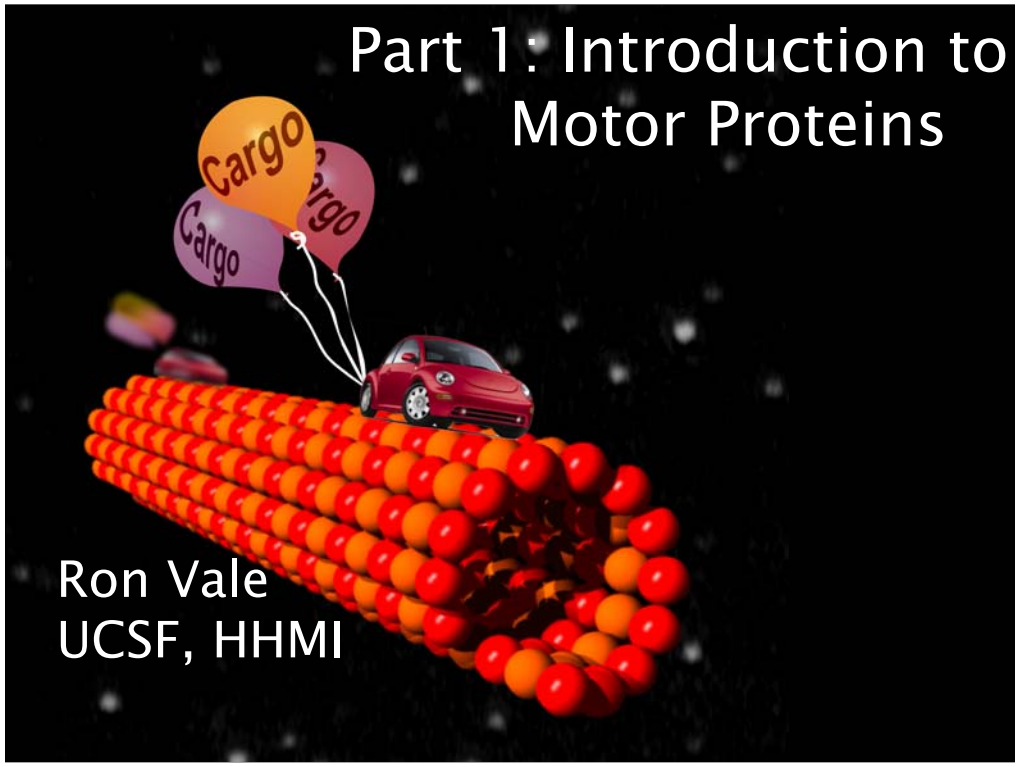
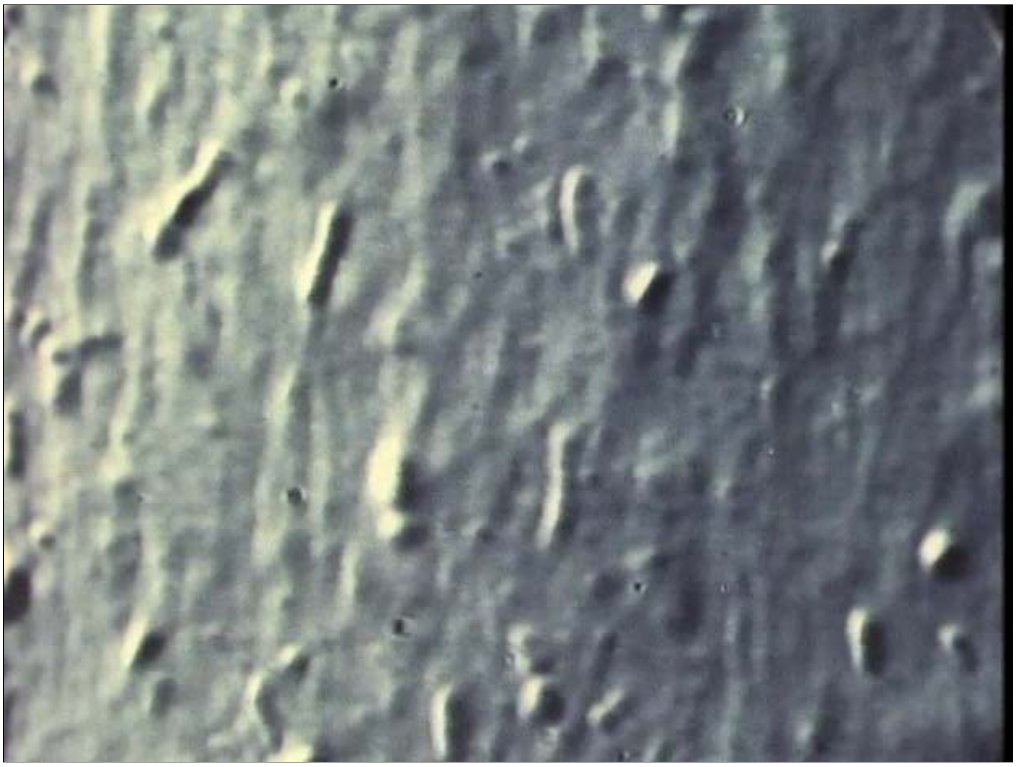
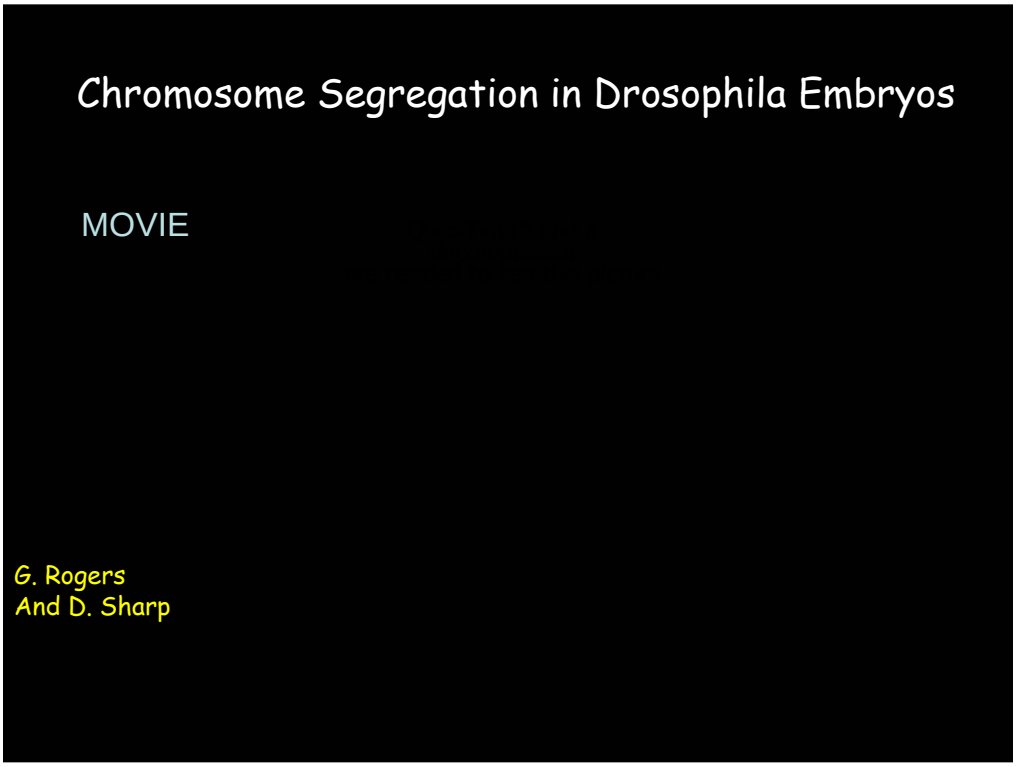


Wecome to iBioSeminars. In my lecture, I discuss cytoskeletal motors, remarkable protein machines that convert chemical energy into motion. Motion is one of the most obvious attributes of living organisms. We are familiar with the contraction of muscle or movement of ciliary and sperm. However, even within the interior of a cell, we find an environment that is teaming with motion.

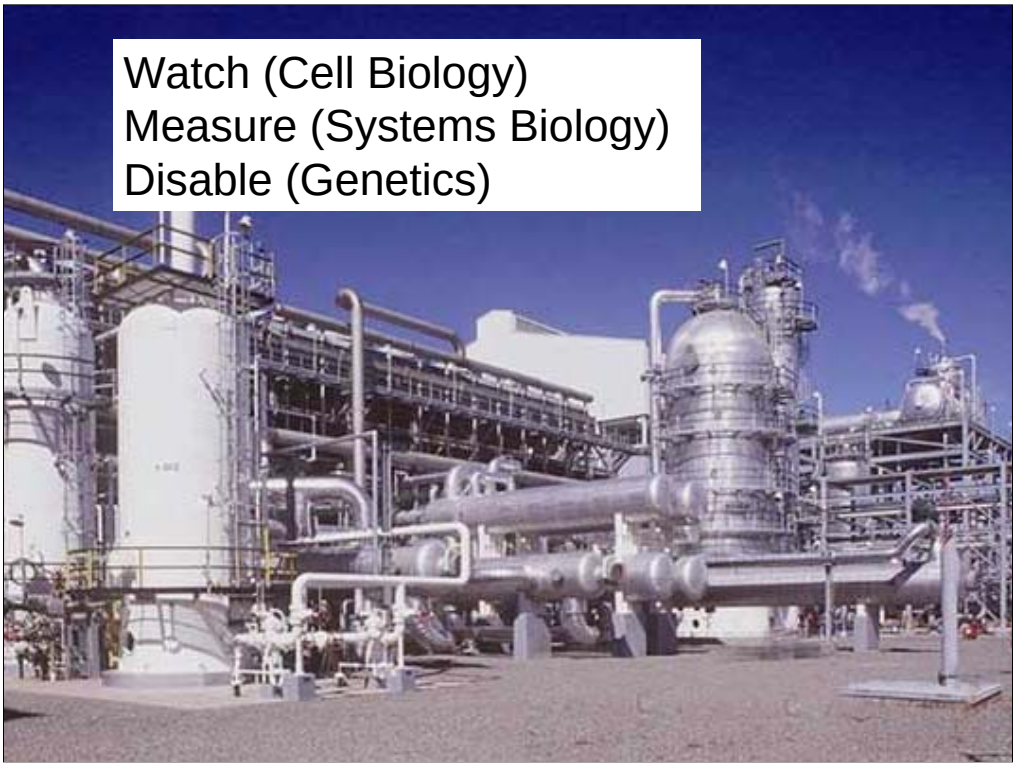


This video showing the classic work of Robert Allen and colleagues shows the interior of a squid giant axon. If we walk into the axon, we can see variety of small organelles and mitochondria that are moving in both directions between the cell body and the nerve terminal. This video shown in real time.



This is an another important example of intracellular movement- the process of mitosis in shown as a time lapse movie of Drosophila embryoes. The chromosomes in green align at the center of the mitotic spindle and then the sister chromatids physically move apart to segregate identical copies of the genetic material.

