BME 42-620 Engineering Molecular Cell Biology

Lecture 12:

The Cytoskeleton (III): Molecular Motors

Mechanical Properties of Cytoskeletal Polymers

Course Administration Notes (I)

- **Correction:** Midterm exam: October 27, 2011; Take-home exam; Instructions handed out at the end of the class.

October 21	F	Undergraduate Mini-1 Exam Day
October 21	F	Mid-Semester Break; No Classes (Mini-1 exams will take place)
October 24	M	Mid-Semester Grades Due by 9 p.m.
October 24	M	Mini-2 Classes Begin (except for Tepper)
October 26	W	Mini-1 Final Grades Due by 6 p.m.
October 26	*W*	*Mini-2 Classes Begin (Tepper only)*

- Midterm exam format:

  1) conceptual and factual questions based on lectures and the textbook;
  2) a literature-based research project;
  3) Due October 31 Monday 12:00Noon at Mellon Institute 403;
  4) Regular lectures will continue as scheduled.

Outline

- Overview of molecular motors
- The myosin superfamily; Myosin motility
- The kinesin superfamily; Kinesin motility
- The dynein family; Dynein motility
- Mechanical properties of cytoskeletal polymers

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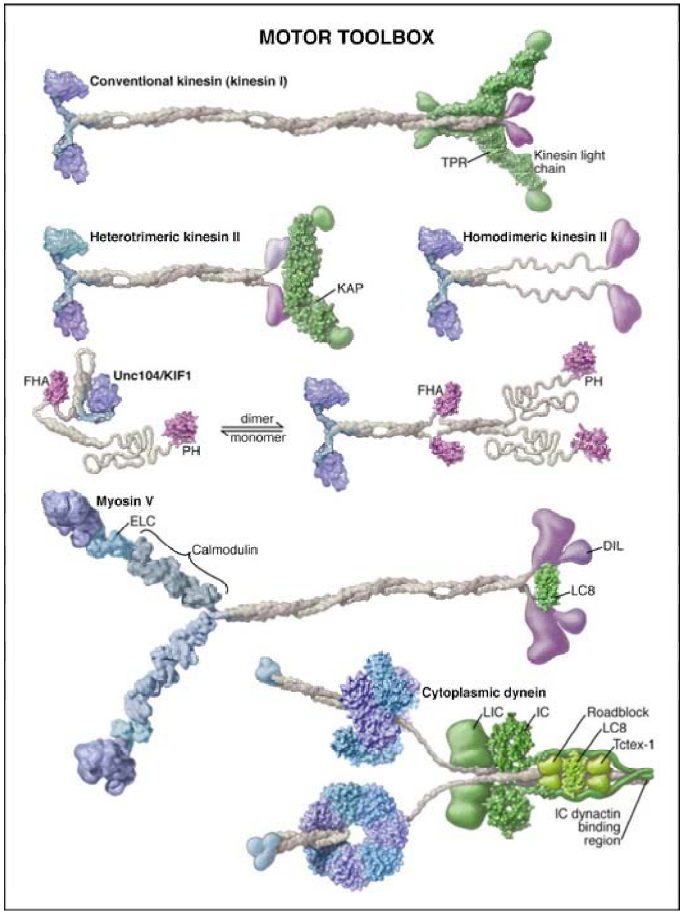
Overview of Molecular Motors

- Myosin walks on actin filaments.
- Kinesin and dynein walks on microtubule.
- Motor (head) domain
  - Produces force and motion
- Tail domain
  - Adapts to different cargoes



Vale RD, *Cell*, 112:467,2003

Motor Behavior Parameters

- Parameters that characterizing motor behaviors
  - processivity: run-length, number of steps
  - step size
  - stall force

- Myosin is nonprocessive.

- Kinesin and dynein are both processive. Processivity of dynein is weaker.

- Motors walk nano-meter scale steps of specific lengths.

- Stall force is on the pico-Newton level.

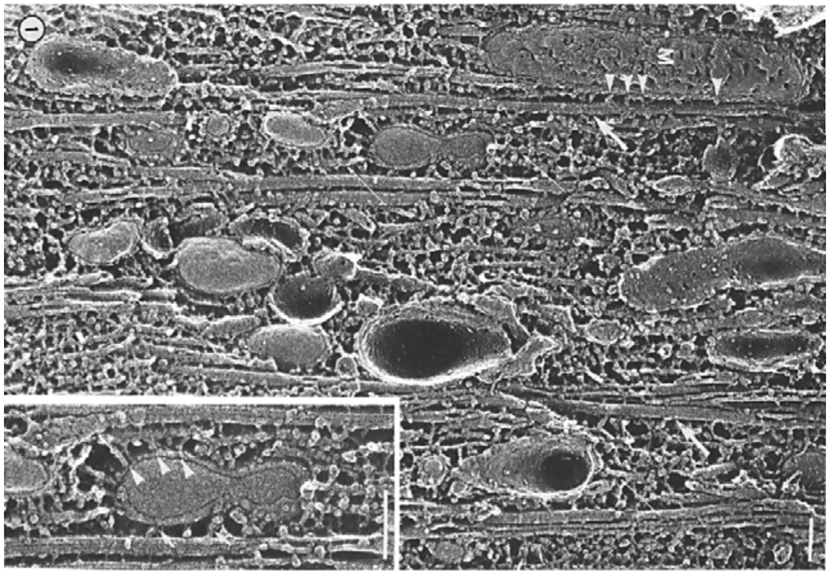
Relations Between Molecular Motors and Cytoskeleton Polymers

- Interactions between motors and cytoskeletal polymers are dynamic and complex.

