BME 42-620 Engineering Molecular Cell Biology

Lecture 11:

Review: Systems Biology

The Cytoskeleton (III): Molecular Motors

Course Administration Notes (I)

- **Correction:** The first group presentation is moved to Oct-18 due to a schedule conflict. No class on Oct-13.

- Substitution lecture for Oct-13: You will be asked to choose and watch a seminar from http://www.ibioseminars.org/. A list will be provided. A one-page summary report is required. More details to come.

- Statistics on the first reading assignment

  MEAN=15.8 (out of 20); STD=1.8; MIN = 12; MAX = 19

- Midterm exam: October 20, 2011; Take-home exam; Instructions handed out at the end of the class.

Course Administration Notes (II)

- Midterm exam format:

  1) conceptual and factual questions based on lectures and the textbook.
  2) a literature-based research project;
  3) Due October 24 Monday 12:00Noon at Mellon Institute 403;
  4) Regular lectures will continue as scheduled.

- Comment on providing learning material to more advanced students.

- General comments on this class

  - A main goal of this course is to help you to develop your scientific (i.e. analytical, rational, and critical) thinking of cell biology.

  - Another main goal of this course is to help you to develop skills for self-learning of cell biology.

Outline

- Review: systems biology
- An overview of molecular motors
- The myosin superfamily; Myosin motility
- The kinesin superfamily; Kinesin motility
- The dynein family; Dynein motility

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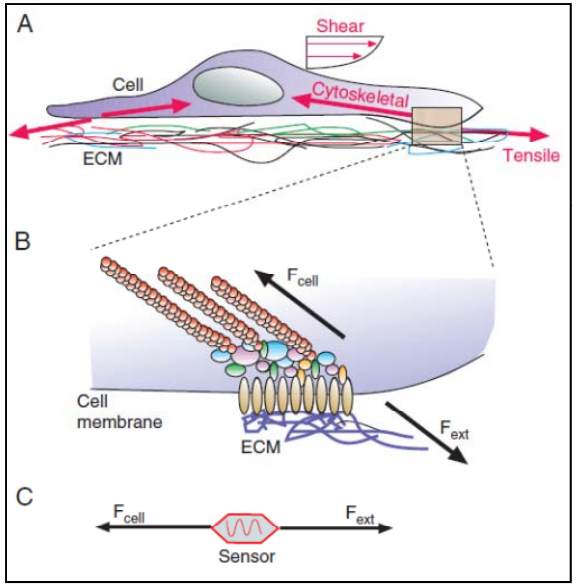
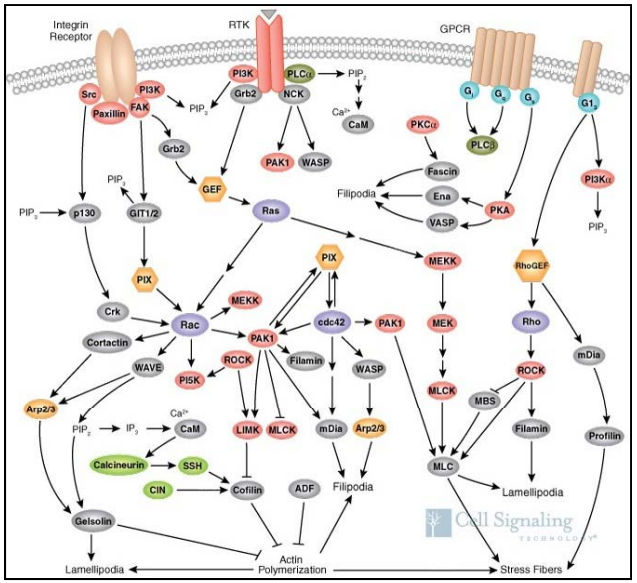
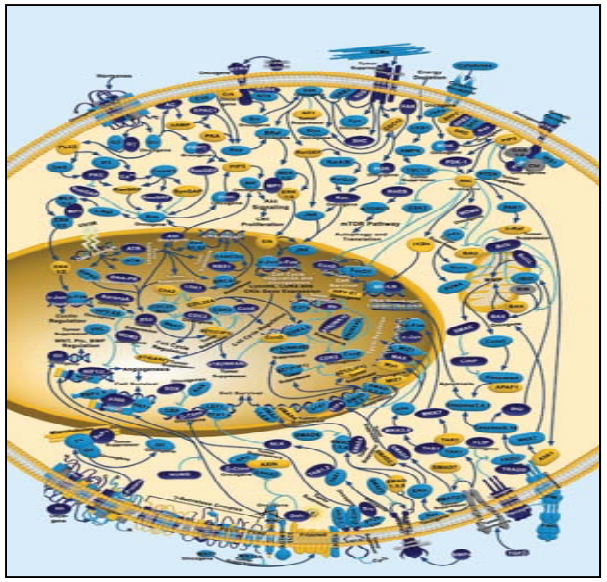
Cells Process Information Using Complex Signaling Pathways



Environment sensing



Actin regulation pathway



Human cancer pathways

Different Perspectives of Systems Biology (I)

- “Although we can connect structure to the gene, we can no longer infer its larger purpose in the cell or in the organism. There are too many purposes; what the protein does is defined by context. The context also includes a history, either developmental or physiological.” -MWK

- “To understand the genetic basis of disease will require not just mapping these genes but an understanding of how the phenotype is created in the first place and the messy interactions between genetic variation and environmental variation.” –MWK

- “Later John Gerhart and I tried to think about the connections among cell biology, biochemistry, development, and evolution. We looked at biology in terms of conserved processes and circuits and asked what features were selected and what changes occurred in evolution. We summarized our findings in a book that took almost a decade to write. By that time I had unconsciously become a systems biologist, awaiting, I assume, merely social acceptance of the term.” -MWK

Different Perspectives of Systems Biology (II)

- …I would simply say that systems biology is the study of the behavior of complex biological organization and processes in terms of the molecular constituents. It is built on molecular biology in its special concern for information transfer, on physiology for its special concern with adaptive states of the cell and organism, on developmental biology for the importance of defining a succession of physiological states in that process, and on evolutionary biology and ecology for the appreciation that all aspects of the organism are products of selection, a selection we rarely understand on a molecular level. -MWK

Different Perspectives of Systems Biology (III)

- "We argue here for the recognition of functional 'modules' as a critical level of biological organization. Modules are composed of many types of molecule. They have discrete functions that arise from interactions among their components (proteins, DNA, RNA and small molecules), but these functions cannot easily be predicted by studying the properties of the isolated components." –LHH et al

- "Other aspects of functional modules are less familiar to engineers. Several can be subsumed under the idea that the rules for a module's function are rigidly encoded in the structures of its proteins, but produce messy, probabilistic intermediates that are then refined to give unique solutions." –LHH et al

Different Perspectives of Systems Biology (IV)