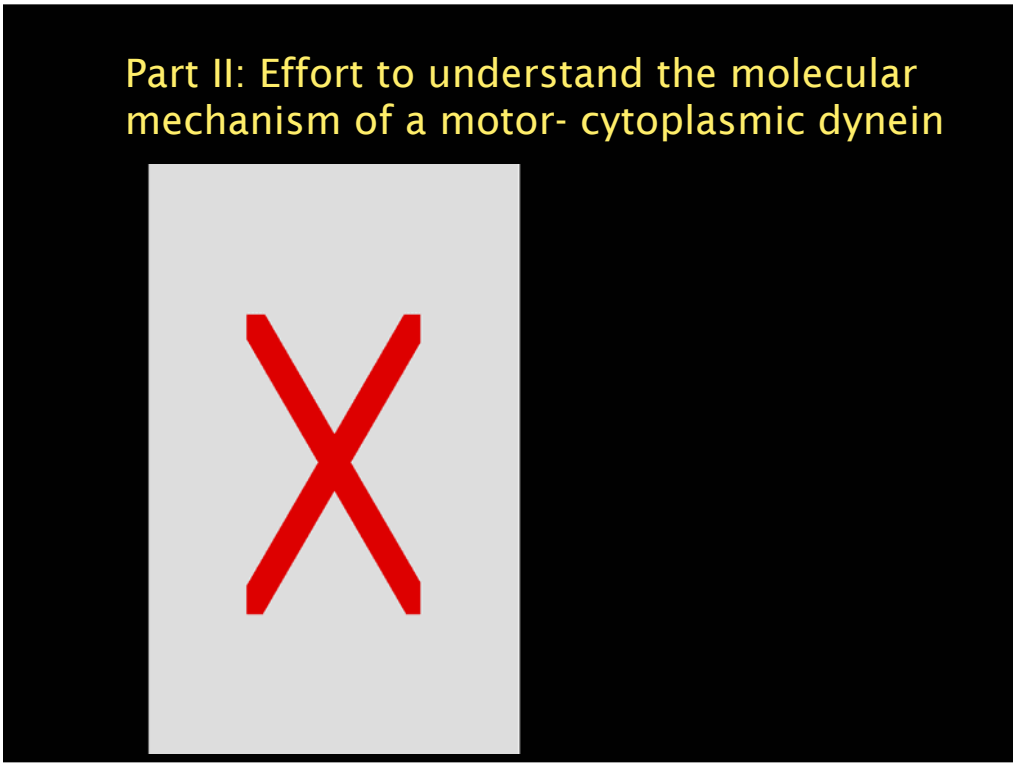
Xvivo Movie

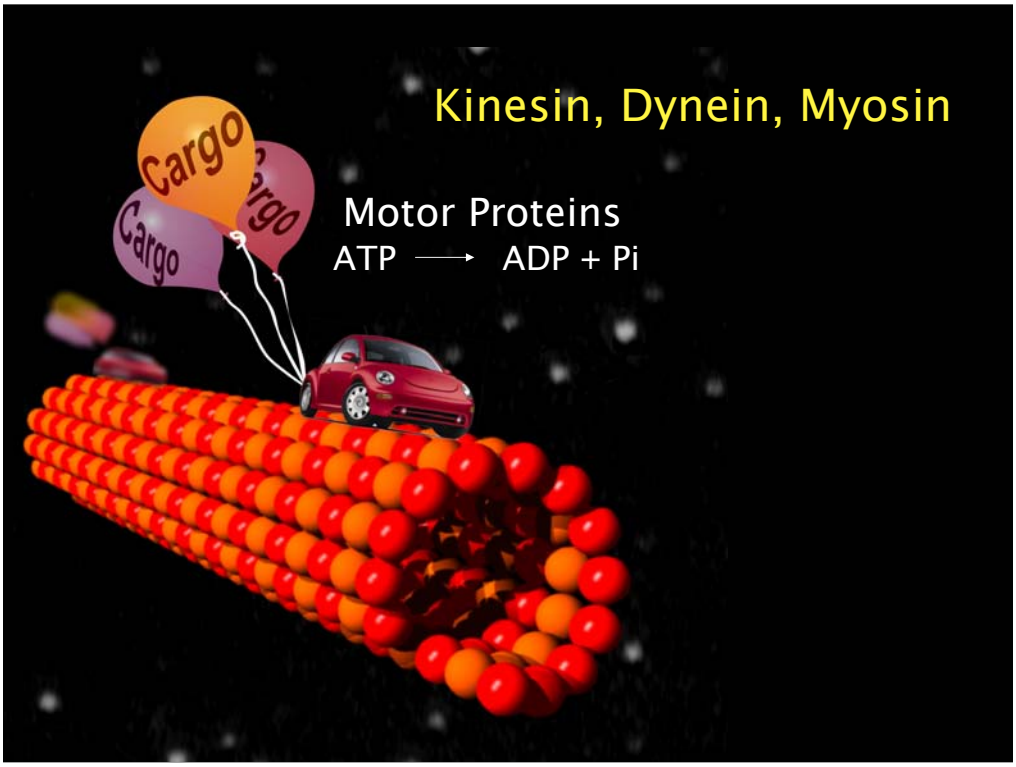

Watch (Cell Biology)
Measure (Systems Biology)
Disable (Genetics)

MOVIE

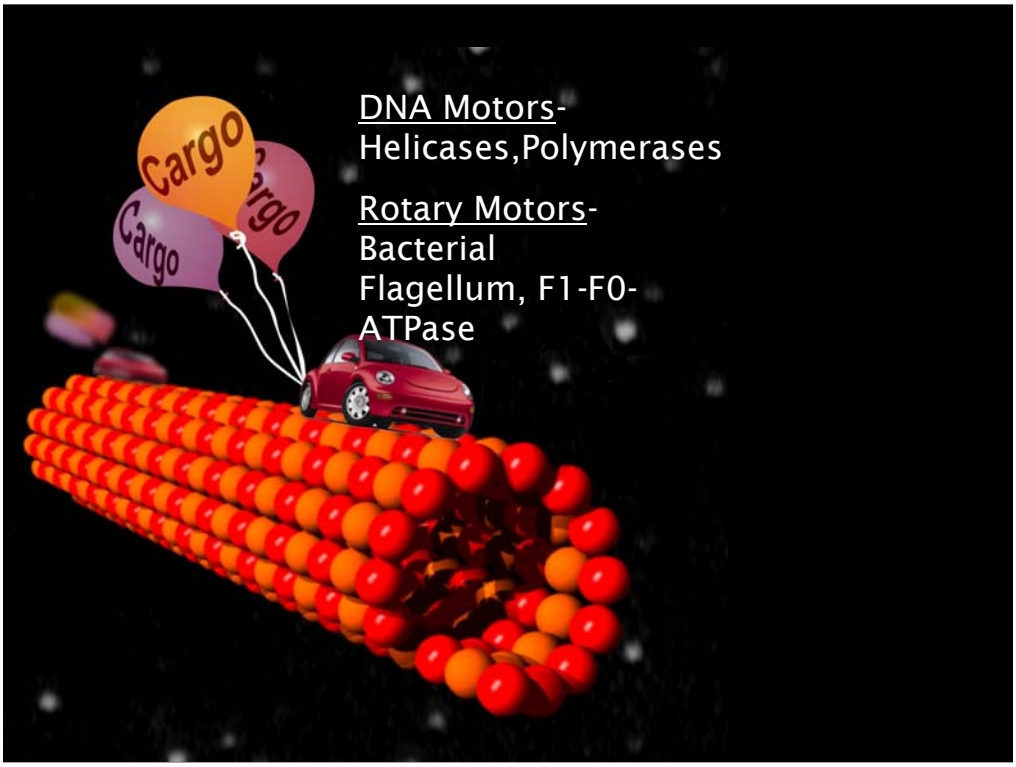
Part 3 will describe efforts to understand the assembly of a complex structure, the mitotic spindle

Understanding the Parts


Part II: Effort to understand the molecular mechanism of a motor- cytoplasmic dynein



Howe does this motion work? The motion that you witnessed in the last two movies are driven by cytoskeletal motor proteins and there are three main classes of these motors which we will discuss in this lecture- kinesin, dynein and myosin. These motor convert energy from ATP hydrolysis to physical motion along a track.



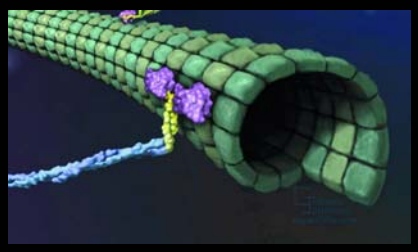
I should add that Kinesin, myosin and dynein are not the only motor proteins in cells. Although I will not discuss other motors here, you should be aware that there are many other kinds of wonderful motors such as ATPases that move along DNA or other that are rotary engines such as the mitochondrial F1 ATPase or the motor that drives the bacterial flagellum.

	Kinesin	Automobile Engine
Size	10^{-8} m	1 m

	Kinesin	Automobile Engine
Size	10^{-8} m	1 m

$\overline{0.03}$ m

	Kinesin	Automobile Engine
Size	10^{-8} m	1 m



0.03 m

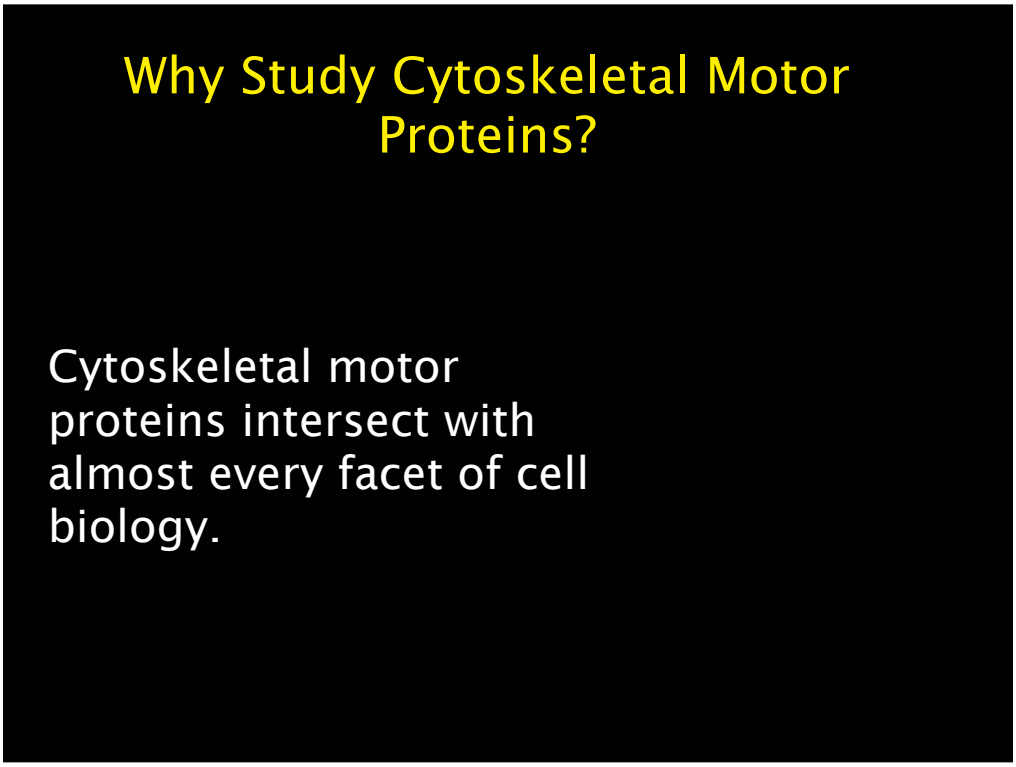
	Kinesin	Automobile Engine
Size	10^{-8} m	1 m
Fuel	ATP	Hydrocarbons

	Kinesin	Automobile Engine
Size	10^{-8} m	1 m
Fuel	ATP	Hydrocarbons
Speed	4 x 10^{-3} m/hr	10^{5} m/hr
	4 x 10^{5} lengths/hr	10^{5} lengths/hr

	Kinesin	Automobile Engine
Size	10^{-8} m	1 m
Fuel	ATP	Hydrocarbons
Speed	4 x 10^{-3} m/hr	10^5 m/hr
	4 x 10^5 lengths/hr	10^5 lengths/hr
Work Efficiency	~60%	~10%

Why Study Cytoskeletal Motor Proteins?

Understanding how living organisms create motion has intrigued scientists for thousands of years.



Biologists from many diverse disciplines including developmental biology, neurosciences, signaling, immunology, cancer biology discover a critical transport process and a molecular motor at the heart of what they are studying.

