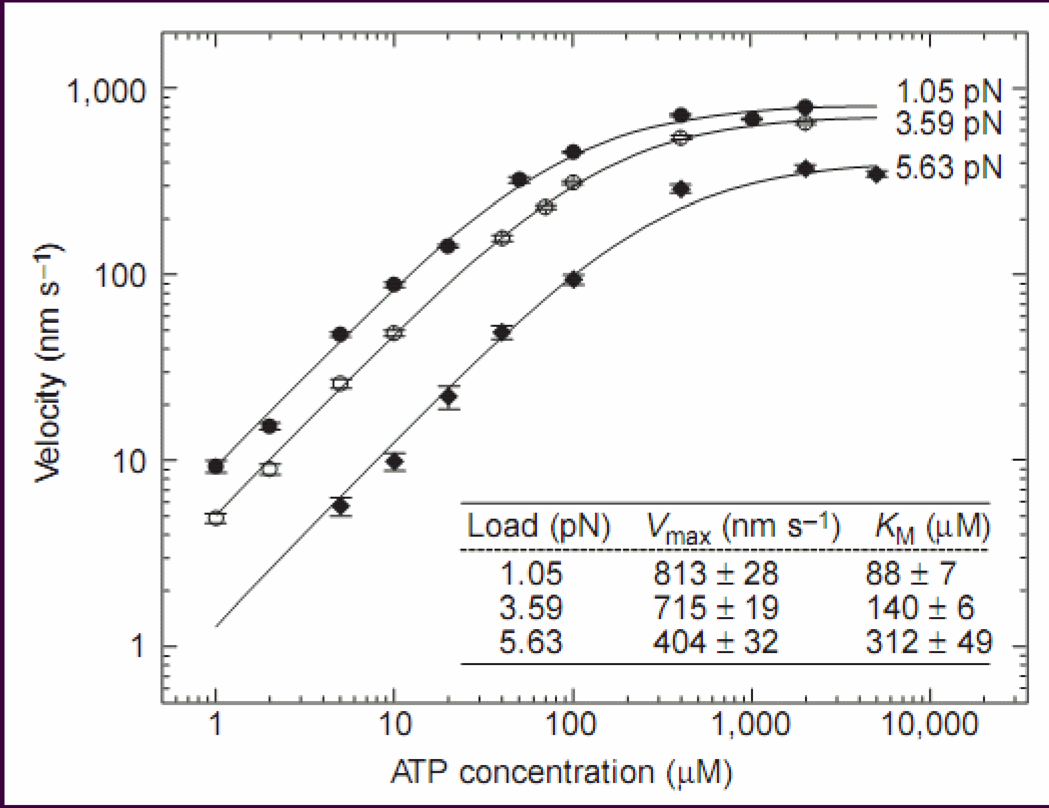
- Apply force to bead using optical trap
- Force clamp = constant force
- Measure bead position as kinesin moves



Visscher, Schnitzer and Block, *Nature* **400**, 184 (1999)

Single-molecule experiments

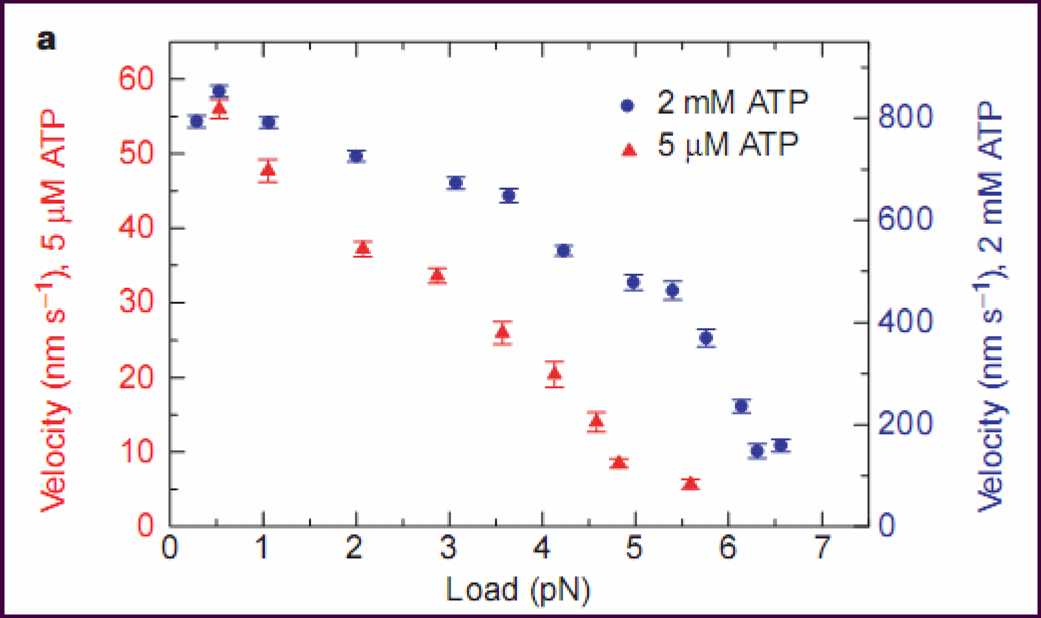
- Velocity saturates as ATP concentration increases=>
- Saturating kinetics
- Maximum velocity depends on load force



Visscher, Schnitzer and Block, *Nature* **400**, 184 (1999)

Single-molecule experiments

- Measure force-velocity relationship
- Result varies with ATP concentration



Visscher, Schnitzer and Block, *Nature* **400**, 184 (1999)