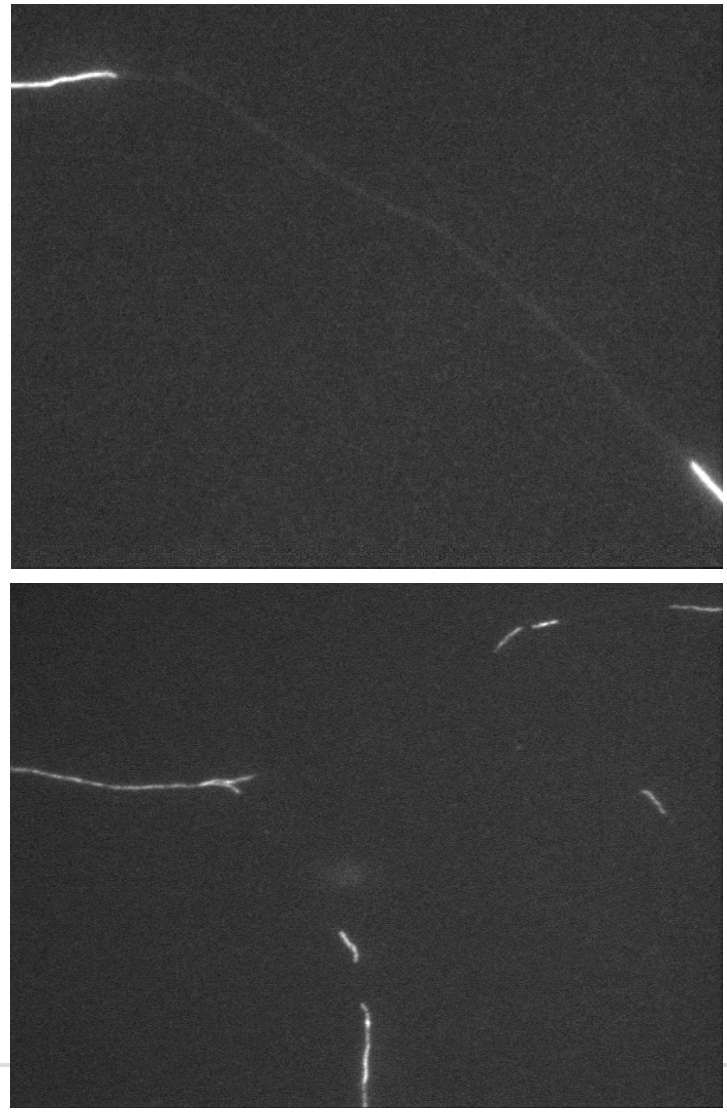
- Cytoskeletal polymers provide dynamic tracks for molecular motors to walk on.

- Molecular motors active interacts with cytoskeletal polymers. For example,
  - Molecular motors transport cytoskeletal polymers, e.g. in neurons.
  - Molecular motors, e.g. MCAK, regulate cytoskeletal dynamics.

Example: Active Transport of Neurofilaments



N. Hirokawa, J. Cell Biol. 94:129, 1982



Wang, L. & Brown, A. (2010). A hereditary spastic paraplegia mutation in kinesin-1A/KIF5A disrupts neurofilament transport. Molecular Neurodegeneration, 5:52

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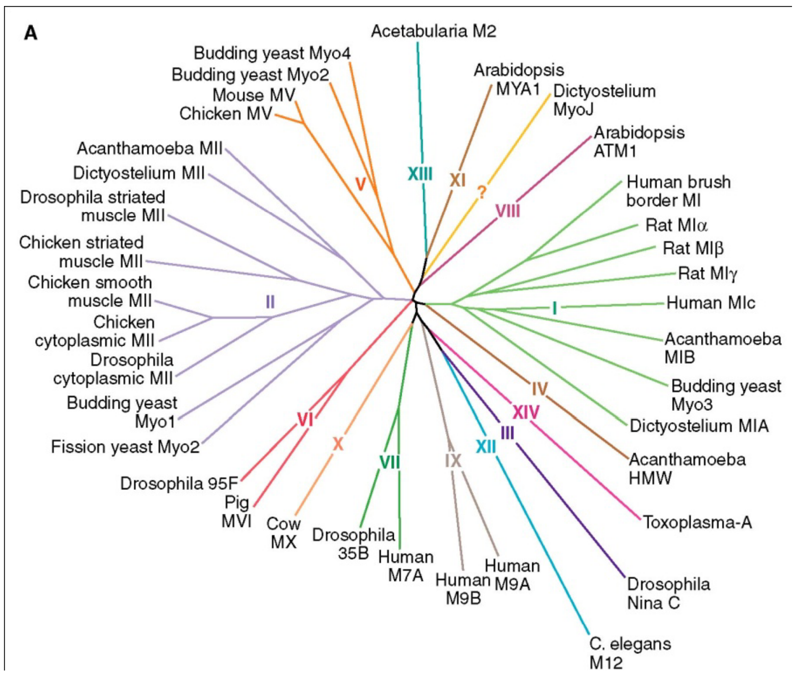
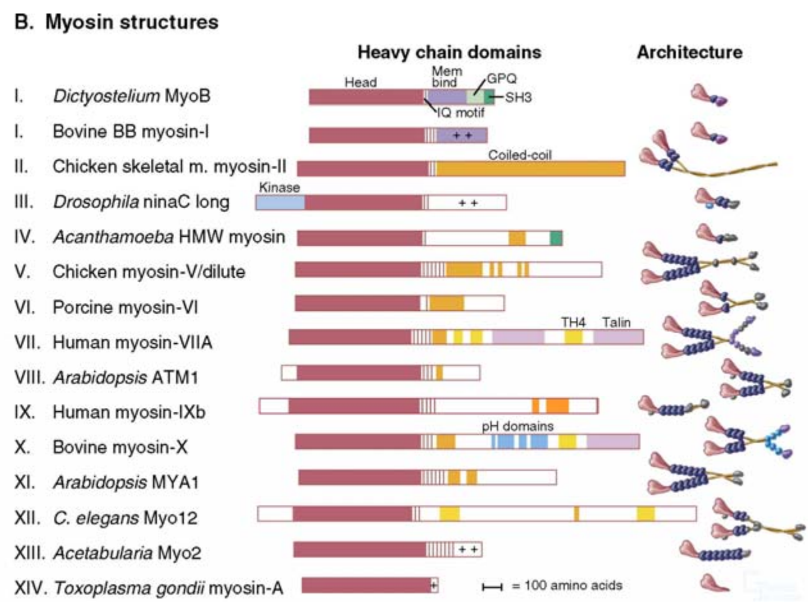
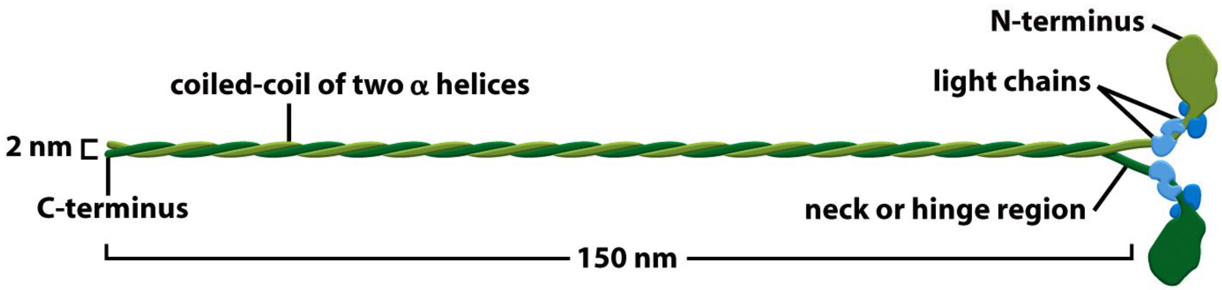
Myosin Family