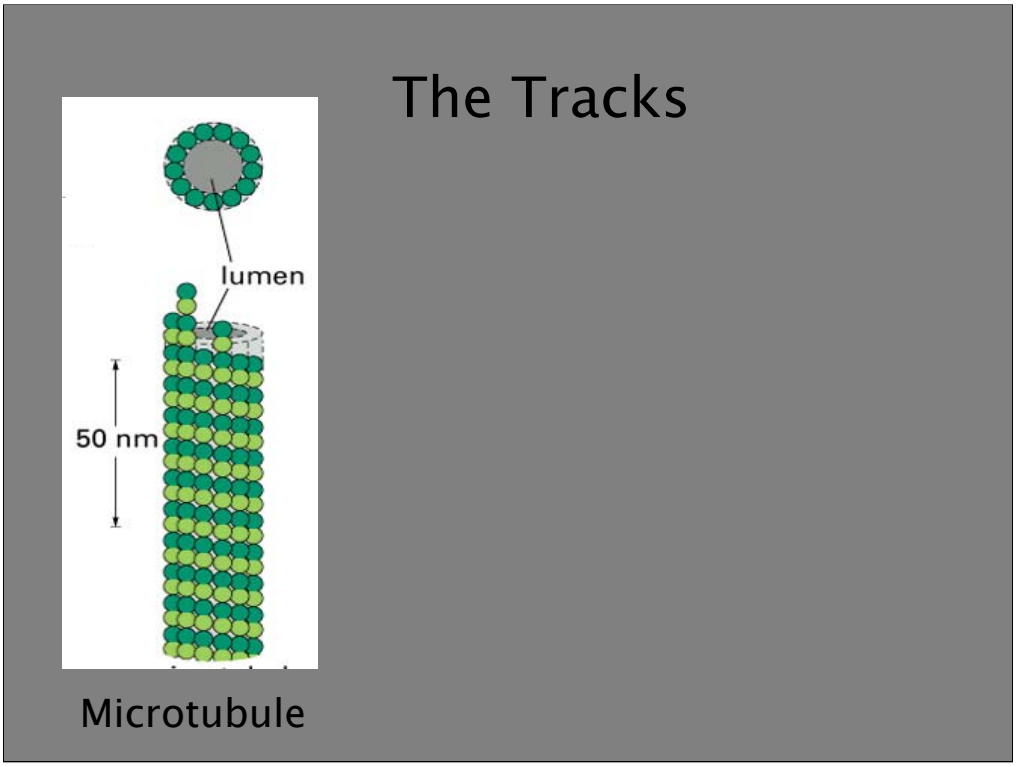
Why Study Cytoskeletal Motor Proteins?

Relevance to medicine:

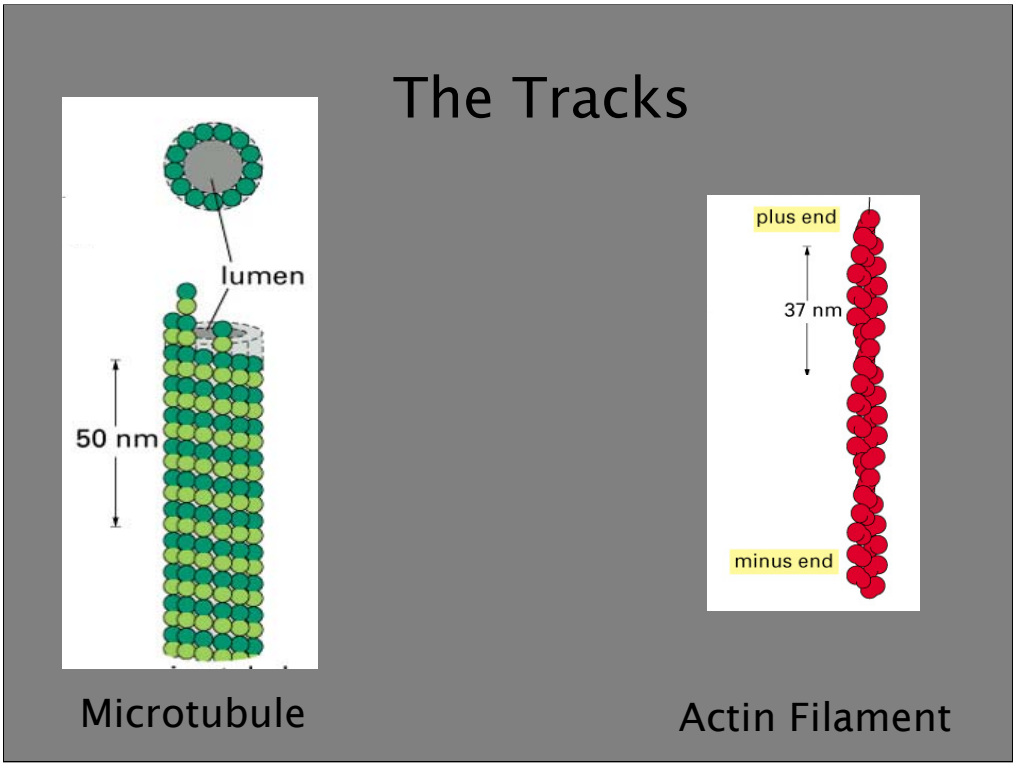
Transport defects can cause disease.

Inhibition or enhancement of motor protein activity may have therapeutic benefit.

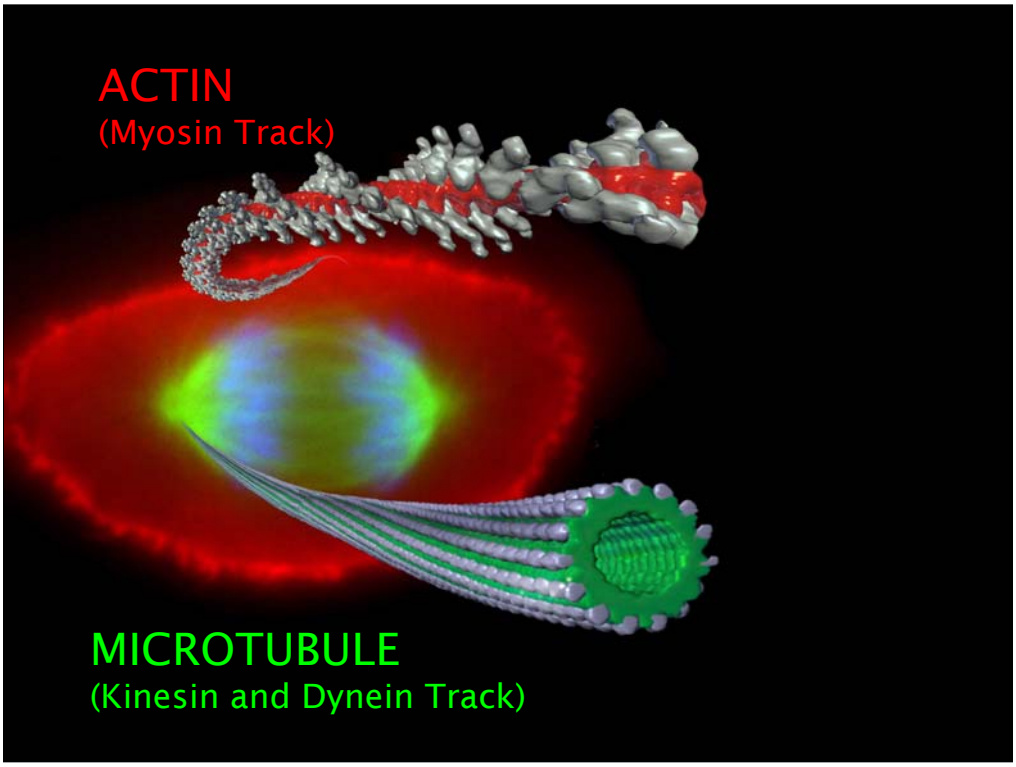
And understanding molecular motors can have impact on medicine and the treatment of human disease



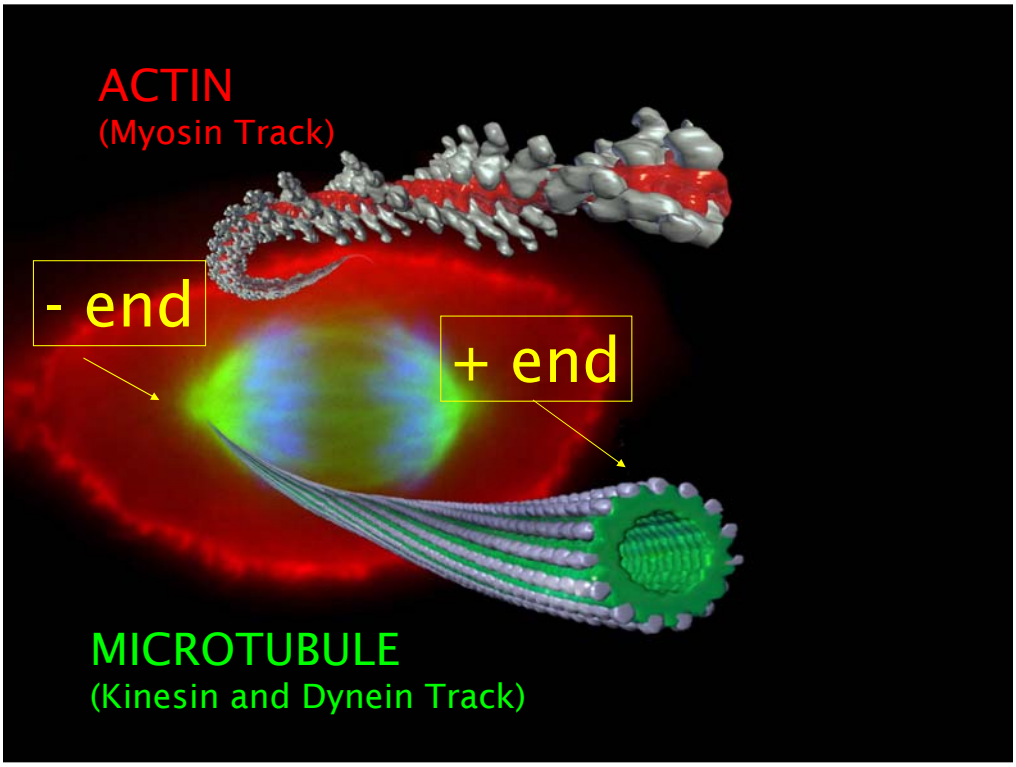
Tracks for the kinesin and dynein motors are microtubules- Hollow cylinders of 25 nm diameter composed of a/b tubulin.



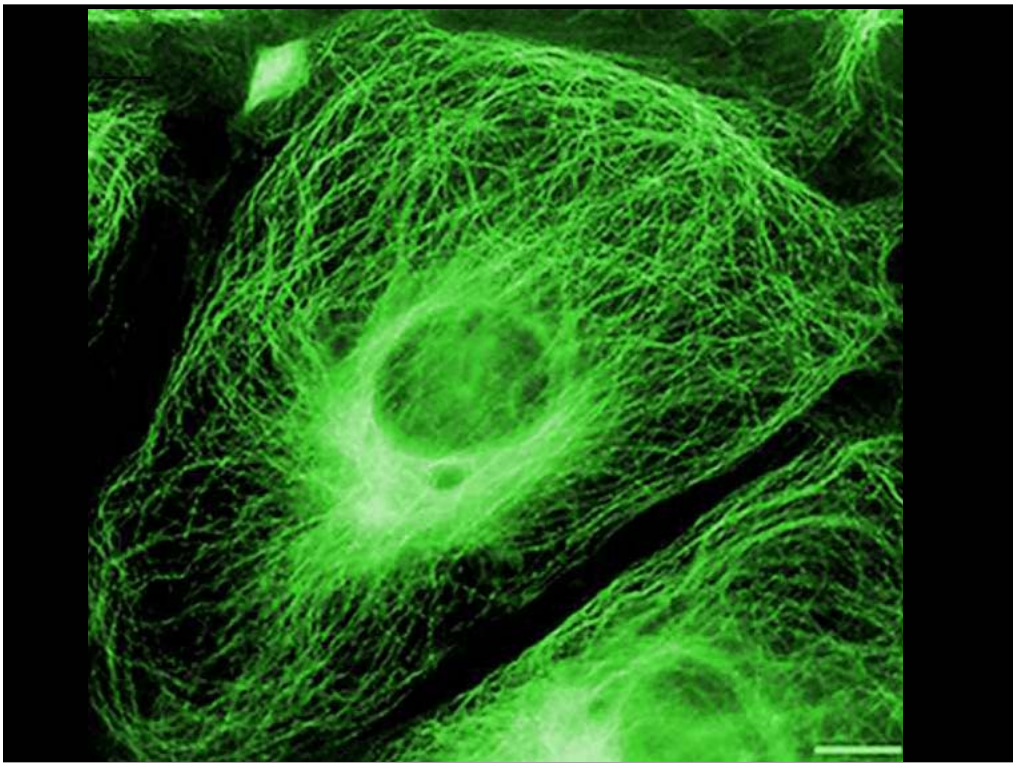
The tracks for myosin motors are actin filaments, which are composed of single subunit (actin) which polymerizes to form a helical polymer of smaller diameter.