Summary

Energy from ATP

Example: kinesin on microtubule

Force and energy scales: pN, nm, pN nm

Efficiency: ~1

Reynolds number: ~0

Thermal fluctuations: important

Heating: ~0

Outline: part 2
Survey of motor phenomena

Spindle assembly

Stereocilia membrane tension

Cutting up collagen

Untangling DNA

Problem #2
Mitotic spindle organization

Newt cells

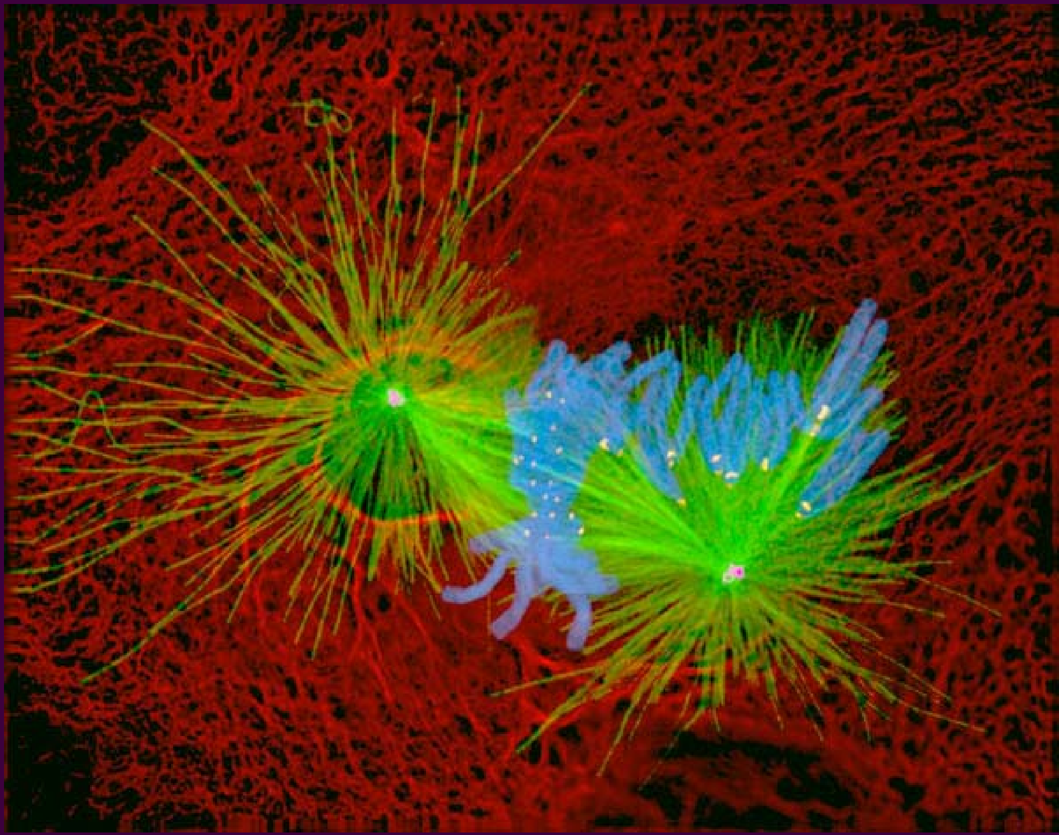
Microtubules-green

Spindle poles-magenta

Chromosomes-blue

Kinetochores-yellow

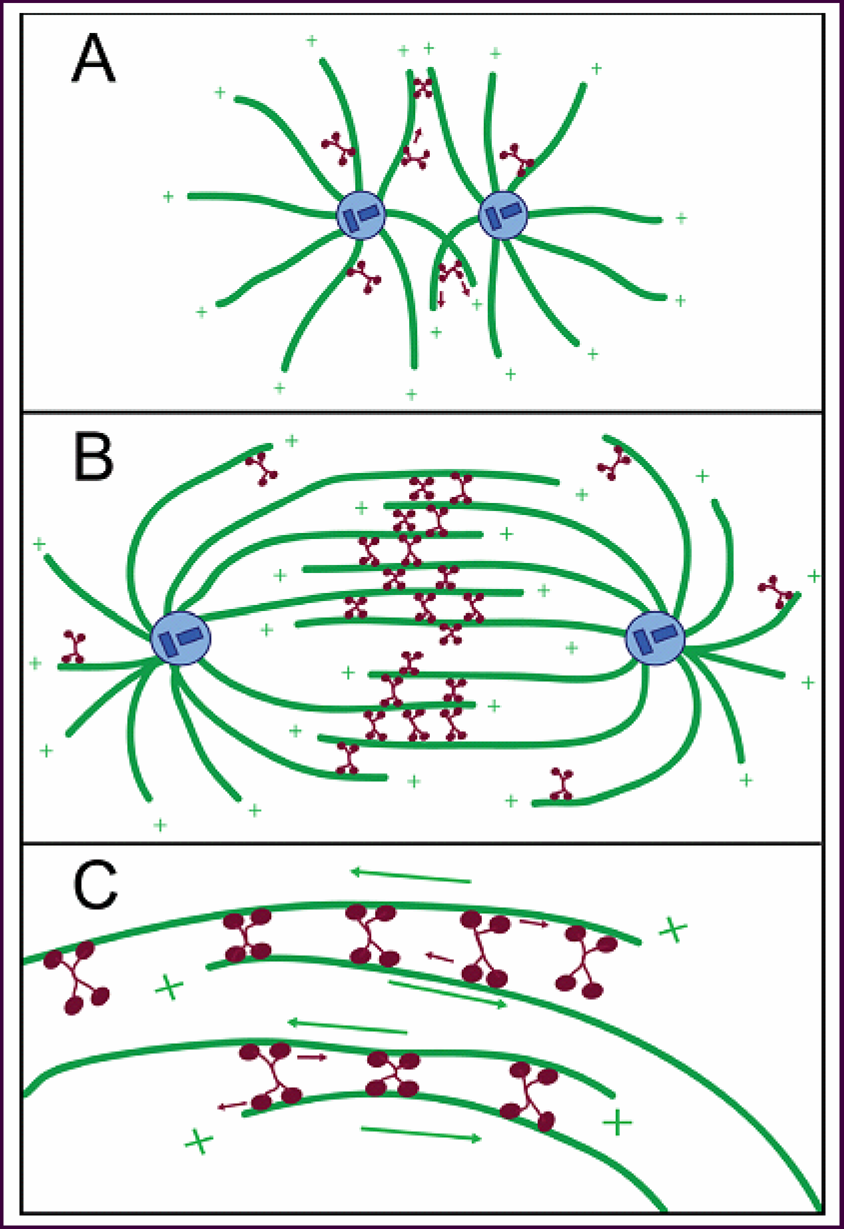
Keratin-red



Alexey Khodjakov, Laboratory of Cell Regulation,
http://www.wadsworth.org/databank/feat-99-12-hq.jpg