- “Finally, we emphasize the importance of integrating experimental approaches with modeling and conceptual frameworks. The best test of our understanding of cells will be to make quantitative predictions about their behavior and test them. This will require detailed simulations of the biochemical processes taking place within the modules. But making predictions is not synonymous with understanding. We need to develop simplifying, higher-level models and find general principles that will allow us to grasp and manipulate the functions of biological modules.” –LHH et al

Different Perspectives of Systems Biology (V)

- “A system-lever understanding of a biological system can be derived form insight into four key properties: 1) system structures; 2) system dynamics; 3) The control method; 4) The design method .” –HK

- “Whether it is to obtain an in-depth understanding of system behavior or to predict complex behaviors in response to complex stimuli, we must first define the scope and abstraction level of the model.” –HK

- “To conduct a systems-level analysis, a comprehensive set of quantitative data is required. Complete system-level analysis of biological regulation requires high throughput and accurate measurements, goals that are perhaps beyond the scope of current experimental practices.” –HK

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- An overview of molecular motors
- The myosin superfamily; Myosin motility
- The kinesin superfamily; Kinesin motility
- The dynein family; Dynein motility

Intracellular Force & Motion Producers

Table 36-1

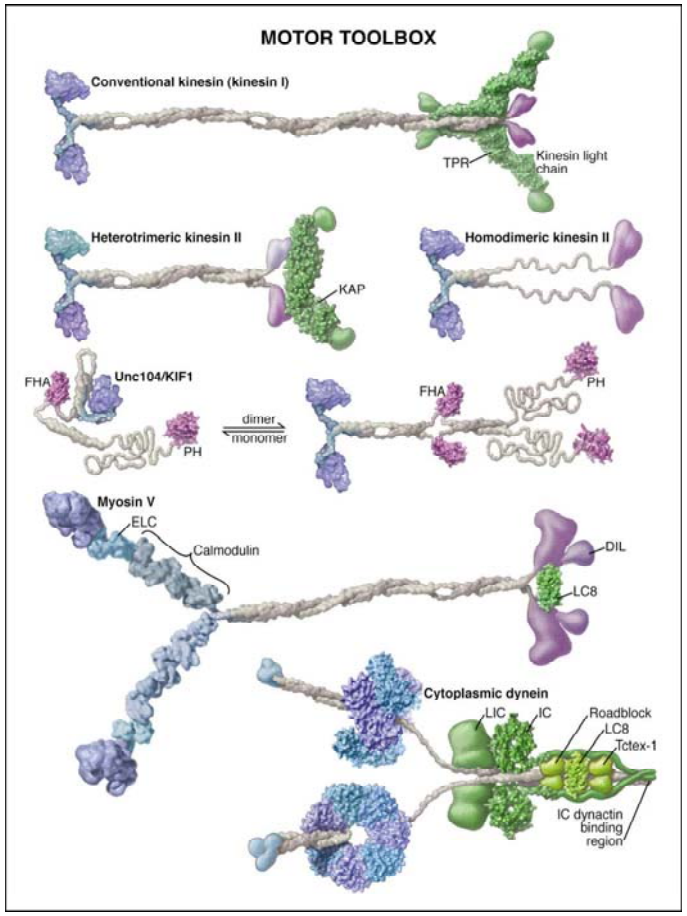
EXAMPLES OF MECHANOCHEMICAL ATPases AND OTHER SYSTEMS

Families	Track	Direction	Cargo	Energy
ATPases				
Myosins				
Muscle myosin	Actin	Barbed end	Myosin filament	ATP
Myosin II	Actin	Barbed end	Myosin, actin	ATP
Myosin I	Actin	Barbed end	Membranes	ATP
Myosin V	Actin	Barbed end	Organelles	ATP
Myosin VI	Actin	Pointed end	Endocytic vesicles	ATP
Dyneins				
Axonemal	Microtubule	Minus end	Microtubules	ATP
Cytoplasmic	Microtubule	Minus end	Membranes, chromosomes	ATP
Kinesins				
Conventional	Microtubule	Plus end	Membranes, intermediate filaments	ATP
Ncd	Microtubule	Minus end	? Microtubules	ATP
Other Mechanochemical Systems				
Polymerases				
Ribosome	mRNA	5′ to 3′	None	GTP
DNA polymerase	DNA	5′ to 3′	None	ATP
RNA polymerase	DNA	5′ to 3′	None	ATP
Conformational System				
Spasmin/centrin	None	None	Cell, basal body	Ca^{2+}
Polymerizing Systems				
Actin filaments	None	Barbed end	Membranes	ATP
Microtubules	None	Plus end	Chromosomes	GTP
Worm sperm MSP	None	Not polar	Cytoskeleton	
Rotary Motors				
Bacterial flagella	None	Bidirectional	Cell	H^+ or Na^+ gradient
F-type ATPase	None	Bidirectional	None	H^+ or ATP
V-type ATPase pump	None		None	ATP

mRNA, messenger RNA; MSP, major sperm protein.

Overview of Molecular Motors

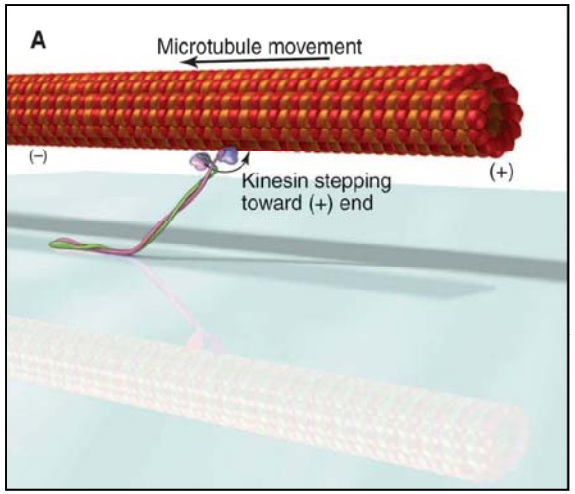
- Myosin walks on actin filaments.
- Kinesin and dynein walks on microtubule.
- Motor (head) domain
  - Produces force and motion
- Tail domain
  - Adapts to different cargoes

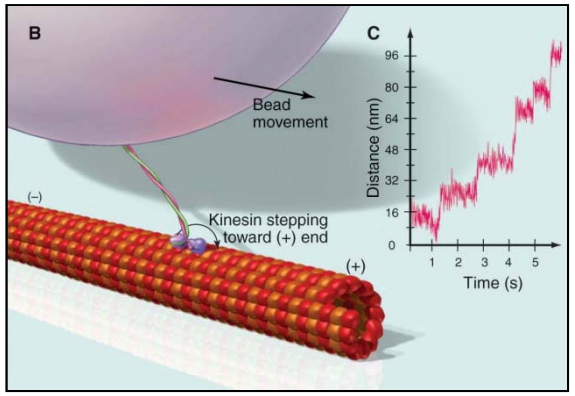


Vale RD, *Cell*, 112:467,2003

Molecular Motors and Motility Assays

- Actin motor
  - myosin
  - Usually for short-distance movement
- Microtubule motors
  - kinesin
  - dynein
  - Usually for long-distance movement
- In vitro motility assays
  - bead assay
  - microtubule sliding assay