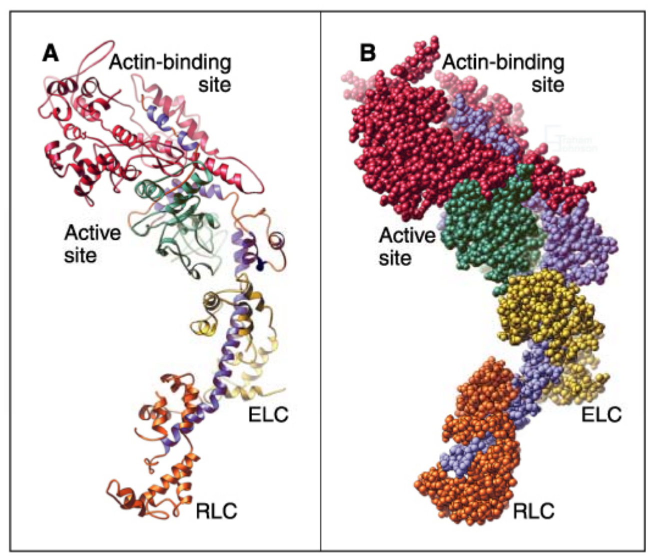


Example: Structure of Head of Myosin-II

- Muscle myosin (Myosin II): 2 heavy chains, 2 light chains

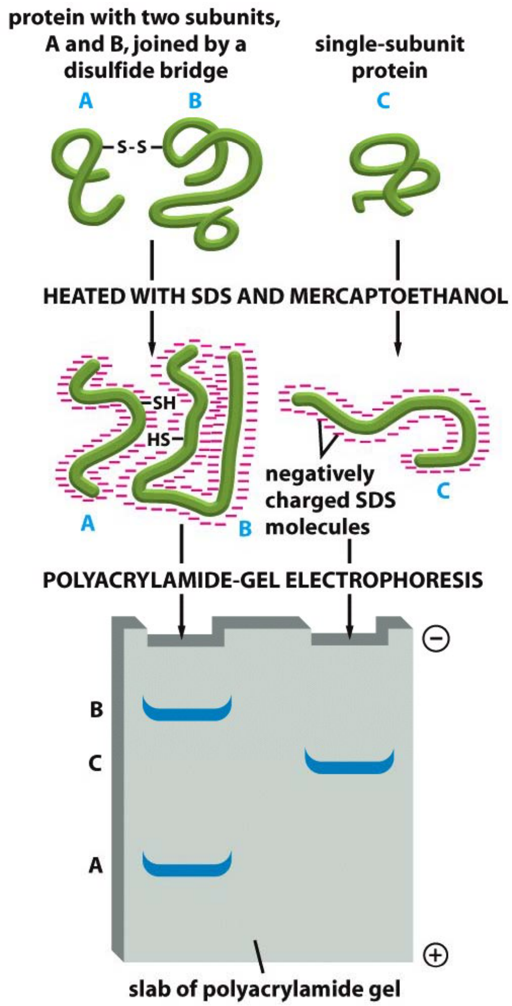


- Light chain stabilizes the heavy chain α-helix.
    - ELC: essential light chain
    - RLC: regulatory light chain