Eg5

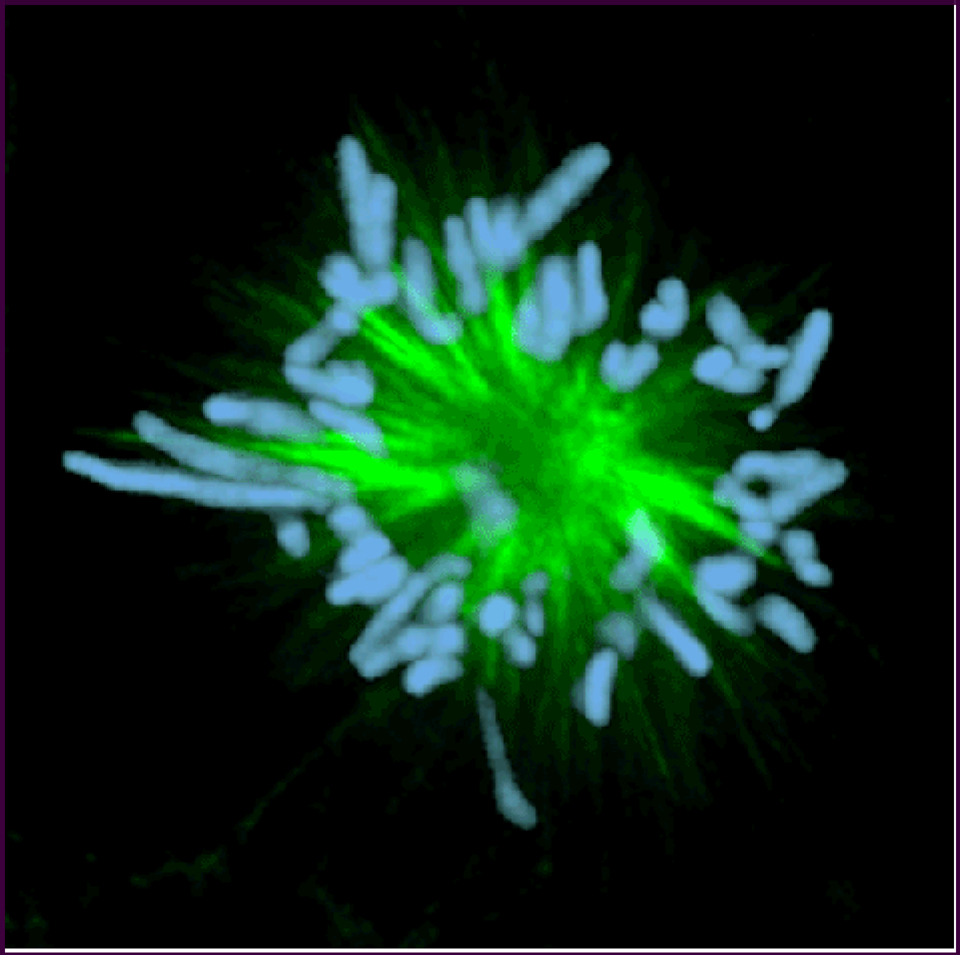
- Tetrameric kinesin
- Can bind two MTs simultaneously
- Moves towards + ends of both MTs
- Slides apart antiparallel MTs
- Helps organize spindle



Valentine, Fordyce and Block, *Cell Division* **1**:31 (2006)

Disruption of Eg5

- Mammalian cell
- Treated with monastrol
- Kills Eg5 function
- Leads to monopolar spindle
- No antiparallel MTs