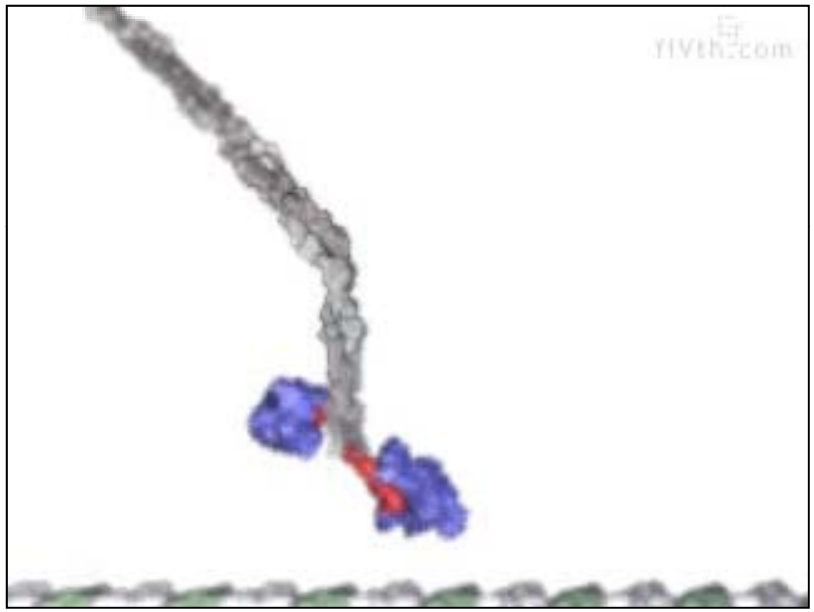


Bead Motility Assay Video

kinesin-coated bead moves along a microtubule

Block Lab, Stanford

How Kinesin-1 Moves?



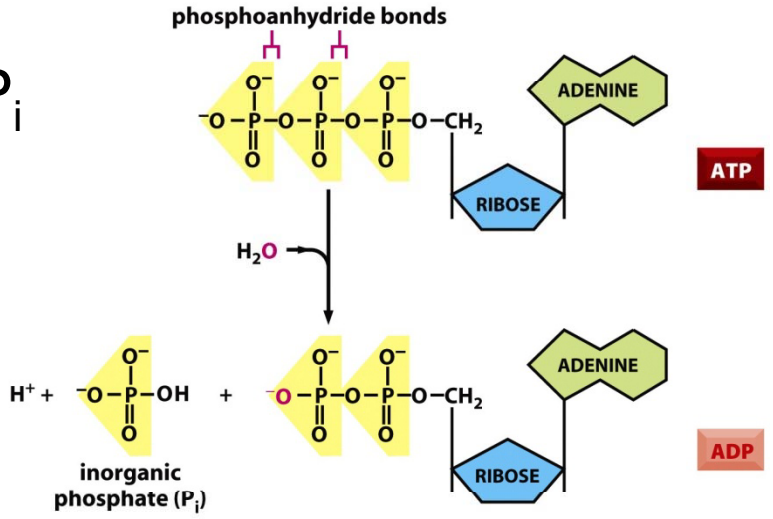
http://www.scripps.edu/cb/milligan/

Molecular Motors Are ATP-Hydrolysis Enzymes

- Molecular motors convert chemical energy derived from ATP hydrolysis directly into mechanical work.

- ATP (adenosine triphosphate) hydrolysis

$$ATP + H_2O \rightleftharpoons ADP + P_i$$



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.

Analyzing Motor Movement at Nanometer Resolution

- Nanometer-resolution measurement of step sizes
  - First implemented in late 1980's
  - Based on fitting of point spread function

  Tracking kinesin-driven movements with nanometre-scale precision. Gelles J, Schnapp BJ, Sheetz MP. *Nature*. 331:450-3 (1988).