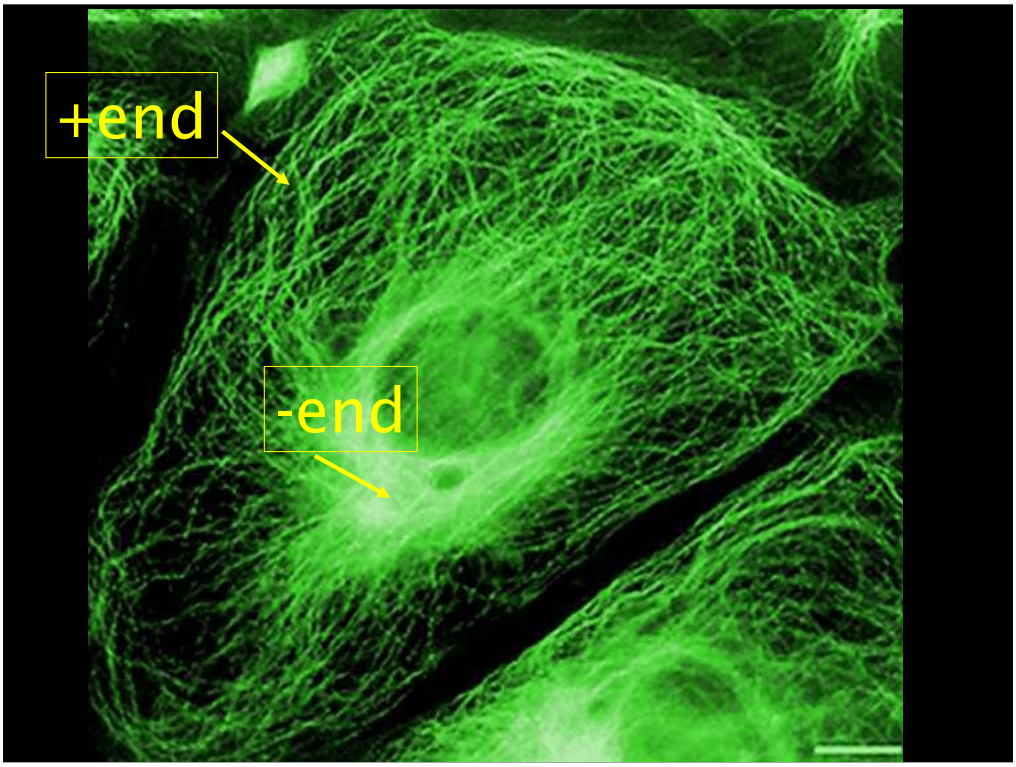
An important attribute of these tracks is that they are polar- since the subunits polymerize in a head-to-tail manner, the two ends of the filaments are different. Motors bind in a defined orientation and move in specific direction along the track. The filaments also tend to be organized with uniform polarity in cells. The so called plus ends of actin point towards the cell membrane



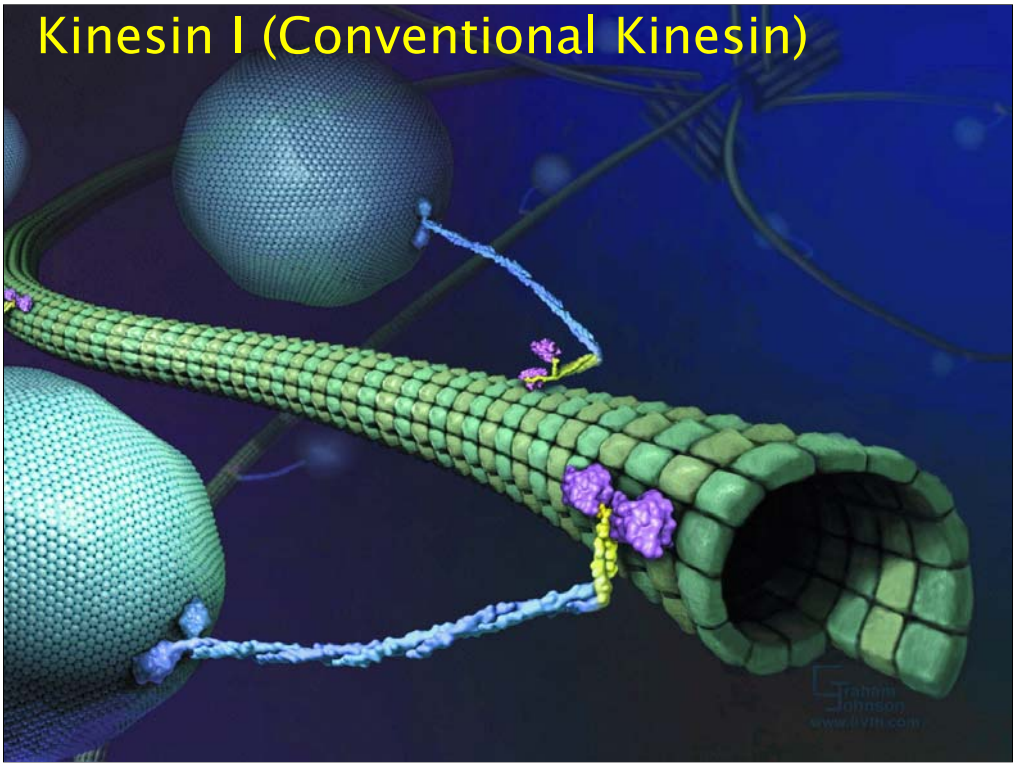
and the plus ends of microtubules in a spindle are directed towards the chromosomes and the minus ends are located at the poles of the spindle.



Organized polarity is also found in interphase cells



Where the minus ends are located at the microtubule nucleating centrosome generally located near the center of the cell while the plus ends extend toward the periphery, thus creating a navigation system between the outside and the inside of the cell.



In this 3D depiction of kinesin moving membranes along microtubules,

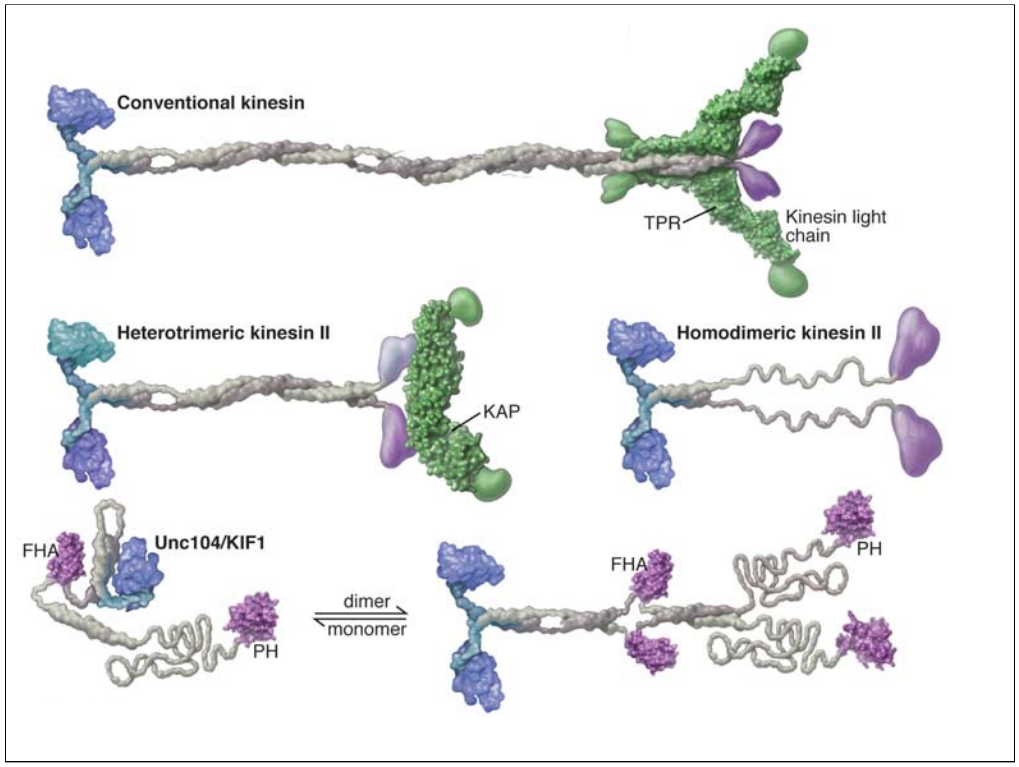
Many Motors, Not Just One

- 45 human kinesin genes
- The different kinesins are specialized for different transport activities

Many Motors, Not Just One

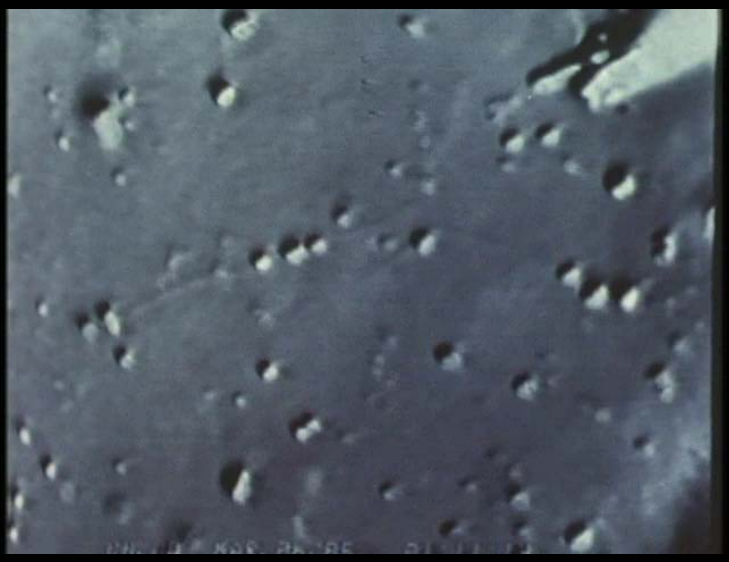
- Kinesin Functions

- Organelle movement
- Transport of RNAs and proteins
- Assembly of cilia/flagella
- Signaling pathways
- Mitotic spindle formation and chromosome movement