Tarun Kapoor, http://www.kapoorlab.com/images/research_2_fig4.gif

Eg5 current understanding

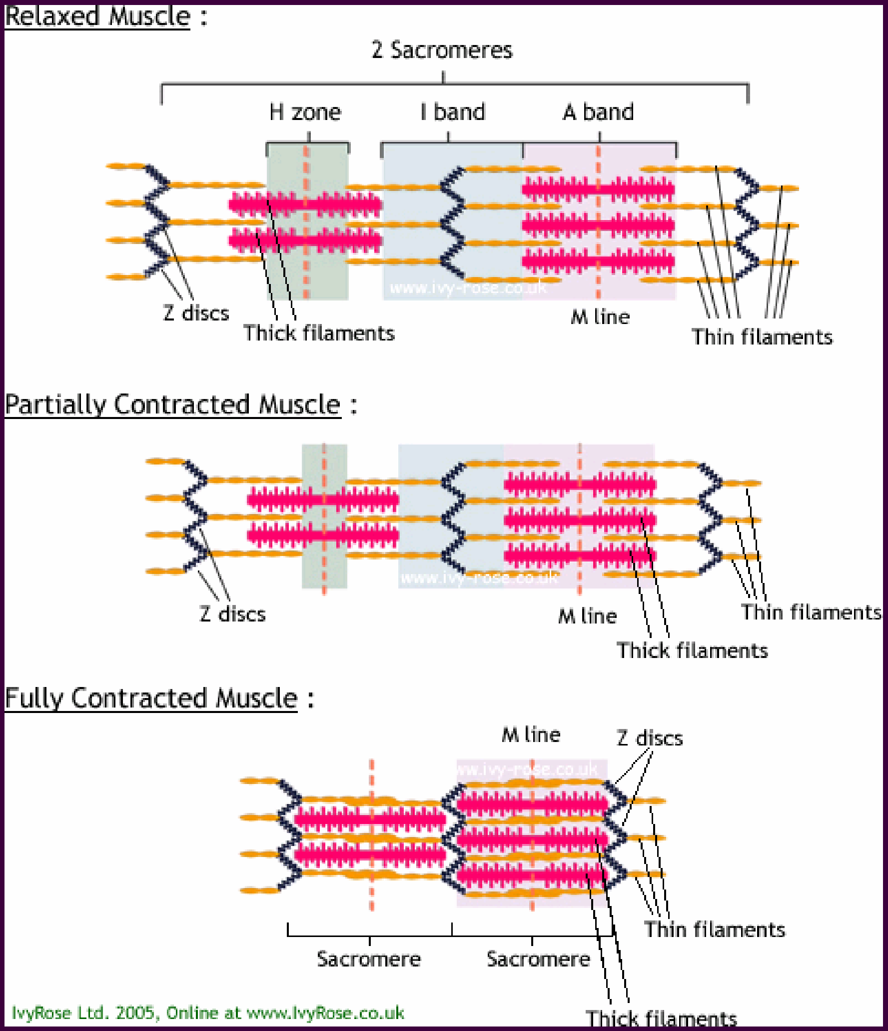
Qualitative model in place

First single-molecule experiments done

No quantitative theory

Problem #3
Muscle contraction

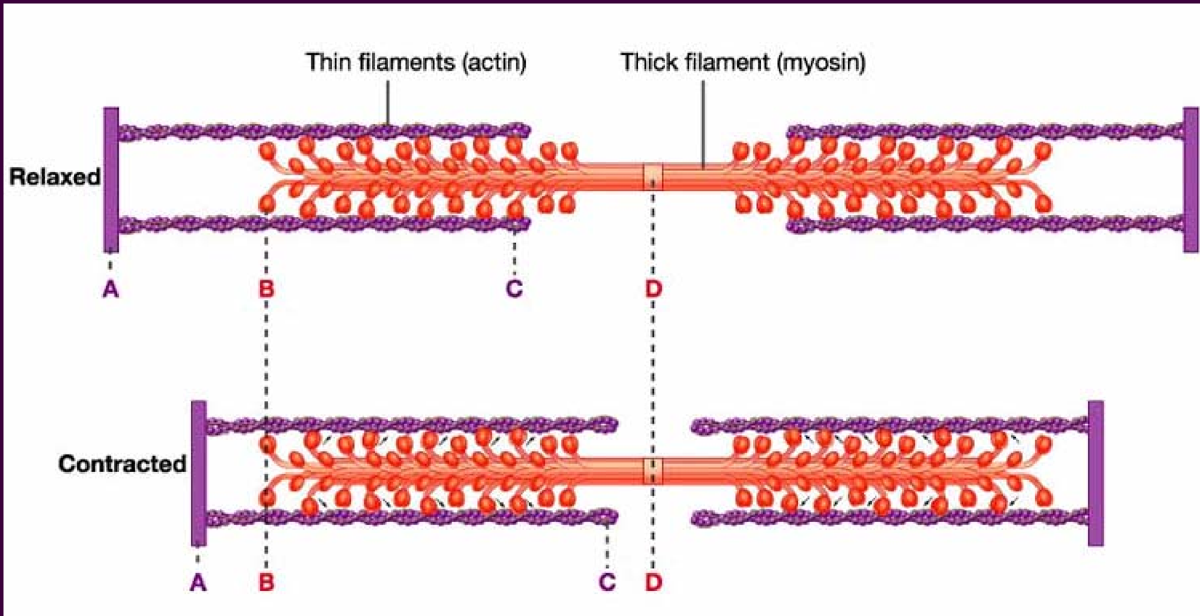
- Thin filaments: actin polymer bundles
- Thick filaments: myosin motor bundles
- Contraction: Motors grab and pull



http://www.ivy-rose.co.uk/Topics/Muscles/Sliding-Filament_Theory.jpg

Thin and thick filaments

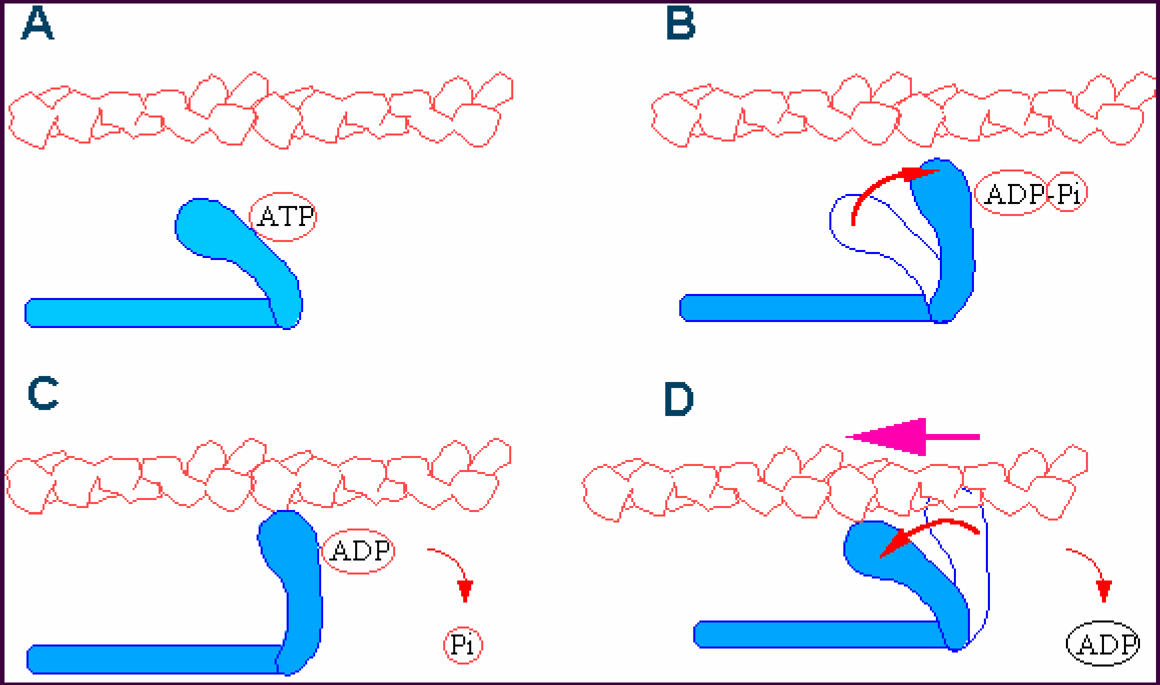
Myosin motors are active cross bridges between the actin filaments