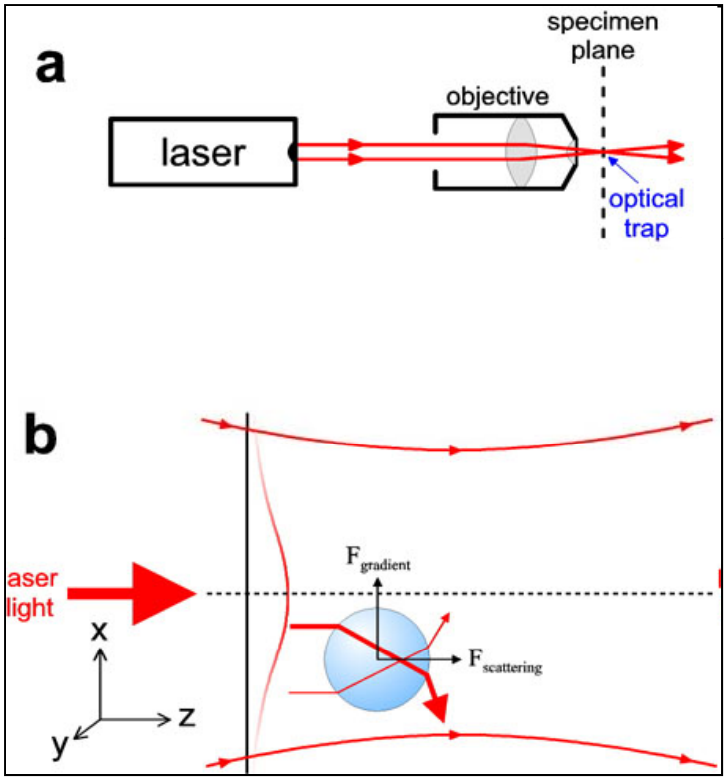
- Further improved by many others

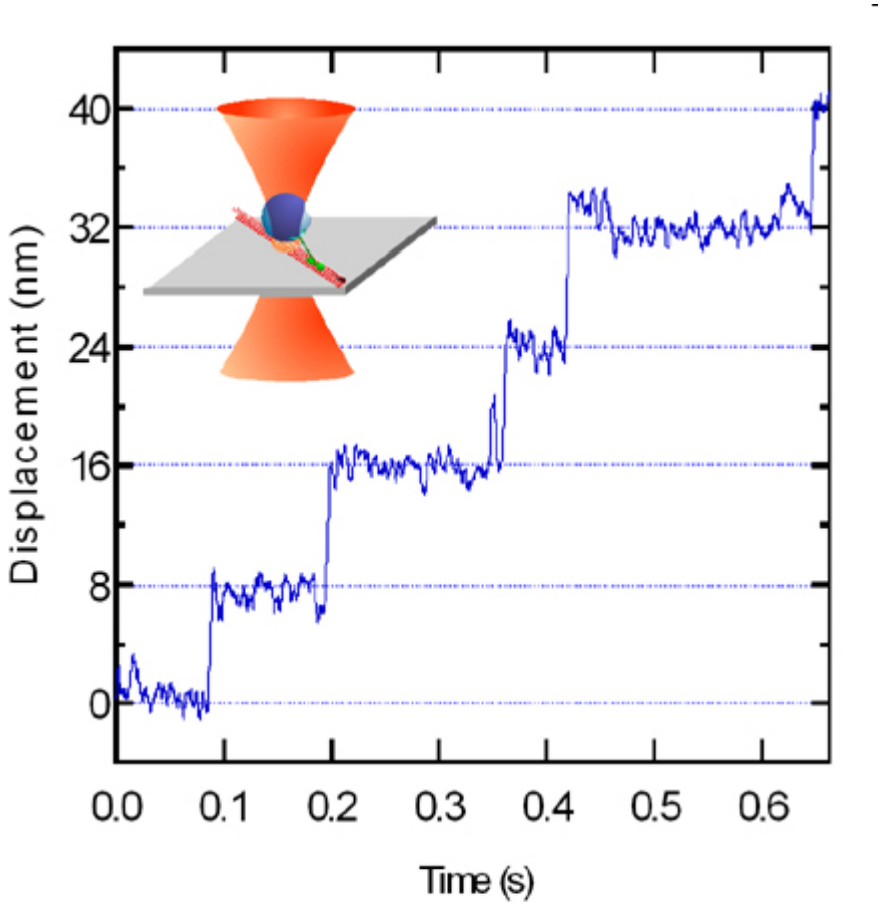
  - Up to 1nm resolution

  Ahmet Yildiz, Paul R.Selvin. Fluorescence Imaging with One Nanometer Accuracy (FIONA): Application to Molecular Motors, *Accounts of Chemical Research*,38(7), 574-82 (2005)

Analyzing Motor Force at Piconewton Resolution

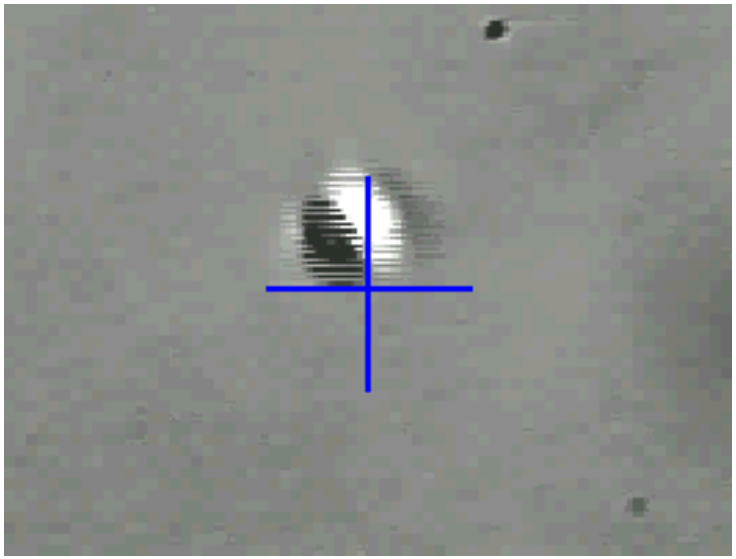
- Optical tweezer is used to generate and measure motor stall force.