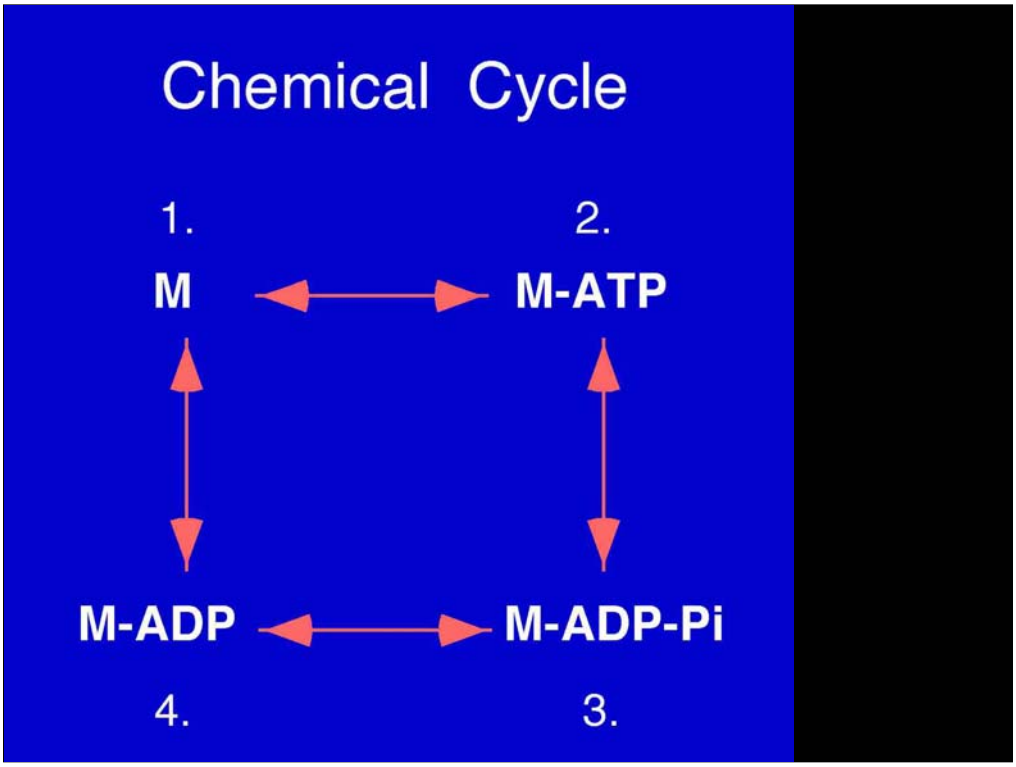
Motors domains in blue are generally similar within the kinesin superfamily, with 30-40% sequence identity. Nevertheless these motors can have very different properties such as velocity and even direction on movement. The nonmotor tail domains, on the other hand, share little or no sequence similarity between different kinesin classes and they can even have very different structures and associated subunits. These differences are critical for cargo attachment and also regulation.

How Do Proteins Produce Motion?



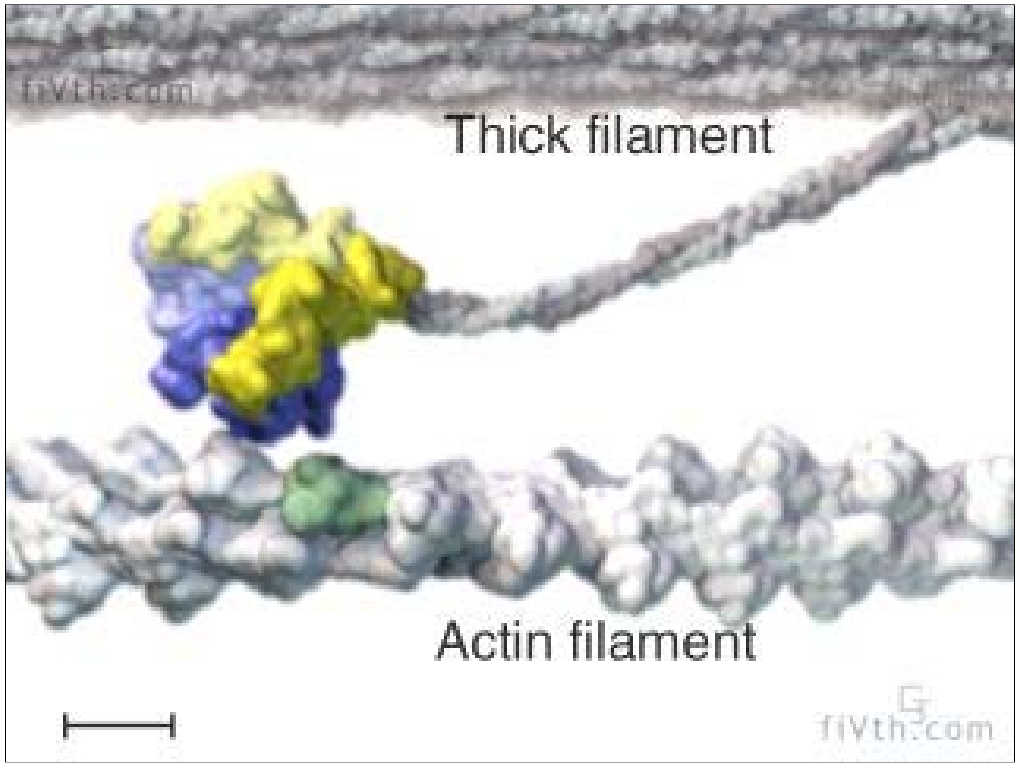
Kinesin transporting 1 um plastic beads

Science should be driven my curiousity. (You should be really interested in the questions that you ask). Fascinating question, but it did not really seem possible to come up with a satisfying answer to this question. Muscle myosin had been around for so long, but it was hard to nail down an answer. (Seemed daunting that so much effort had completely solved the problem).

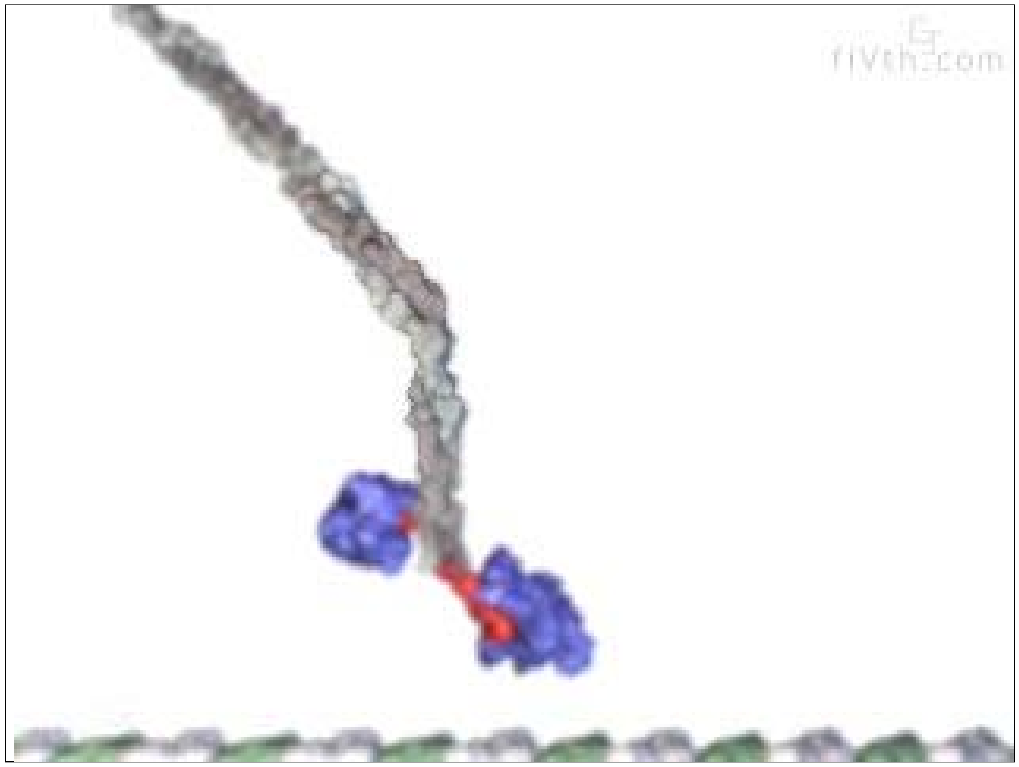


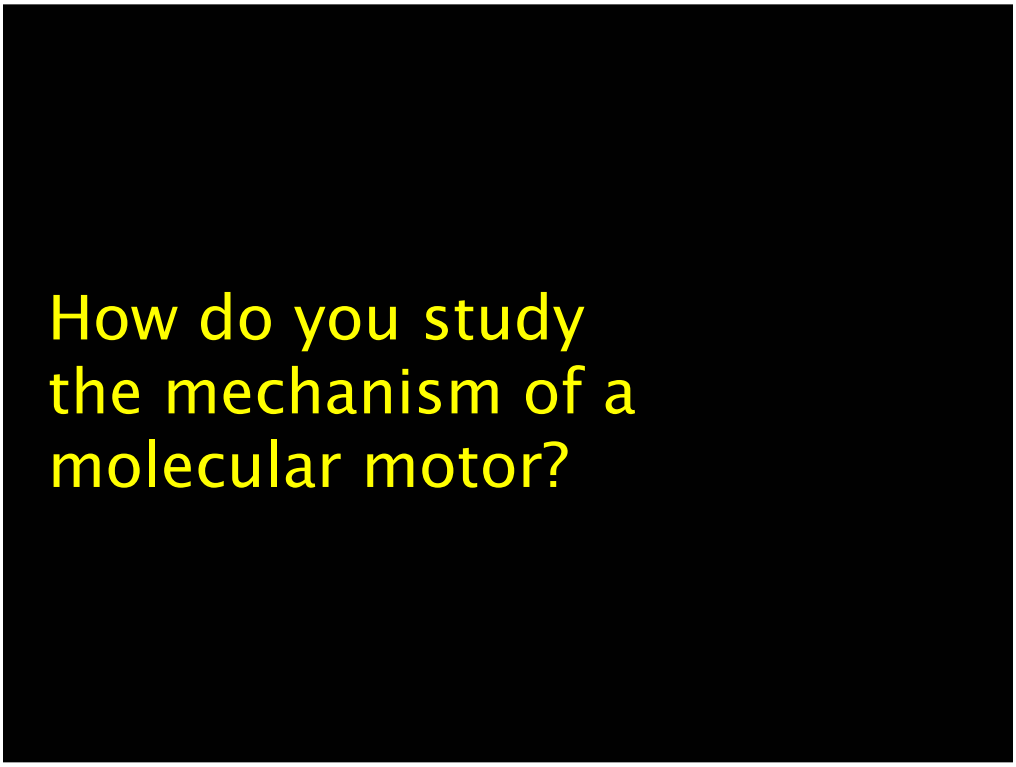
Now we come to the main topic of this lecture- how do motor proteins work?

First, motor proteins are ATPases- they bind, hydrolyze ATP and then release the products sequentlially. And during these transitions, protein conformational changes occur that produce motility.

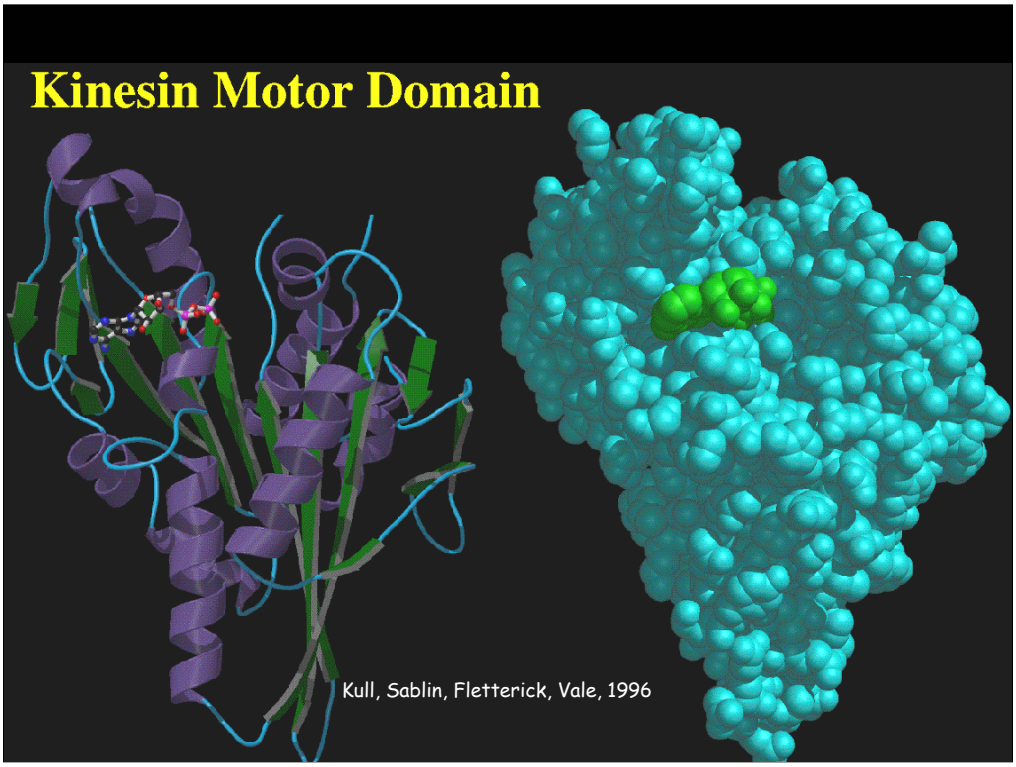


These movie animations, made by Graham Johnson, and based upon actual crystal structures and decades of research on myosin illustrate howthe force generating cycle of myosin is thought to work.