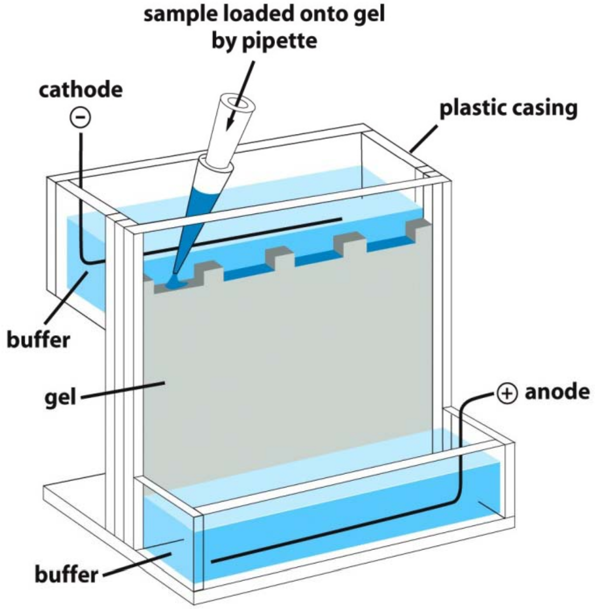


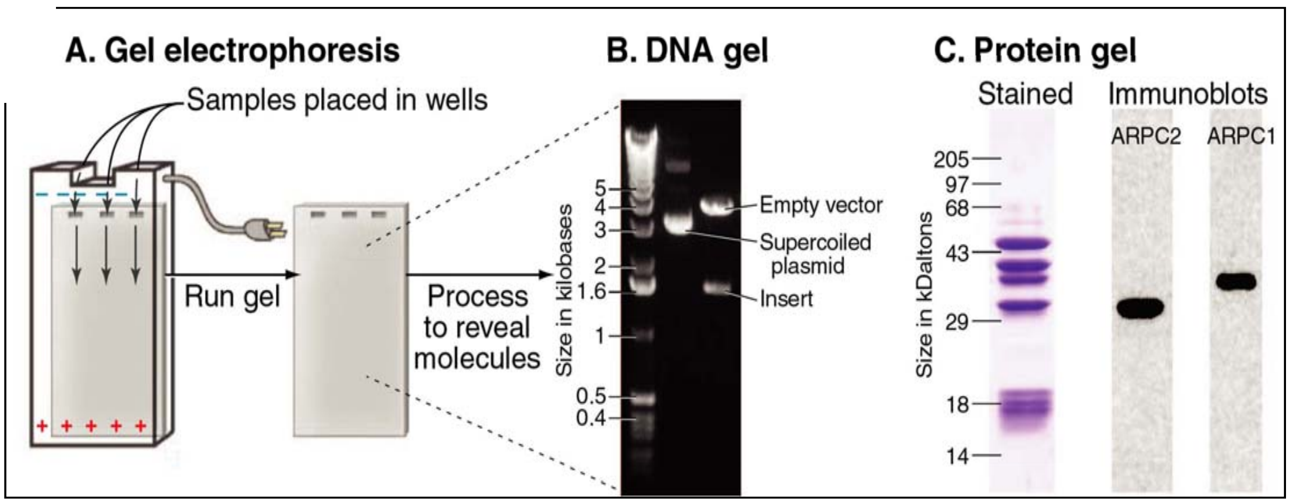
Protein Analysis Using Gel Electrophoresis

- Proteins can be separated based on their molecular weights and analyzed using gel electrophoresis
- Gels (e.g. polyacrylamide-gel) are used to generate a viscous matrix through which protein molecules move.
- Ionic detergent such as SDS (sodium dodecyl sulfate) binds to hydrophobic regions of proteins so that they unfold and negatively charged.
- SDS-PAGE: SDS polyacrylamide-gel electrophoresis



Identification of Heavy Chain & Light Chain





Mechanical Parameters of Myosin

- Velocity
  - Varies substantially between different families
    Myosin II: 6000 nm/sec
    Myosin V: 200 nm/sec
- Force
  - Ranging between 1~10 pN
- Step
  - Myosin II: 5 nm
  - Myosin V: 36 nm
- Run-length
  - up to several hundred nm