http://fig.cox.miami.edu/~cmallery/150/neuro/sf43x16.jpg

Crossbridge model of myosin motion

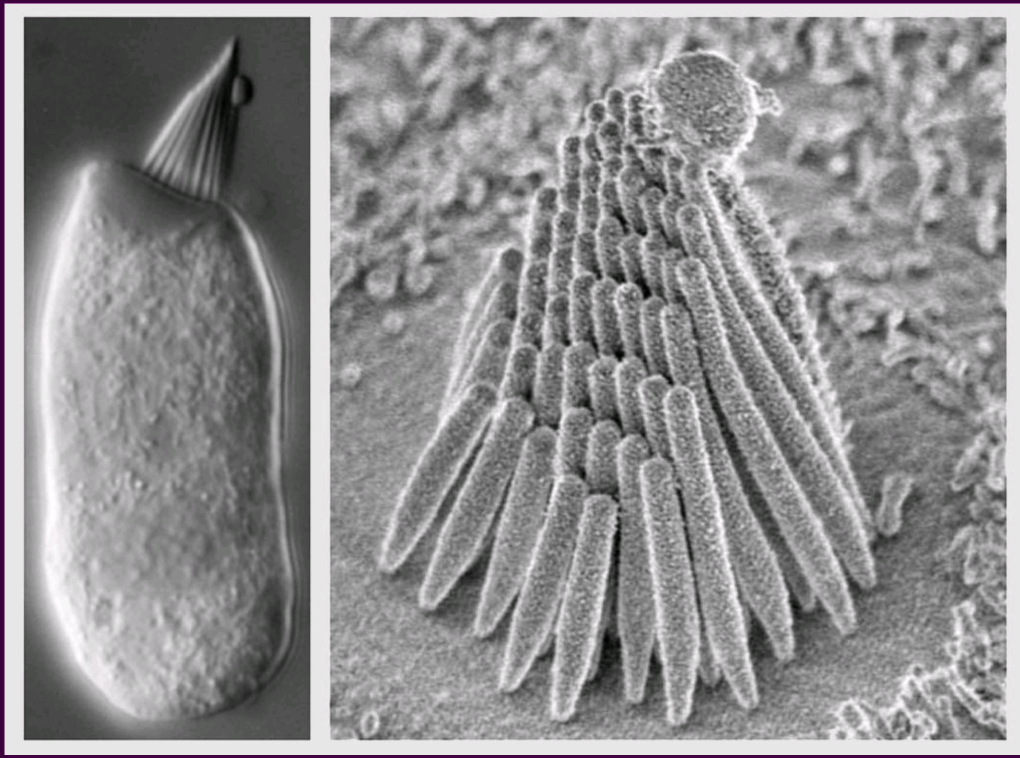
- Myosin head changes conformation
- Depends on nucleotide binding



http://www.bms.ed.ac.uk/research/others/smaciver/Cycle.gif

Problem #4
Hair cells and myosin VI

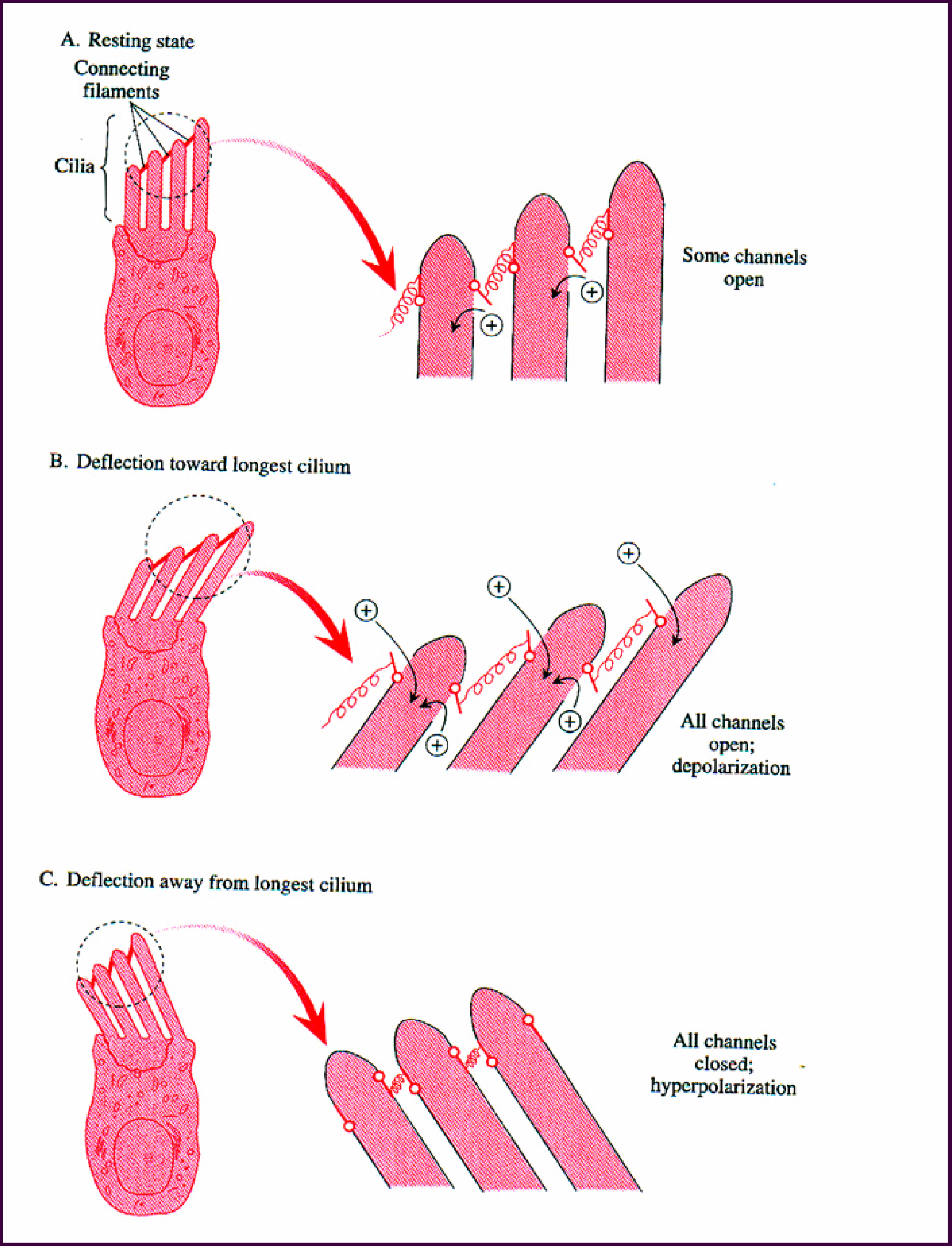
- Inner ear contains the basilar membrane
- Hair cells sit on basilar membrane
- Hair cells detect vibrations using stereocilia