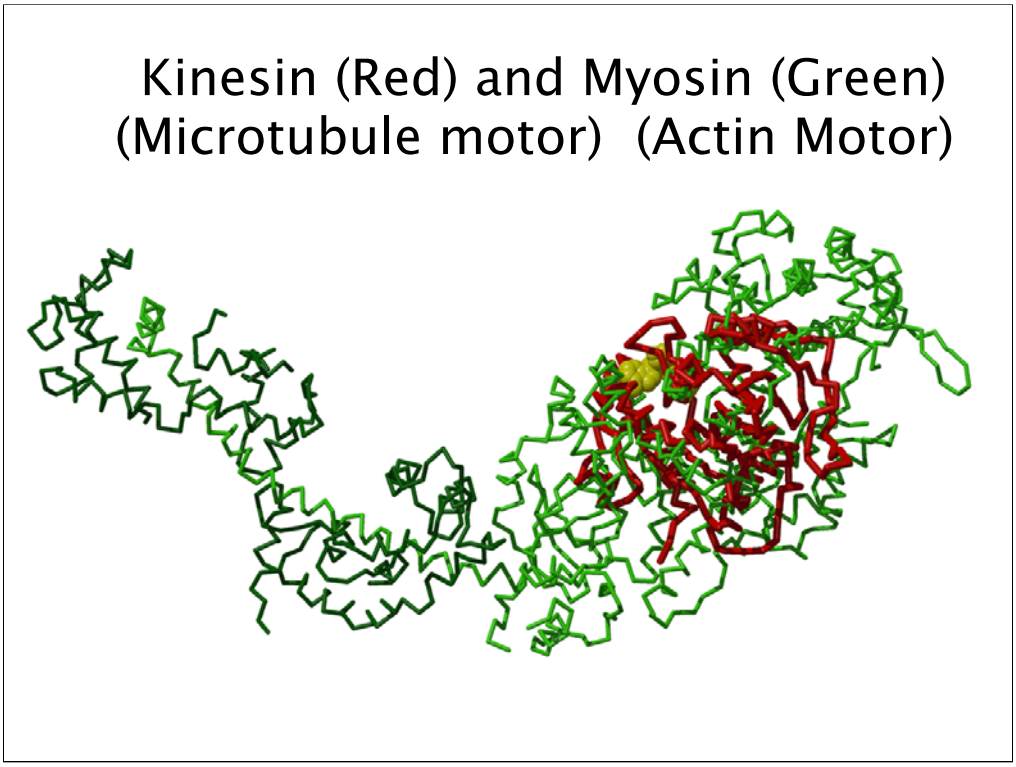




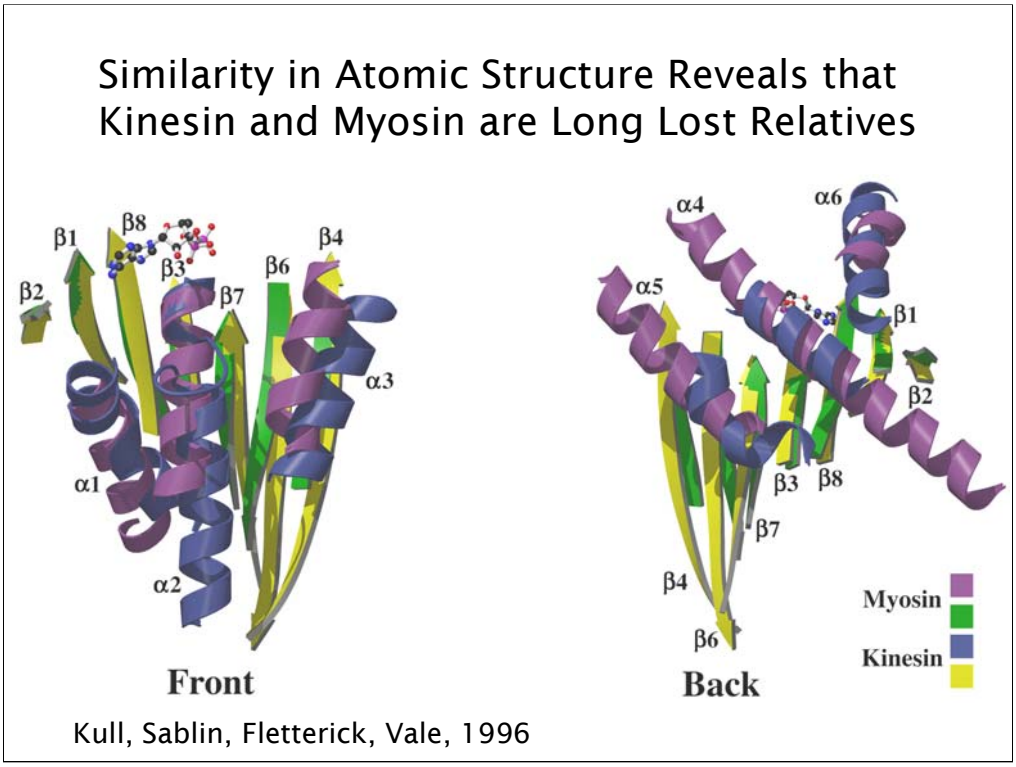
You have seen two models for motor proteins, but now I would like to illustrate the actual experimental tools that are used to understand how motors work and are used to help derive these models.



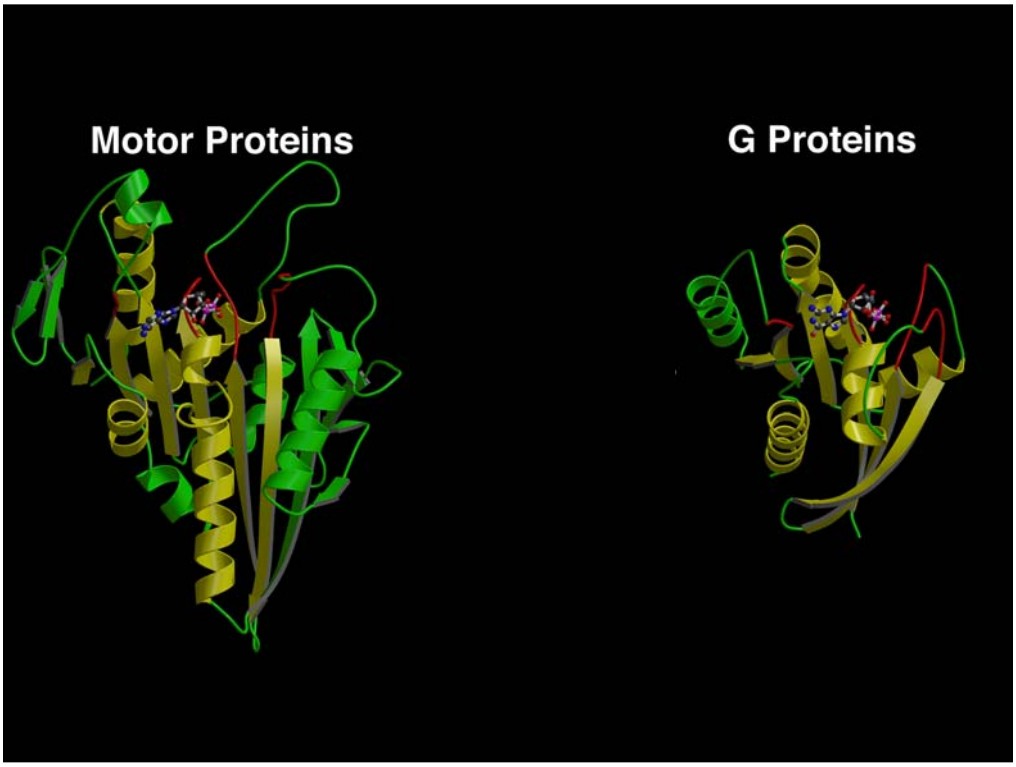
First, critical information on mechanism is derived from resolving the 3D structure of the motor at atomic resolution by X-ray crystalllography. Ribbon diagram and space filling and here is the nucleotide in the active site. At this bird's eye view, it may not seem that informative.



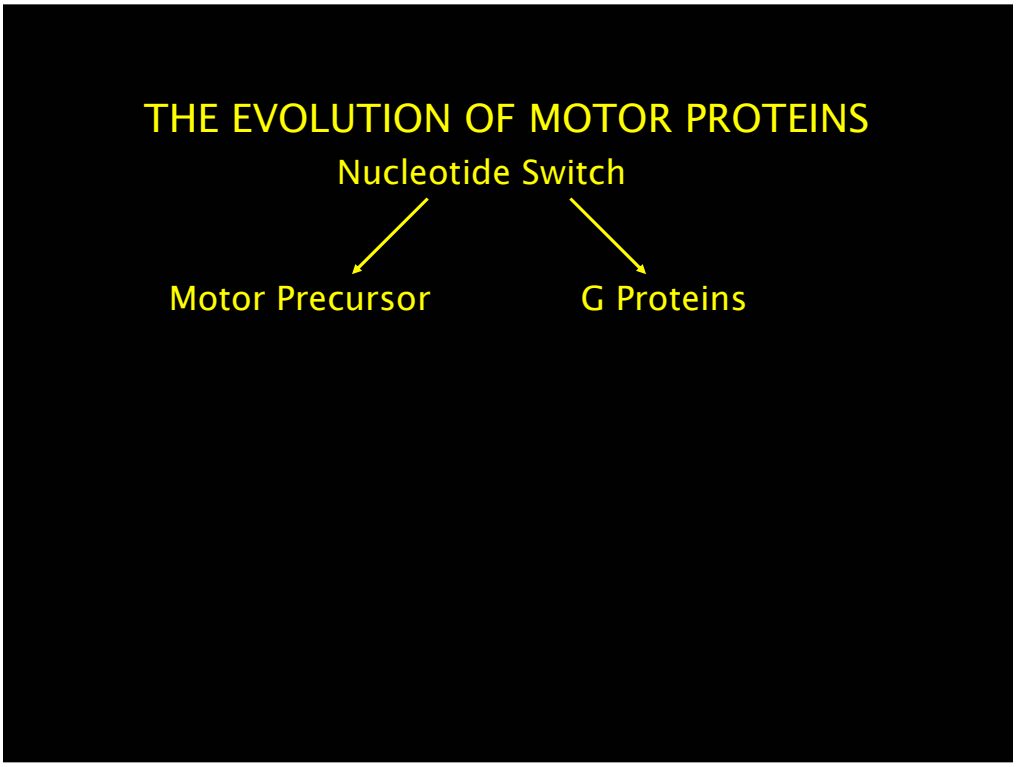
To understand the parts of the motor, we were aided considerably by evolutionary comparisons and specifically by comparing kinesin and myosin. Before the kinesin crystal structure, no one thought that kinesin and myosin were related. After all, one was microtubule motor and the other was an actin motor and they are completely different sizes, and computers found no sequence similarity.