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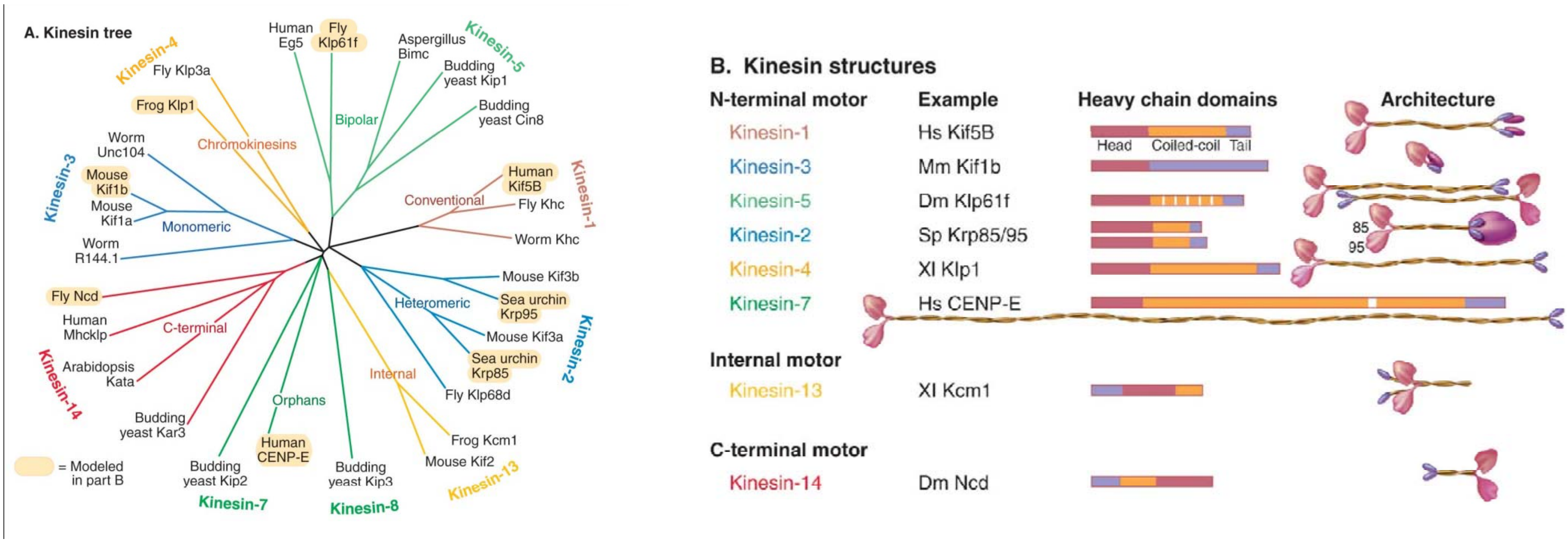
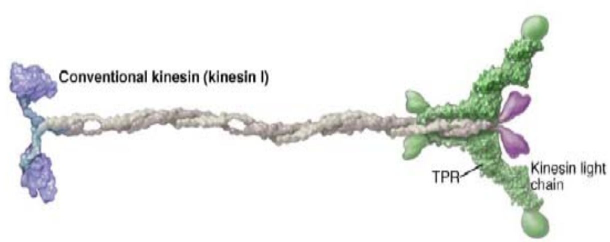
Kinesin Families

Standardized Name	Example Sequences[a]	Other Names for this Group of Sequences[b] *Endow et al*[c]	*Hirokawa*[d]	*Lawrence et al.*[e]	*Other Names*
Kinesin-1	KHC (J05258) KIF5A (AF067179) KHC (L47106) K7 (U41289)	KHC	N-I	Kinesin-I	Conventional
Kinesin-2	KRP85 (L16993) KRP95 (U00996) KIF3A (D12645) KIF3B (D26077) FLA10 (L33697)	KRP85/95	N-IV	Kinesin-II	Heterotrimeric
Kinesin-3	KIF1A (D29951) UNC104 (M58582) KIN (L07879) Unc104 (AF245277)	Unc-104/Kif1	N-III	Unc104	Monomeric
Kinesin-4	KIF4 (D12646) CHRKIN (U18309) XKLP1 (X82012) F11C1.80 (AB061676) AY224568 (AY224568) K8 (U69985)	Chromokinesin/Kif4	N-V	Chromokinesin (upper clade)	
Kinesin-5	KIF11 (AB001427) EG5 (X54002) BIMC (M32075) KRP125 (AC005896)	BimC	N-II	BIMC	Eg5 Bipolar Tetrameric
Kinesin-6	MKLP1 (X67155) KIF20A (NM_009004) K12 (AY484465)	MKLP1	N-VI	MKLP (lower clade)	Cho1
Kinesin-7	CENP-E (Z15005) KIF10 (AB001426) KIP2p (Z11963) AB028470 (AB028470)	CENP-E	N-VII	CENP-E	
Kinesin-8	KIF19A (AB054026) KIP3p (Z72739) T9C5.240 (NM_114825)	Kip3	N-IIX	Kip3	
Kinesin-9	KIF9 (AJ132889) KLP1 (X78589)			MKLP (middle clade)	
Kinesin-10	Nod (M38195)[f] KID (AB017430) KIF22 (NM_145588) T1E22.130 (NM_120315)			Chromokinesin (lower clade)	Kid
Kinesin-11	VAB-8 (NM_070062) KIF26A (XM_138275) SMY1p (M69021)[f]			Divergent Kinesin-I	
Kinesin-12	KIF15 (AJ560623) Xklp2 (X94082) PaKRP1 (NM_117492) 3g23670 (NM_113271) JGI_3356[g]			MKLP (upper clade)	
Kinesin-13	MCAK (U11790) KIF2 (D12644) 3g16060 (NM_112476) DSK1 (U51680)	MmKif2, MCAK/Kif2	M	I-Type	KinI
Kinesin-14	NCD (X52814) KIFC1 (D49544) KAR3p (M31719) KATA (D11371) KCBP (L40358)	C-Terminal Motor	C	C-Type	
Orphan Kinesins[h]		Orphans		Orphans	Ungrouped

Structure of Different Kinesin Families





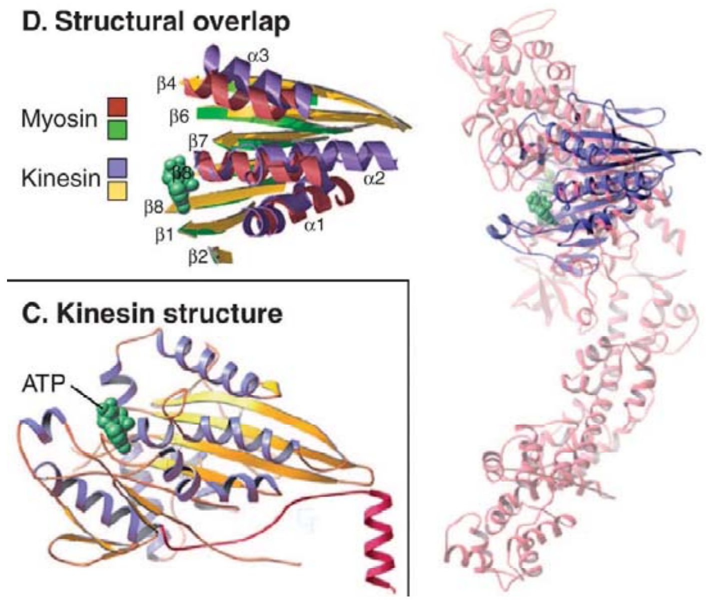
Vale RD, *Cell*, 112:467,2003

Mechanical Parameters of Kinesin

- Velocity
  - typically ~ 1 μm/sec
- Stall force
  - up to 7 pN
- Step
  - 8nm (size of tubulin heterodimer)
- Run length
  - typically ~1 μm