From Steven Block Lab webpage
http://www.stanford.edu/group/blocklab/

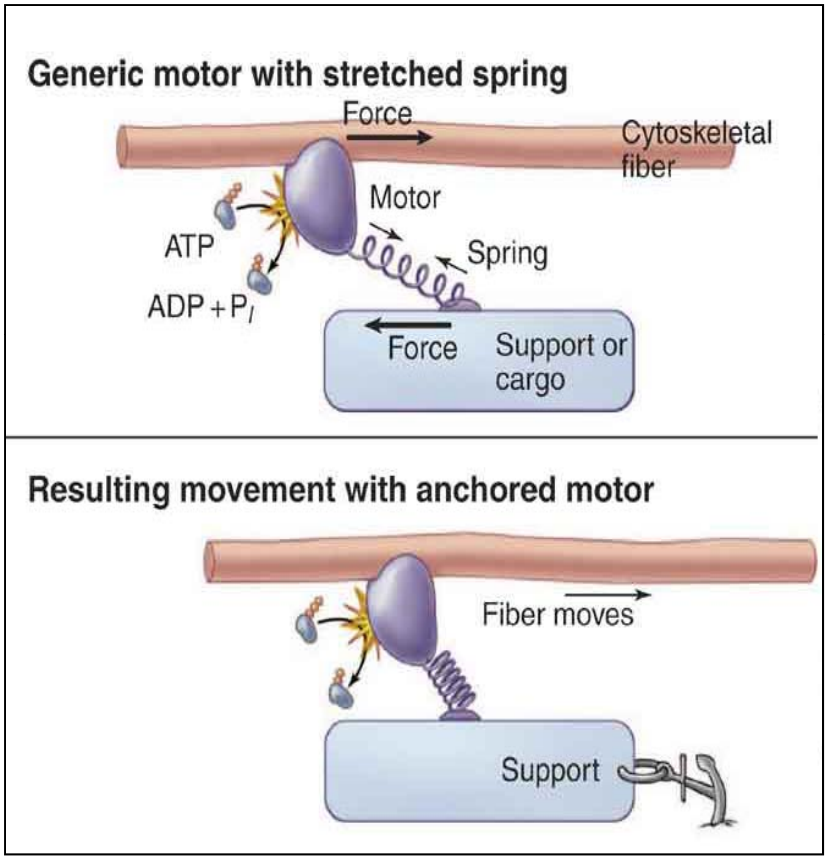
Laser Force Trap Video

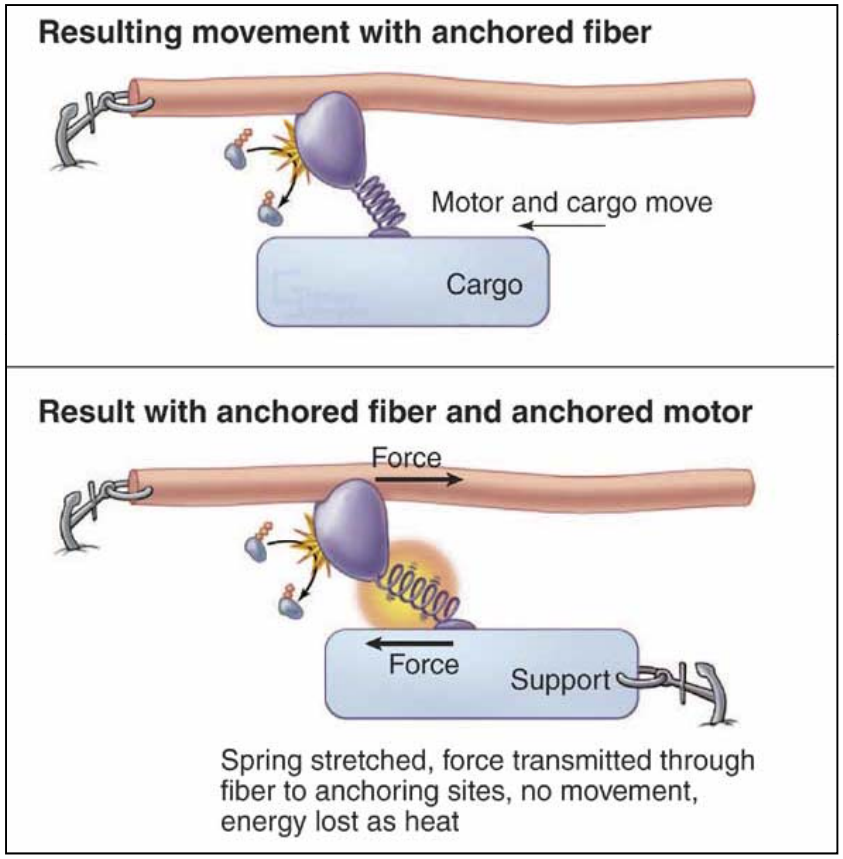


kinesin-coated bead moves in a force trap

Block Lab, Stanford

Different Motility Schemes