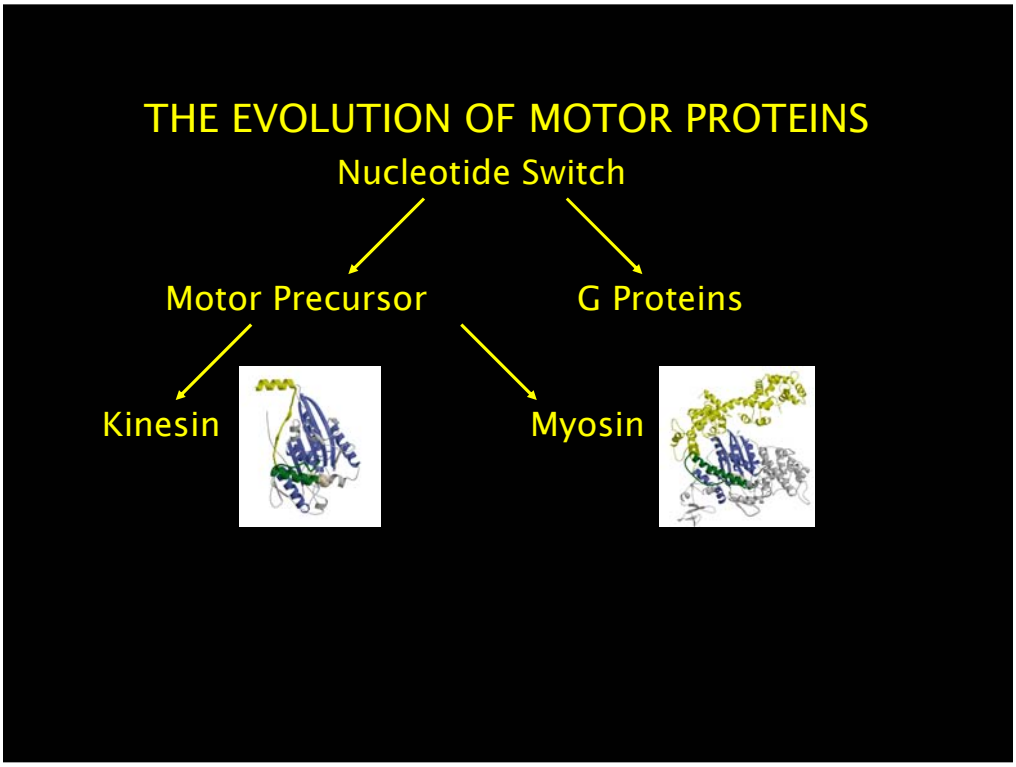
However, if one looks at the core of the motor, one now finds very clear structural similarity of overlapping beta strands and helices that could only occur if the proteins were evolutionarily related and their sequences diverged over time.

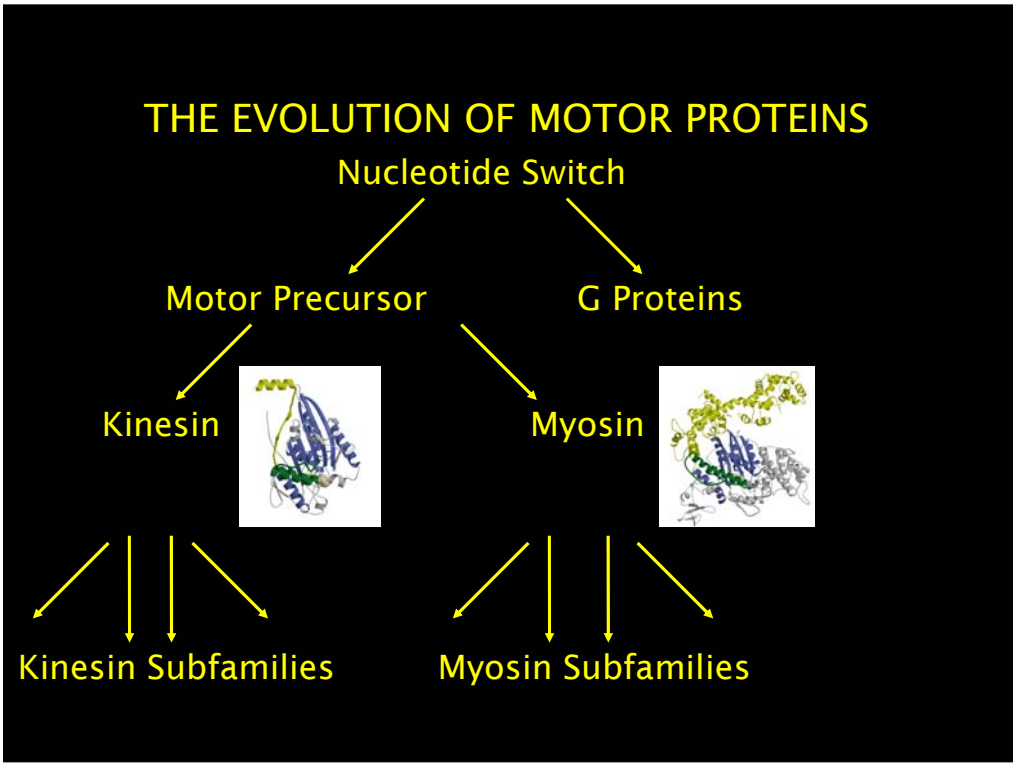


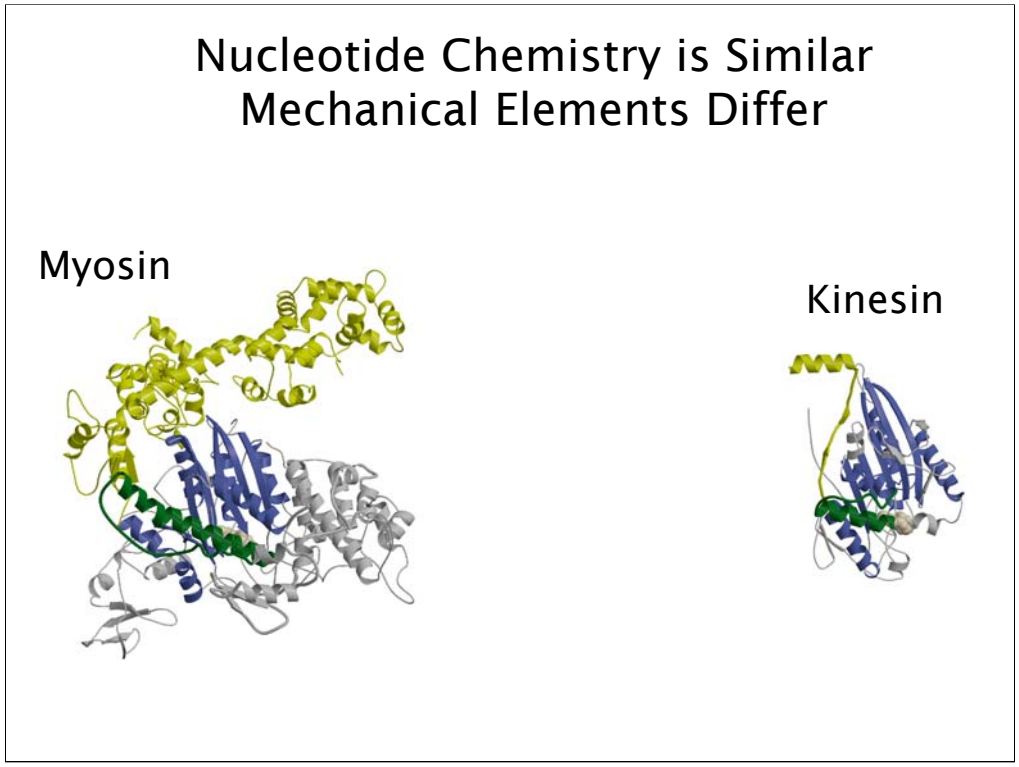
Morever, not only were the motors related, but structural comparisons also found clear common structural elements between the motor proteins and a large class of molecular swtiches that change conformation between GTP and GDP and are known as G proteins.



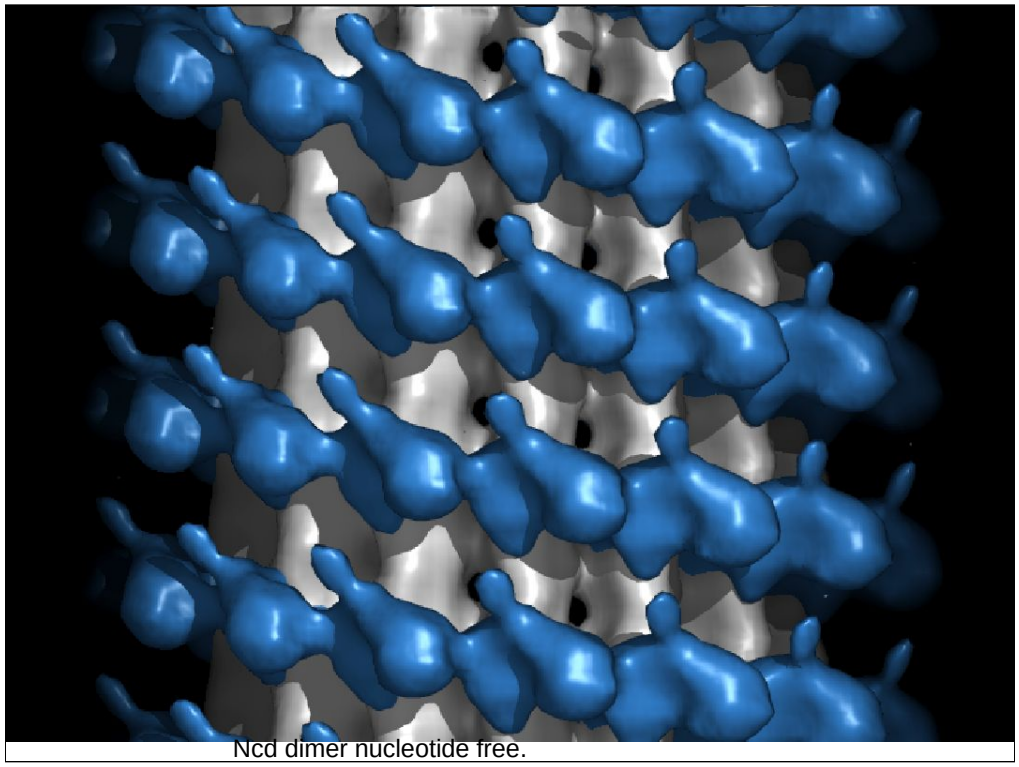
From the structures, we can piece together the following evolutionary history.


THE EVOLUTION OF MOTOR PROTEINS
Nucleotide Switch
Motor Precursor
G Proteins
Kinesin
Myosin

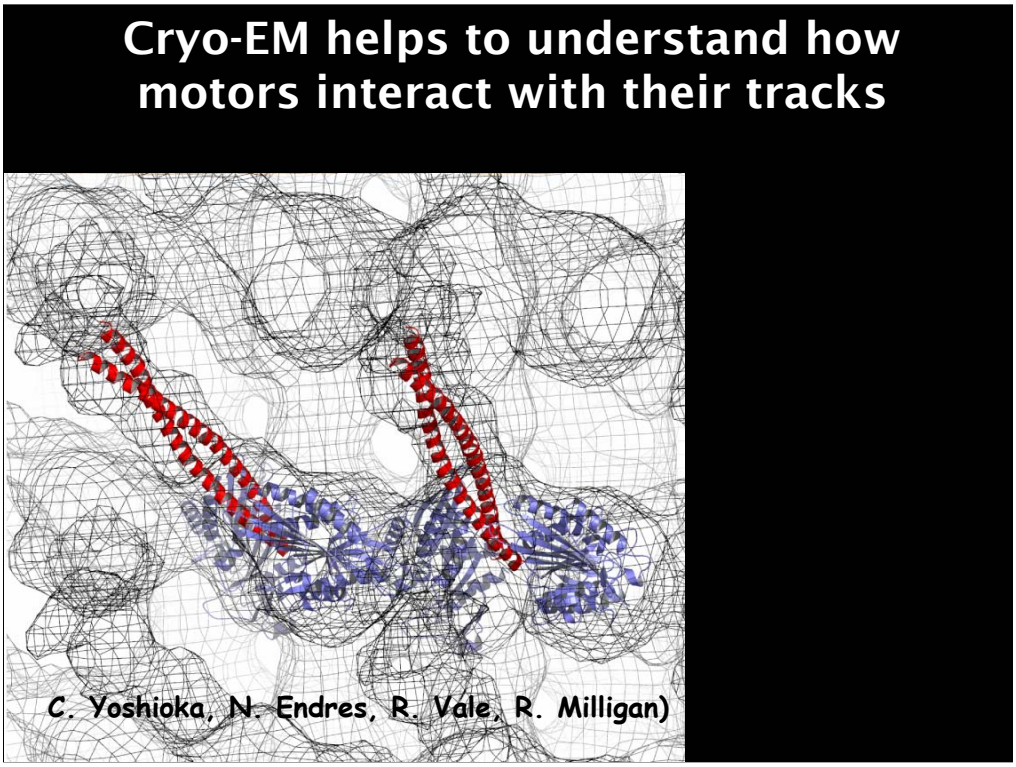

THE EVOLUTION OF MOTOR PROTEINS
Nucleotide Switch
Motor Precursor
G Proteins
Kinesin
Myosin
Kinesin Subfamilies
Myosin Subfamilies



While the y-phosphate sensing mechanism has been well preserved, other elements must be different. The mechanical elements for myosin and kinesin are very different shown in yellow.