Kinesin and Myosin are Structurally Similar

- Kinesin head is less than half of the size of a myosin head.
- Kinesin and myosin lack similarity in amino acid sequence.
- Kinesin head is folded in a way similar to the ATP binding core of the myosin head.



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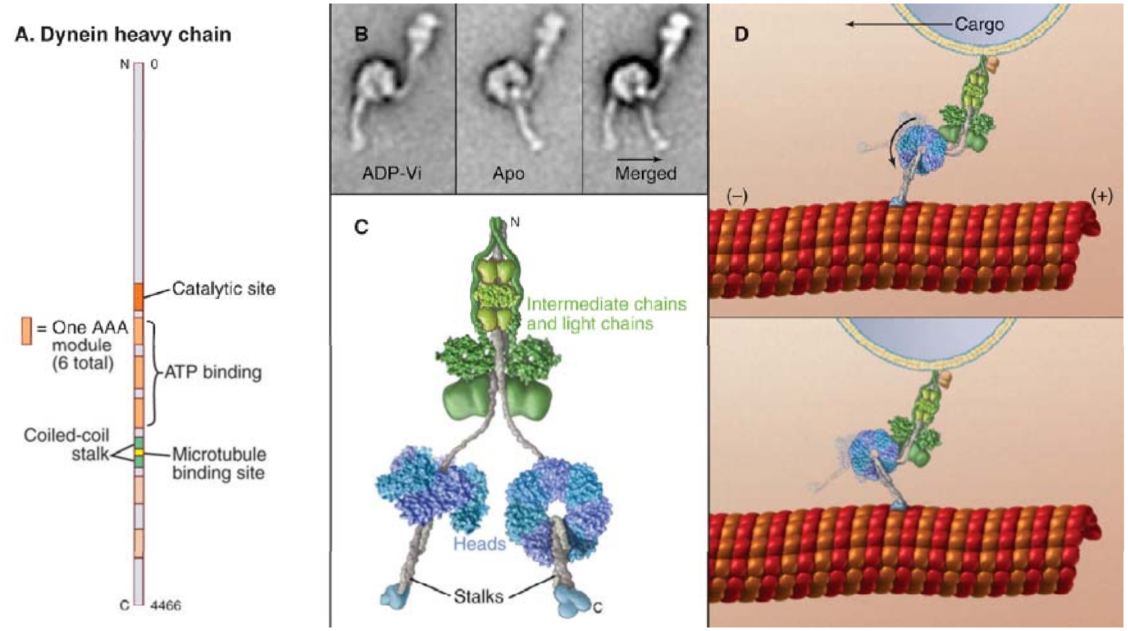
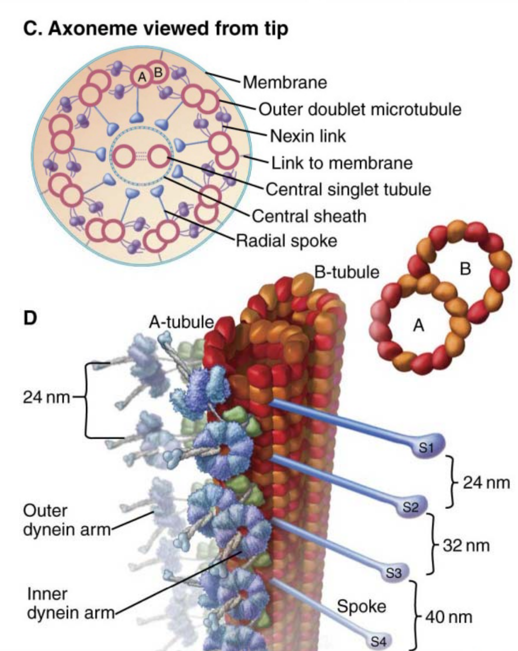
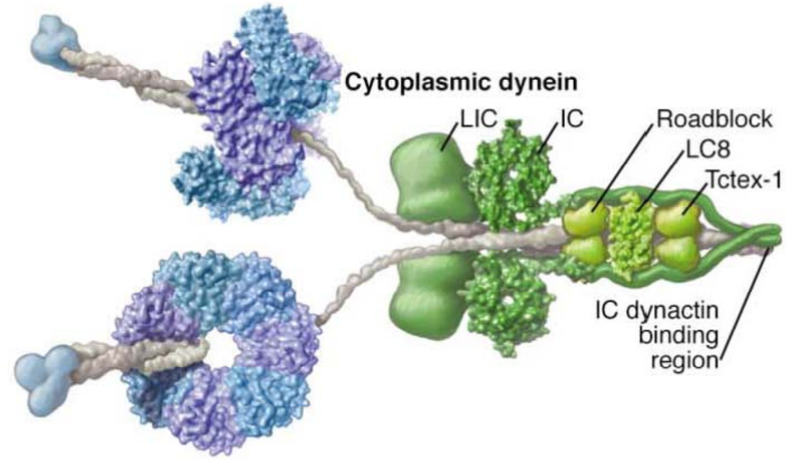
Dynein