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.

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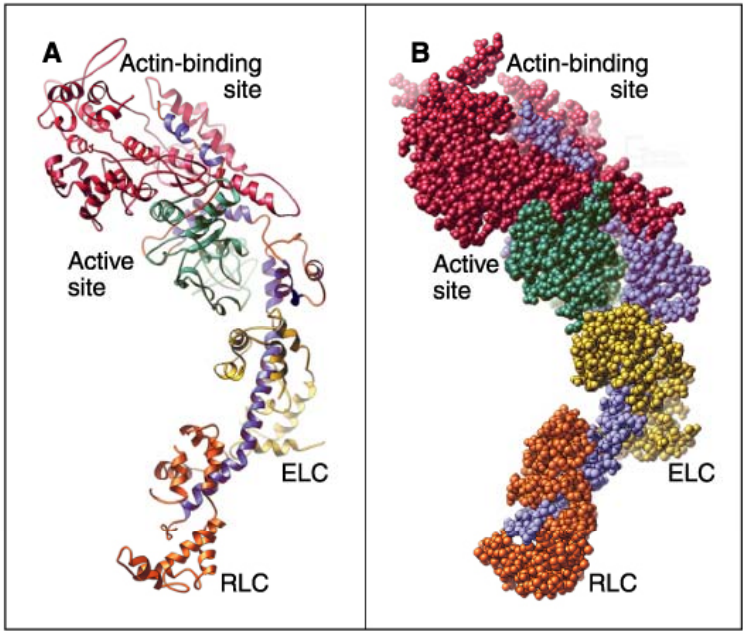
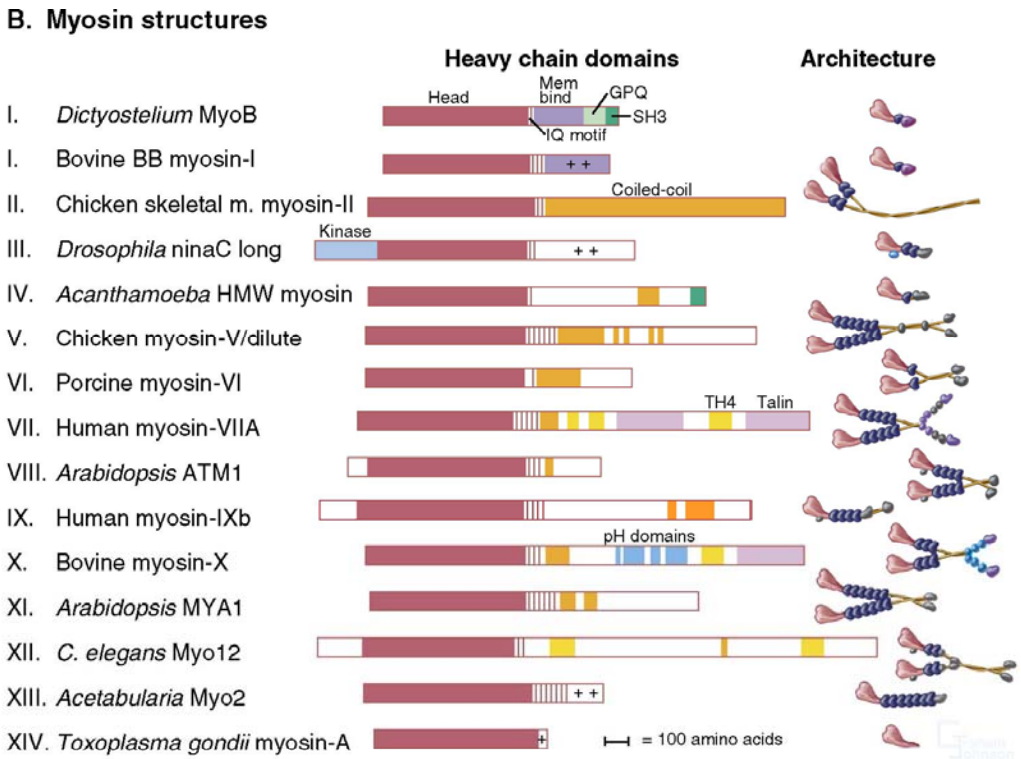
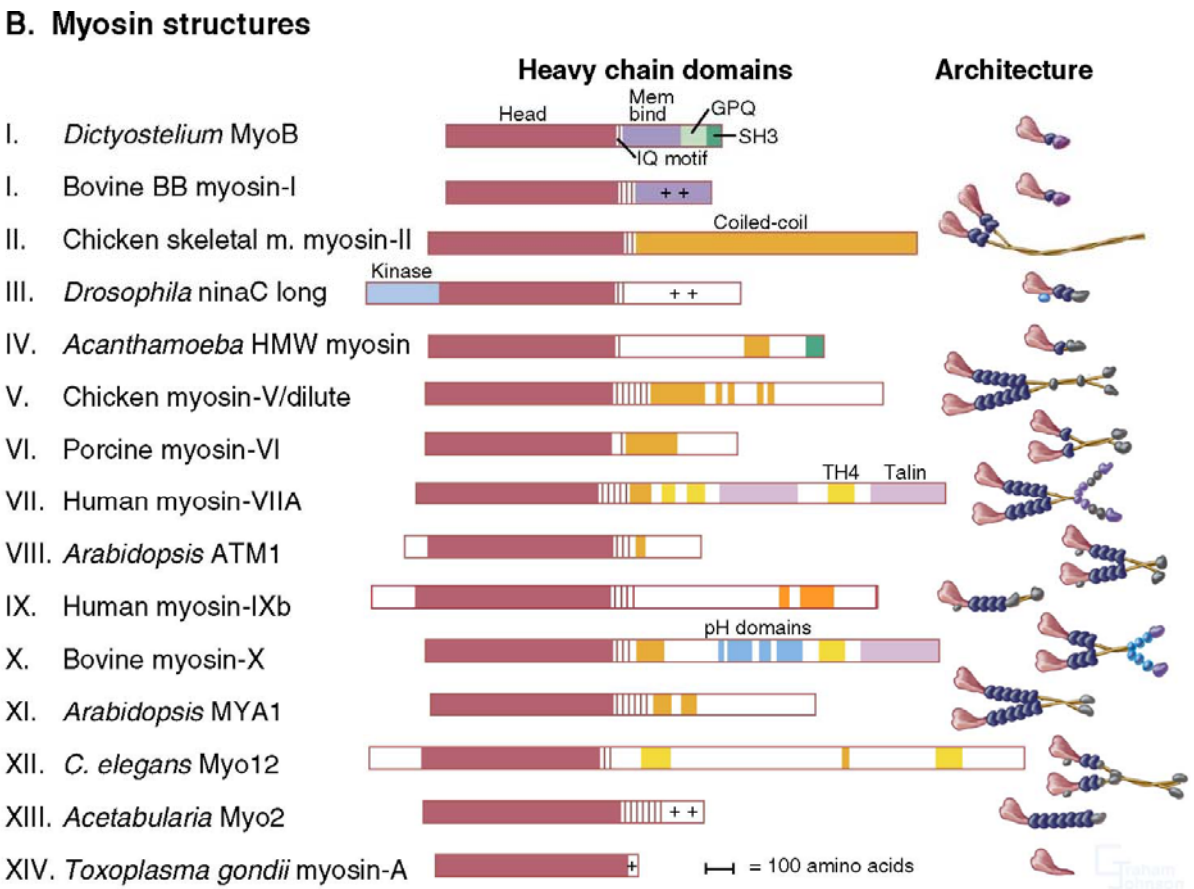
Atomic Structure of Head of Myosin-II