http://www.ohsu.edu/ent/gen/haircells.jpg

Stereocilia

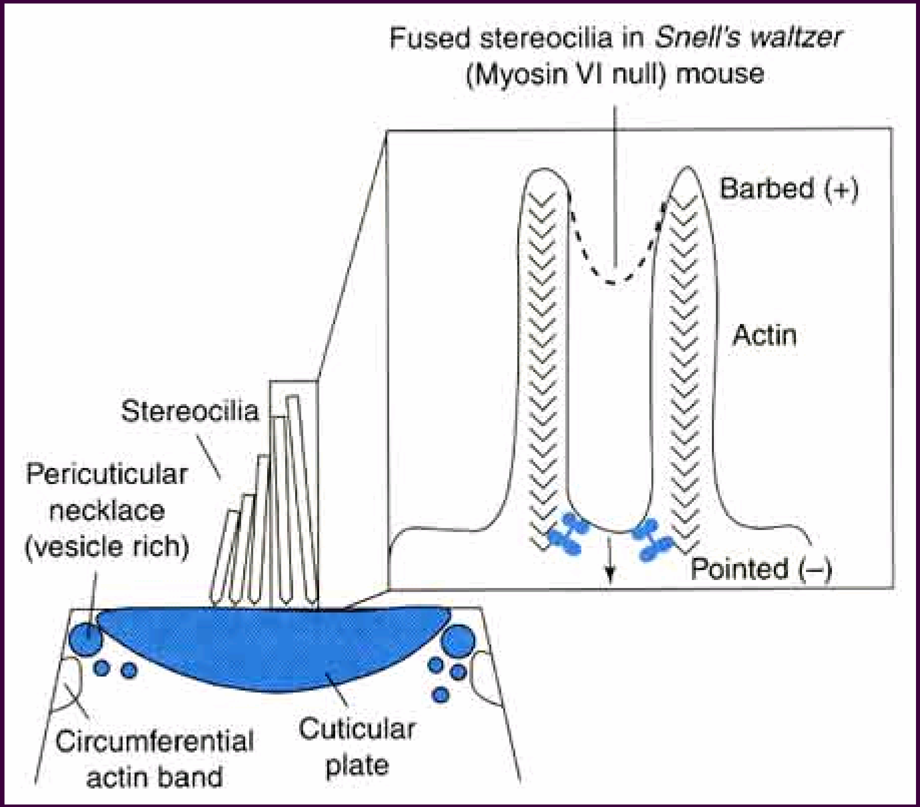
- Motion of stereocilia
- Triggers mechanically sensitive ion channel opening
- Causes polarization of the cell and nerve impulse



http://www.du.edu/~kinnamon/3640/hearing/matthews18.4.jpg

Stereocilia and myosin VI

- Defective myosin VI leads to deafness and balance problems
- Proposed role: tension membrane between stereocilia
- May act as motor and clamp



http://www.bio.umass.edu/vidali/web/cell_motil/myosin_vi_4.jpg

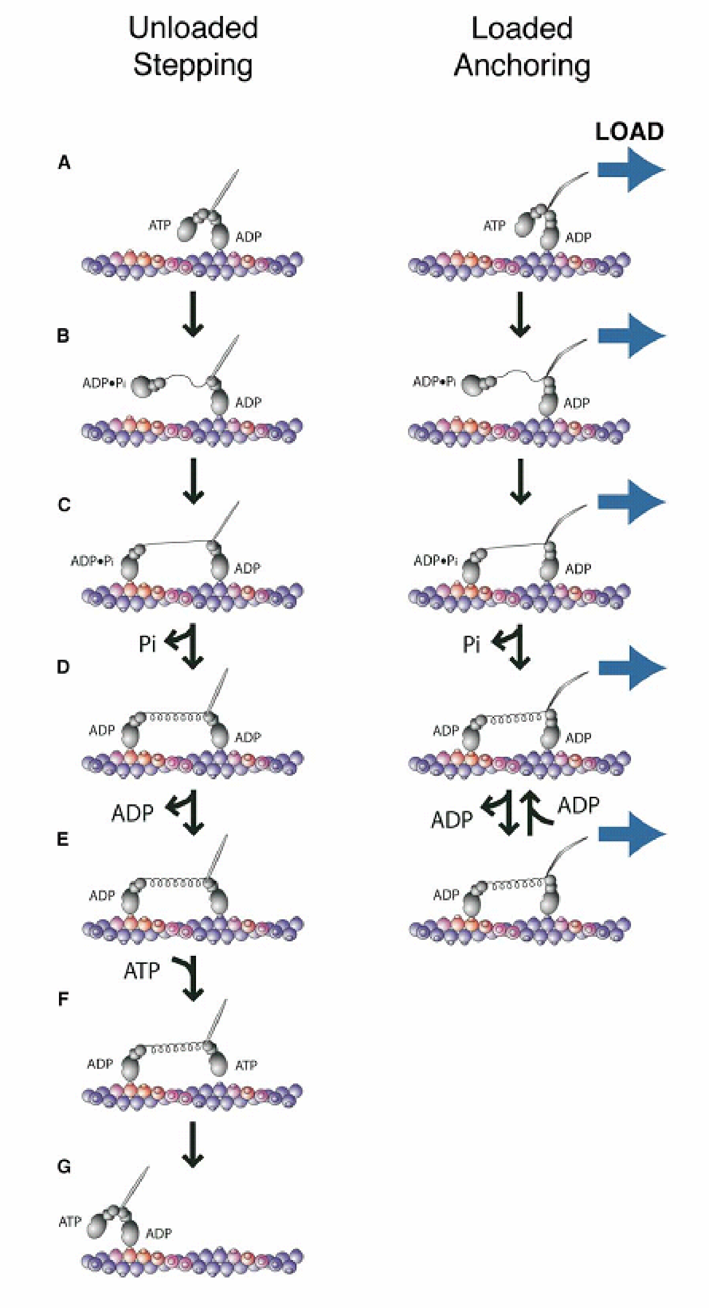
Myosin VI model

Different kinetics and binding with load

Proposed model:

No/low load: stepping

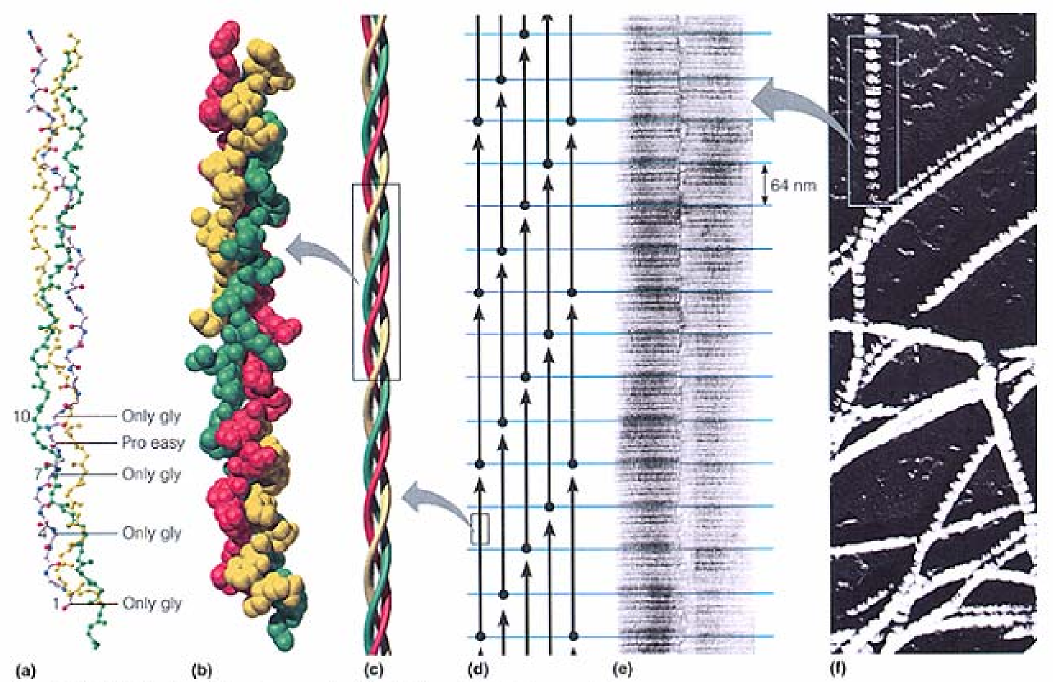
Load: anchoring



Altman, Sweeney and Spudich, *Cell* **116**, 737 (2004)

Problem #5
Collagen cutting

- Collagen fibrils:
- Extracellular matrix (ECM)
- Connective tissue
- 25% of mammalian protein
- Monomer is 300 nm long



http://wwwfac.mcdaniel.edu/Chemistry/CH3321JPGs/Proteins/Collagen.jpg

Collagenase

Motor protein: moves with bias

Moves on and cuts up collagen fibrils

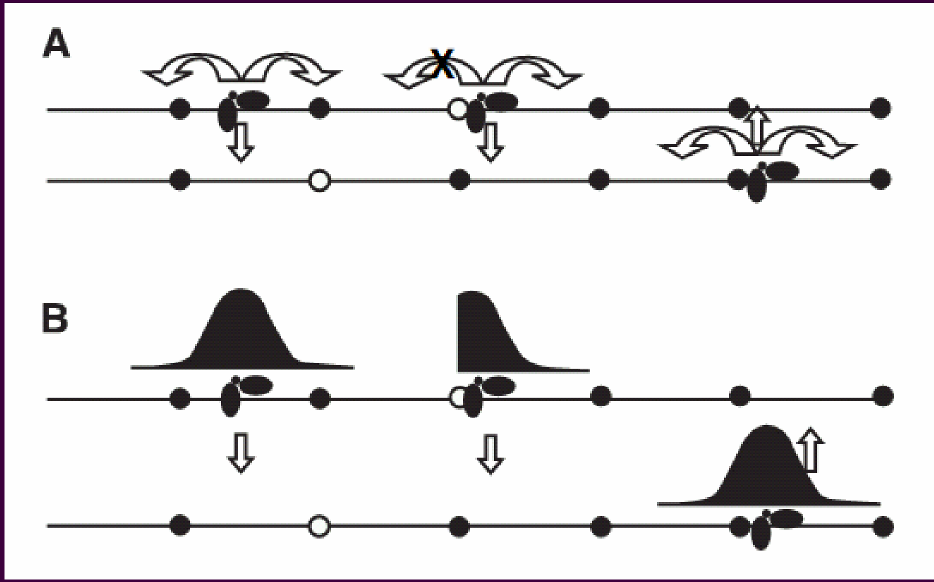
Does not consume ATP

Requires proteolysis of collagen

Saffarian et al., *Science* **306**, 108 (2004)