- Two classes
  - axonemal dynein: cilia and flagella
  - cytoplasmic dynein
- Subunits
  - DLC
  - DLIC
  - DIC
  - DHC

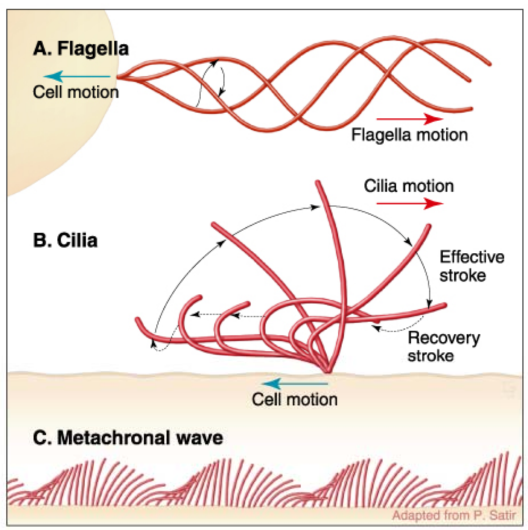


Axonemal and Cytoplasmic Dynein





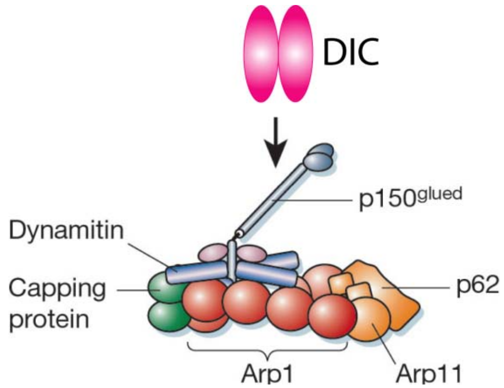
Vale RD, *Cell*, 112:467,2003



Dynein: Basic Parameters

- Function in vivo requires dynactin

Schroer TA, Dynactin, Annu. Rev. Cell Dev. Biol., 20:759-79, 2004



Adapted from Schliwa & Woehlke, *Nature*, 422:759, 2003

- Velocity

  axonemal: can be up to 7 µm/sec

  cytoplasmic: typically ~ 1 µm/sec

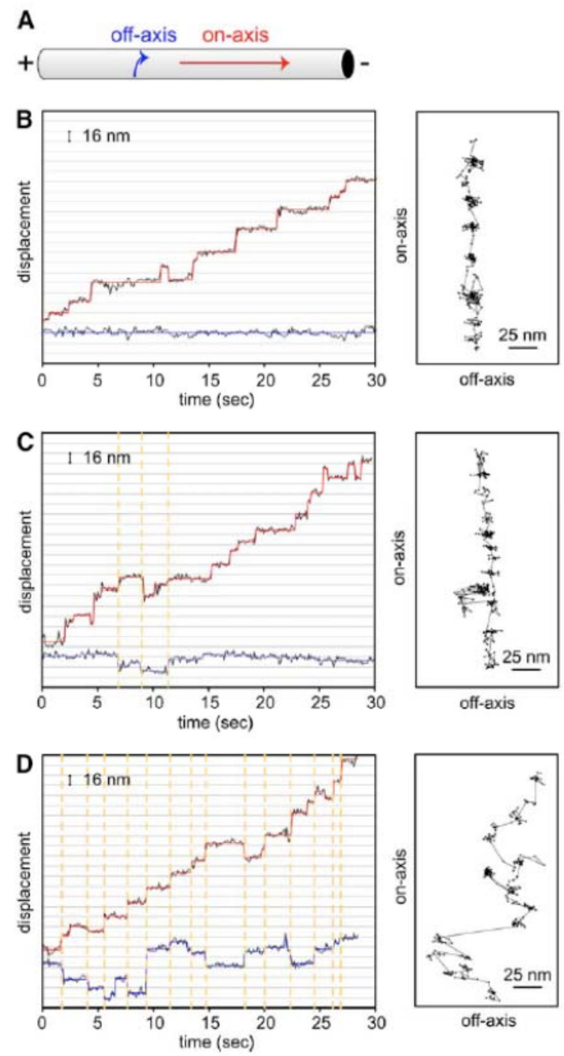
- Stall force

  up to 7 pN

- Step: multiples of 8nm

Dynein: Processivity

- Processivity of dynein is relatively poor.
- Dynein can undergo lateral and backward motion on microtubule.



Gennerich, A., Carter, A.P., Reck-Peterson, S.L. and Vale, R.D. (2007) Force-induced bidirectional stepping of cytoplasmic Dynein. *Cell* 131: 952.

The Rotating Crossbridge Model

- Motors cycle through attached and detached states.
- Motors undergo amplified conformational change during attached state.
- Motors undergo conformational recovery during detached state.