- Heavy chain
- Light chain
  - ELC
  - RLC





Myosin Family



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.

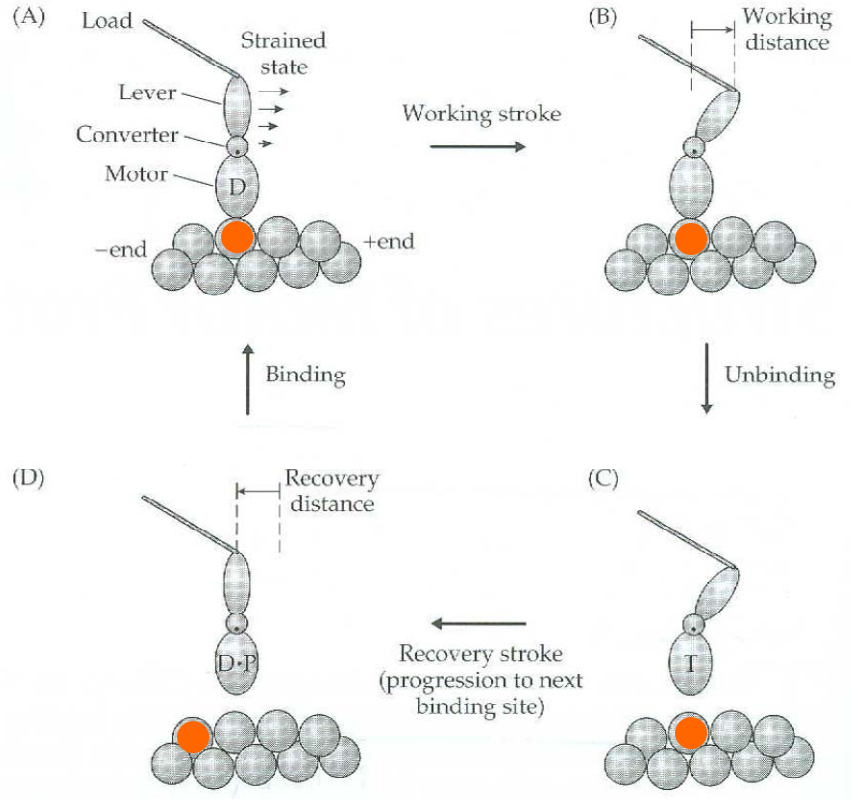


Figure 12.1 The rotating crossbridge model for myosin
(A) The binding of myosin to the actin filament catalyzes the release of phosphate from the motor domain and induces the formation of a highly strained ADP state. (B) The strain drives the rotation of the converter domain, which is connected to a 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

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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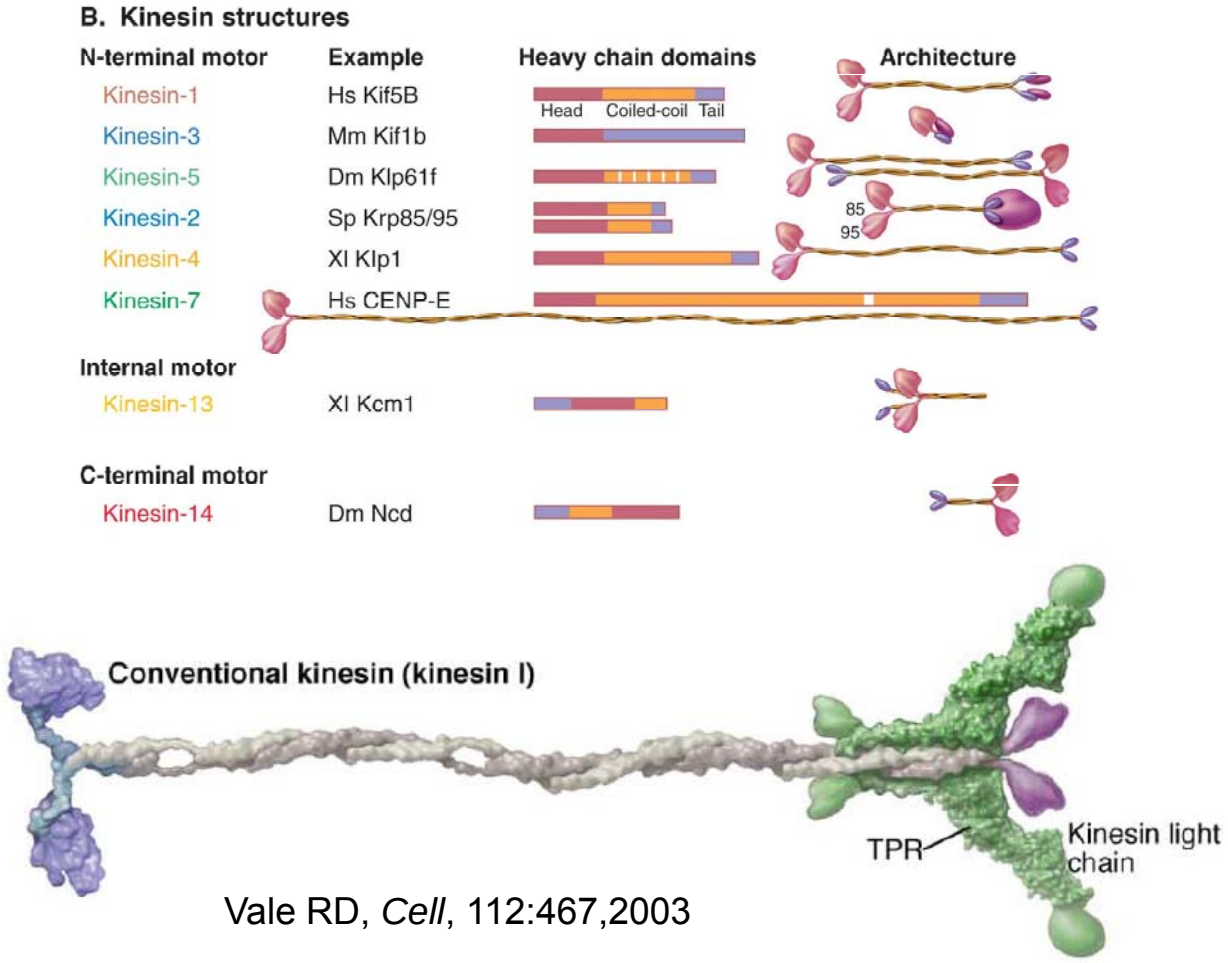
Kinesin Families