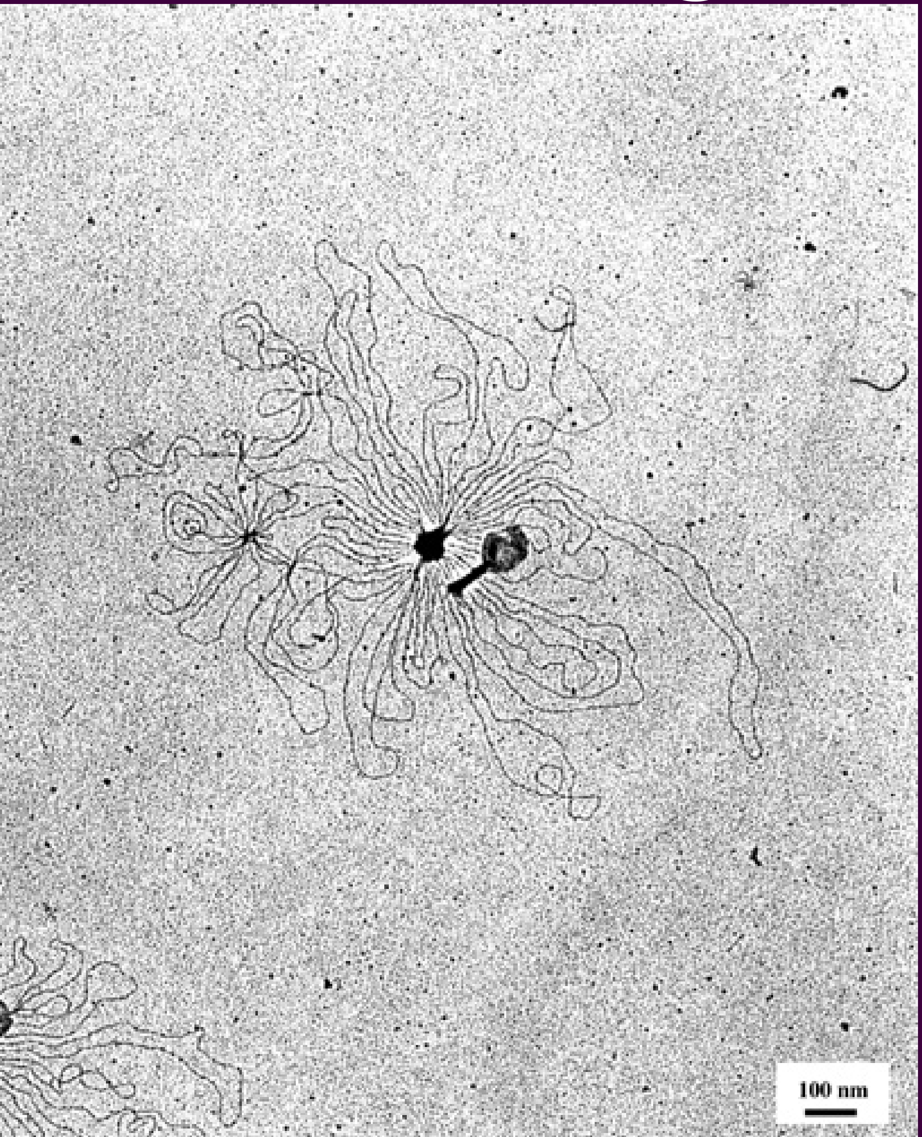
Burnt bridges model

- Brownian ratchet:
- 1D diffusion on filament
- Cleave filament at specific site
- Always end up to right of cleavage site
- Can't jump gaps
- Can switch to other filament



Saffarian et al., *Science* **306**, 108 (2004)

Problem #6
Dealing with DNA tangles

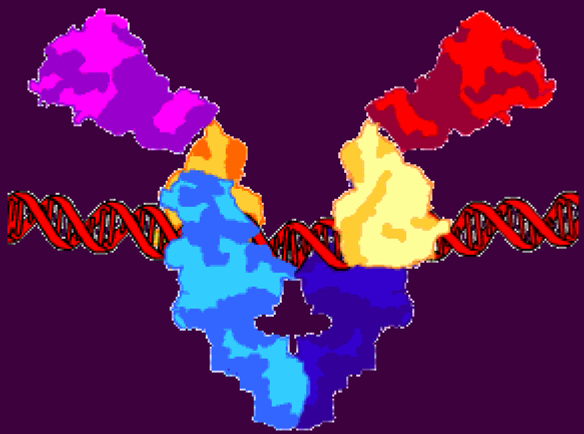


Length of DNA

Virus: 20 µm

Human: 2 meters

DNA management example



DNA Topoisomerase

Eats “topoisomers”

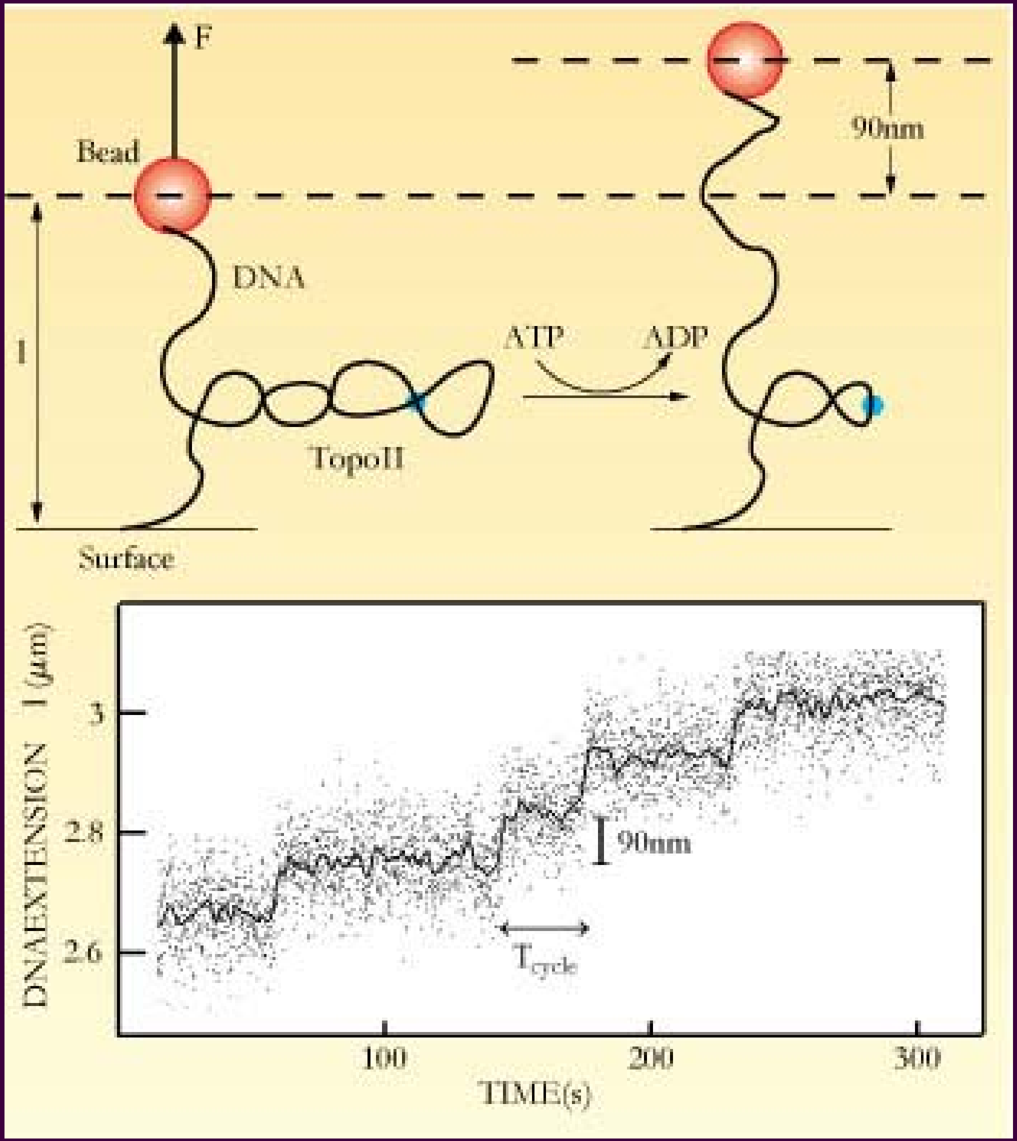
Undoes tangles

Mechanism of Topoisomerase II

- Bind two strands of DNA
- Cut one strand
- Pass other strand through cut
- Seal cut

Joaquim Roca Bosch, http://www.cid.csic.es/homes/roca/TOPOII.html

Single-molecule Topo II experiments



Strick *et al.*, Physics Today, February 1997

Pull on DNA

Twist DNA

Measure:

Length

Elasticity/Twist elasticity

Action of **single proteins**