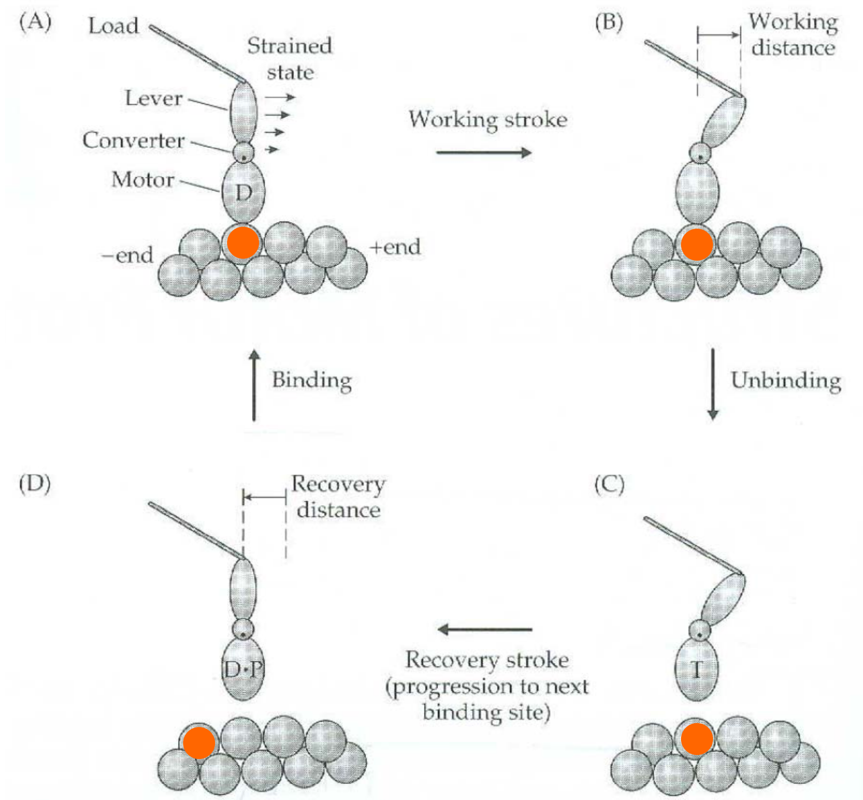


Figure 12.1 The rotating crossbridge model for myosin
(A) The binding of myosin to the actin filament catalyzes the release of phosphate from the motor domain and induces the formation of a highly strained ADP state. (B) The strain drives the rotation of the converter domain, which is connected to a lever domain that amplifies the motion, moving the load through the working distance. (C) Following ADP release, ATP binds to the motor domain and causes dissociation of myosin from the actin filament. (D) While dissociated, the crossbridge recovers to its initial conformation, and this recovery moves the motor toward its next binding site on the filament. T = ATP, D = ADP, P = Pi.

Jonathon Howard, Mechanics of Motor Proteins and the Cytoskeleton, Sinauer Associates, 2001

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Polymer Mechanics (I)

- Rationale: characterizing mechanical properties of individual filaments as a starting point for understanding mechanical properties of tissues and organs.

- Polymer mechanics is an established research field.
  - A classic treatment: M. Doi & S.F. Edwards, *The theory of polymer dynamics*, Oxford University Press, 1986.

- Investigating the mechanics of biopolymers in cells is a very active research field.

Polymer Mechanics

- We will take a simplified approach here.
- Theory of elasticity holds at the scale of single filaments.
- Cytoskeleton polymers are modeled as thin and slender beams.

Basic Properties

- Bending rigidity
- Drag coefficient
- Buckling force
- Persistence length

Bending Rigidity

- Basic equation

$$M = EI\frac{1}{R}$$

R: Radius of curvature
M: Torque; bending moment

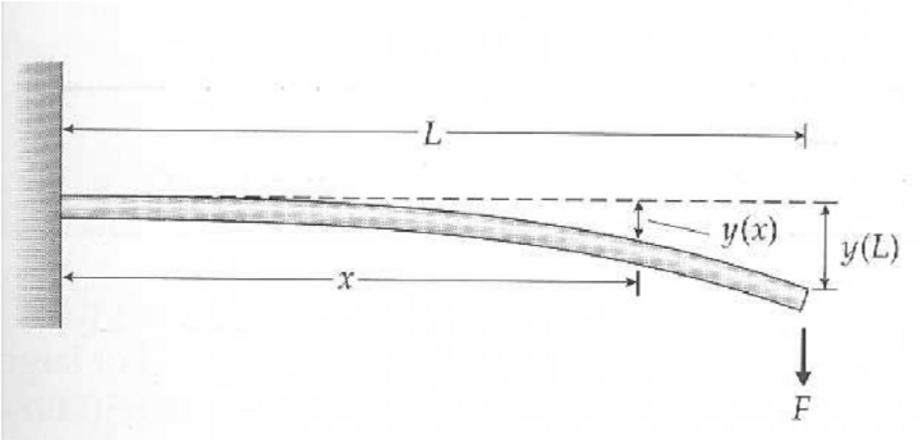
E: Young's modulus
I: second moment of inertia
EI: bending (flexural) rigidity

- Bending rigidity of cytoskeletal filaments is generally independent of bending direction since cytoskeletal filaments have approximately circular or helical symmetry.

Cantilever Beam Under Small-Angle Bending

- Deflection

$$y(x) = \frac{F}{EI}\left(\frac{Lx^2}{2} - \frac{x^3}{6}\right)$$



- Spring constant

$$k = \frac{F}{y(L)} = \frac{3EI}{L^3}$$

Mechanics of Materials, 7th ed., J. M. Gere & B. J. Goodno, 2008

Examples: cantilever beam models

- Glass cantilever beam

  $r = 0.25\mu m$, $L = 100\ \mu m$
  E=70 GPa, $I=(\pi/4)r^4=3\times10^{-27}$

  k=0.64 pN/nm

- Microtubule

  $EI=30\times10^{-24} N\cdot m^2$. $L = 10\ \mu m$
  $k = 0.00009$ pN/nm

- Coiled coil

  $EI=400\times10^{-30} N\cdot m^2$. $L = 8$ nm
  $k = 2.34$ pN/nm

Questions ?