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 (M36195)[f] KID (AB017430) KIF22 (NM_145588) T1E22.130 (NM_120315)			Chromokinesin (lower clade)	Kid
Kinesin-11	VAB-8 (NM_073662) 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



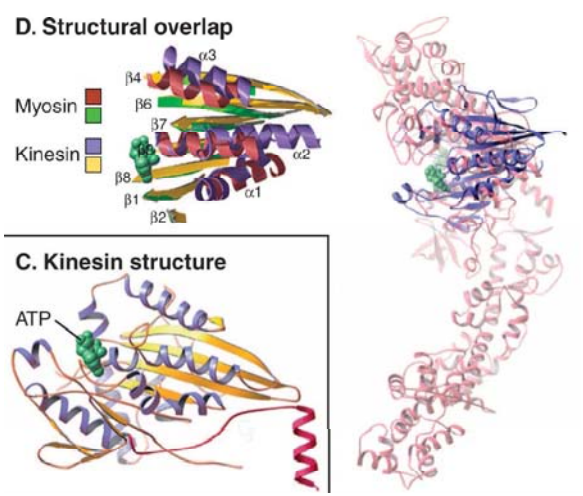
Vale RD, *Cell*, 112:467,2003

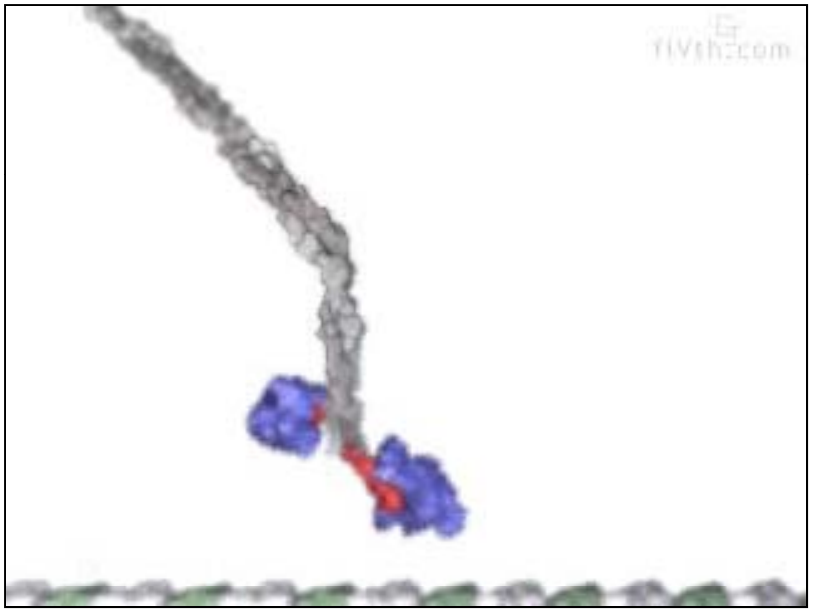
Mechanical Parameters of Kinesin

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

Structure and Motion of Kinesin-1

- Structural similarities between kinesin and myosin

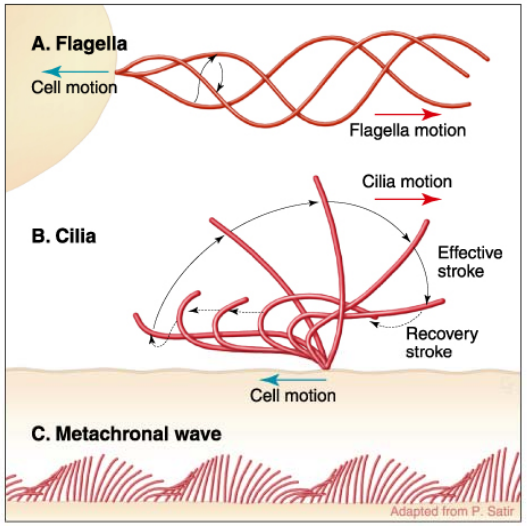




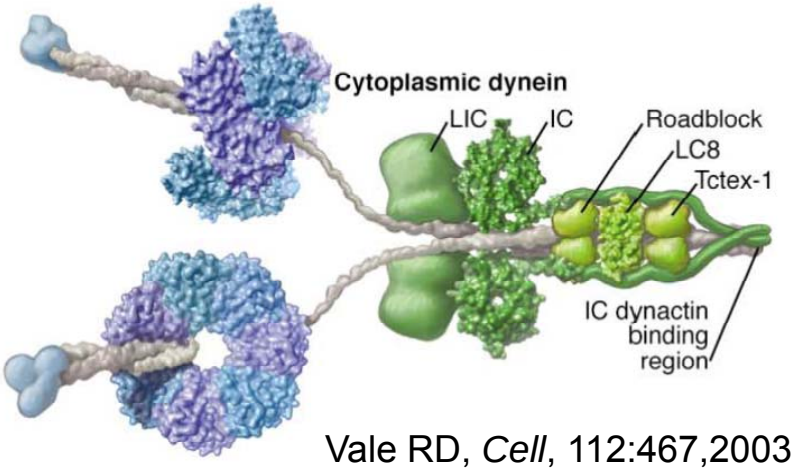
http://www.scripps.edu/cb/milligan/

Dynein

- Two classes
  - axonemal dynein: cilia and flagella
  - cytoplasmic dynein

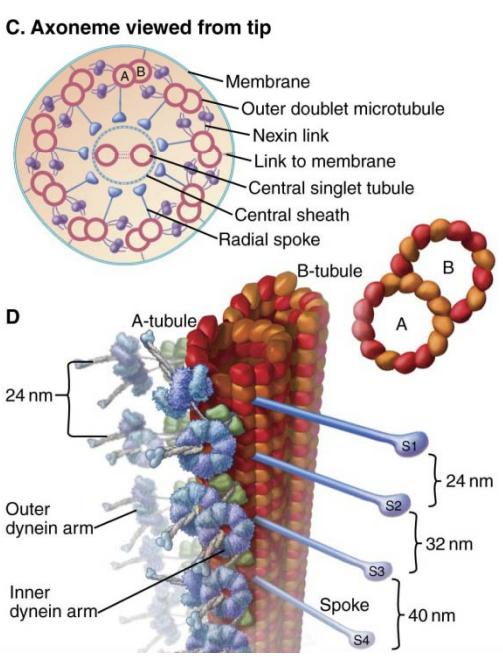


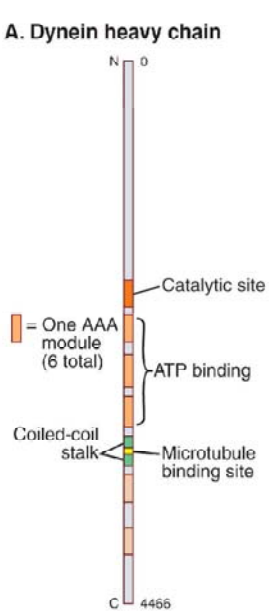
- Subunits
  - DLC
  - DLIC
  - DIC
  - DHC

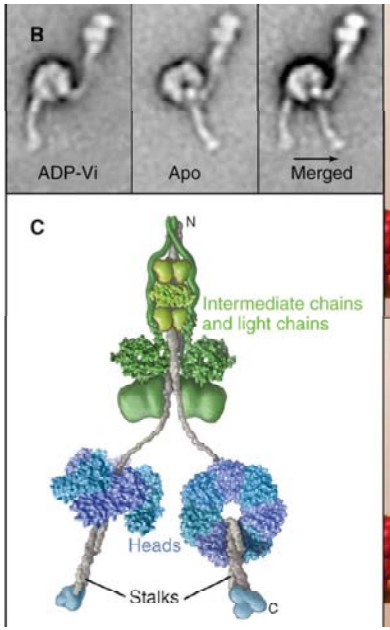


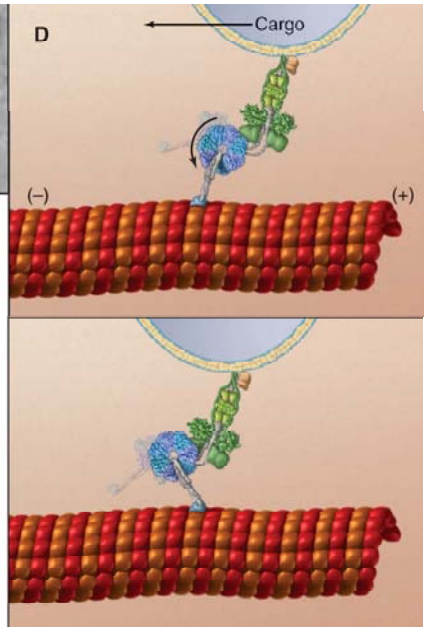
Vale RD, *Cell*, 112:467,2003

Axonemal and Cytoplasmic Dynein



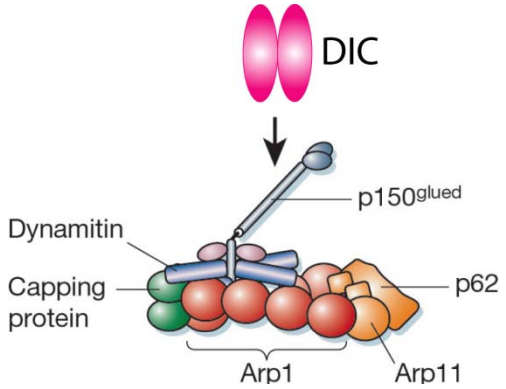






Dynein: Basic Parameters

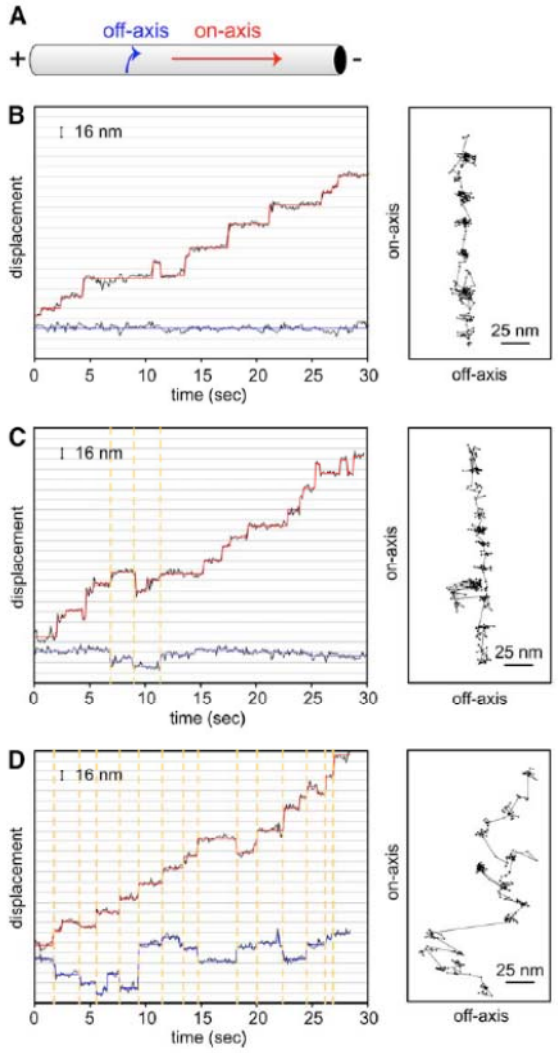
- Function in vivo requires dynactin
- Velocity
  - axonemal: can be up to 7 μm/sec
  - cytoplasmic: typically ~ 1 μm/sec
- Stall force
  - up to 7 pN
- Step: multiples of 8nm



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

Dynein: Processivity

- Poorly processive
- Frequent lateral and backward motion



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

Questions ?