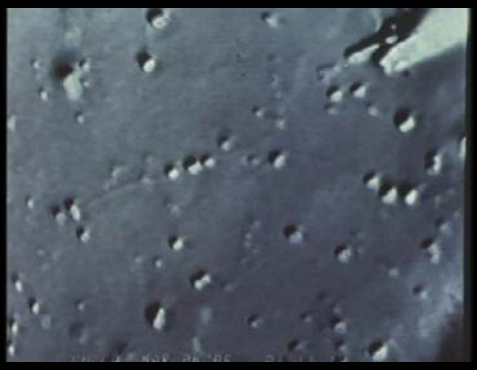


Ncd dimer nucleotide free.

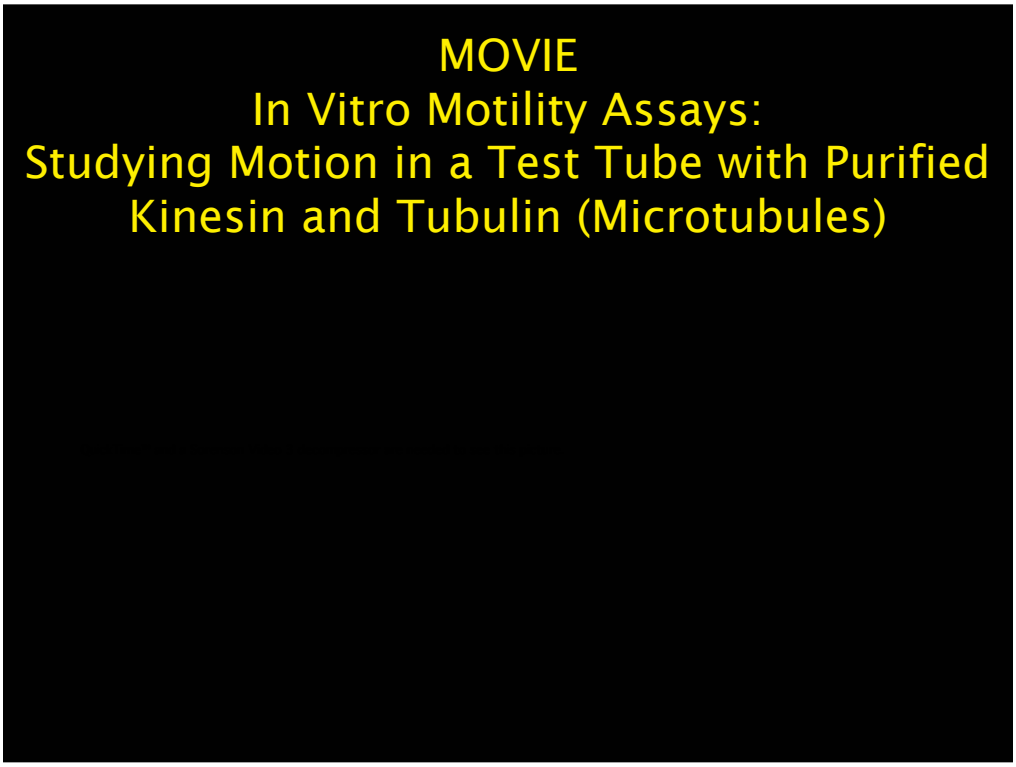


Of course, the motors are only part of the story since they work as intimate partners with the filament track and it has not been possible to date to solve a x-ray structure of a motor bound to tubulin or actin. So this is the domain of cryo-EM, where it is possible to obtain an electron density map of a motor bound to a polymer, here shown a kinesin-related motor Ncd bound to microtubules with an atomic structure docked into the motor density. From this, you can understand the motor-filament interface and also how the track and nucleotide influence the position of the mechanical element.s

In Vitro Motility Assays: Studying Motion in a Test Tube with Purified Kinesin and Tubulin (Microtubules)



Vale, Schnapp, Reese, Sheetz
Cell 1985



Transition in: Of course, x-ray and cryo provide static images and we can only make inferences on the dynamics of motor movement. So we need motility assays for this purpose.

We can study motility under a microscope with a simplified system involved purified motor proteins, microtubules made from purified tubulin and ATP

Transition out: How many motor proteins are driving the movement of these microtubules or beads

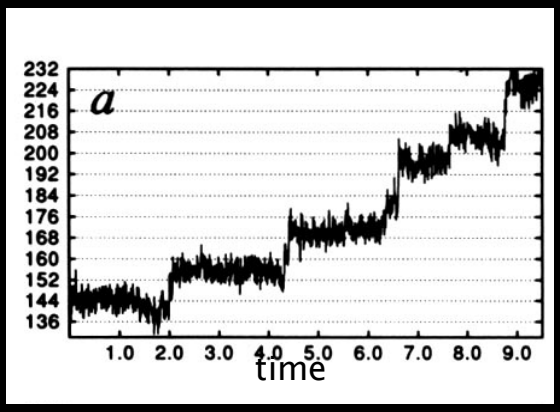
MOVIE

One Can Study the Motion of Single Motor Proteins!

Low Kinesin Density on Glass

J. Howard, J. Hudspeth, R. Vale Nature, 1989

Single Molecule Fluorescence and Optical Trap Microscope Detect the Motion of Single Molecules!



K. Svoboda, S. Block et al.
Nature, 1993

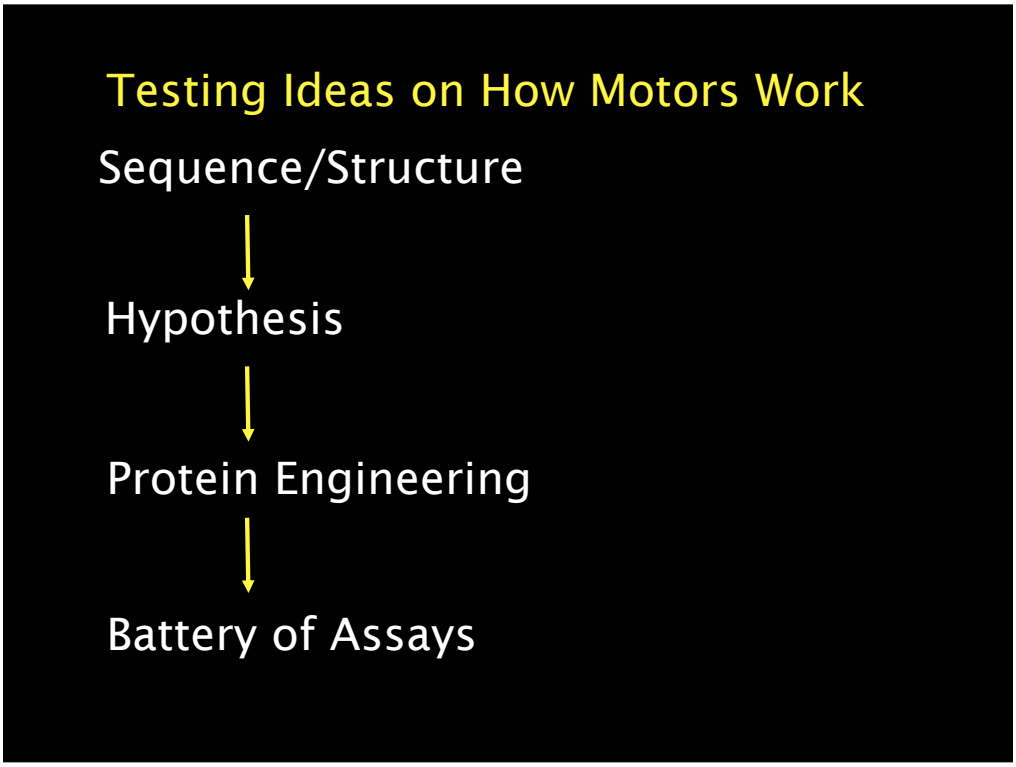
Show both of these techniques later in my lecture

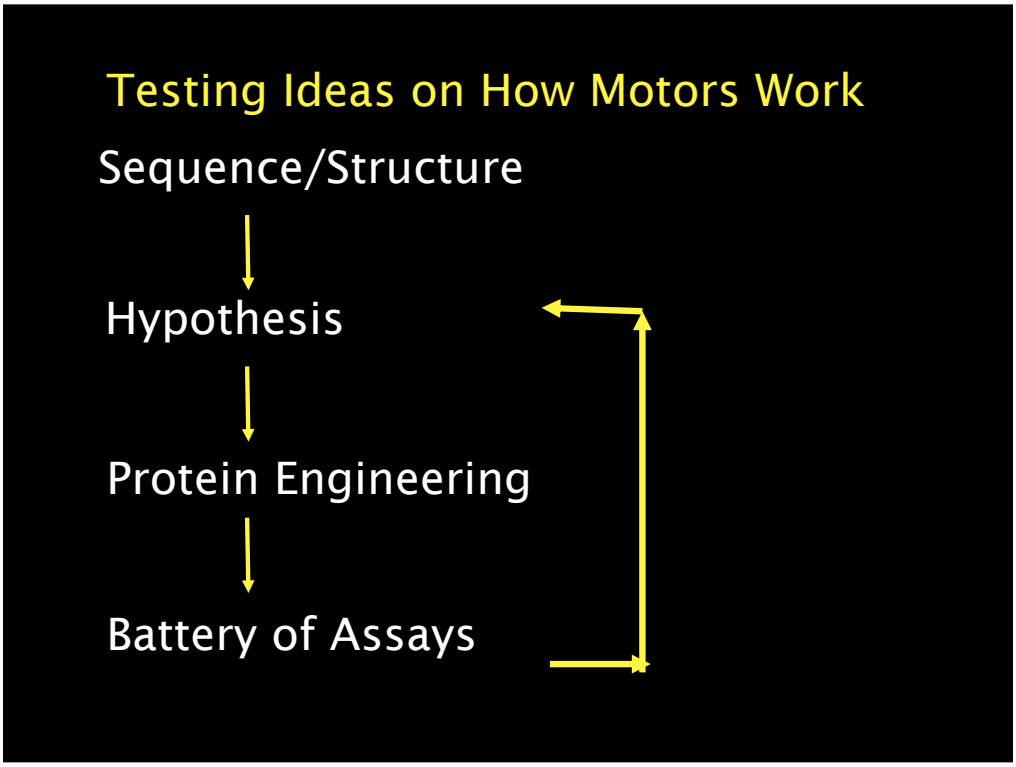
MOVIE
Single Molecule Fluorescence and Optical Trap Microscope Detect the Motion of Single Molecules!

Vale, Yanagida et al.
Nature 1996

What I cannot create,
I do not understand

-Richard Feynman


Testing Ideas on How Motors Work
Sequence/Structure
Hypothesis
Protein Engineering
Battery of Assays

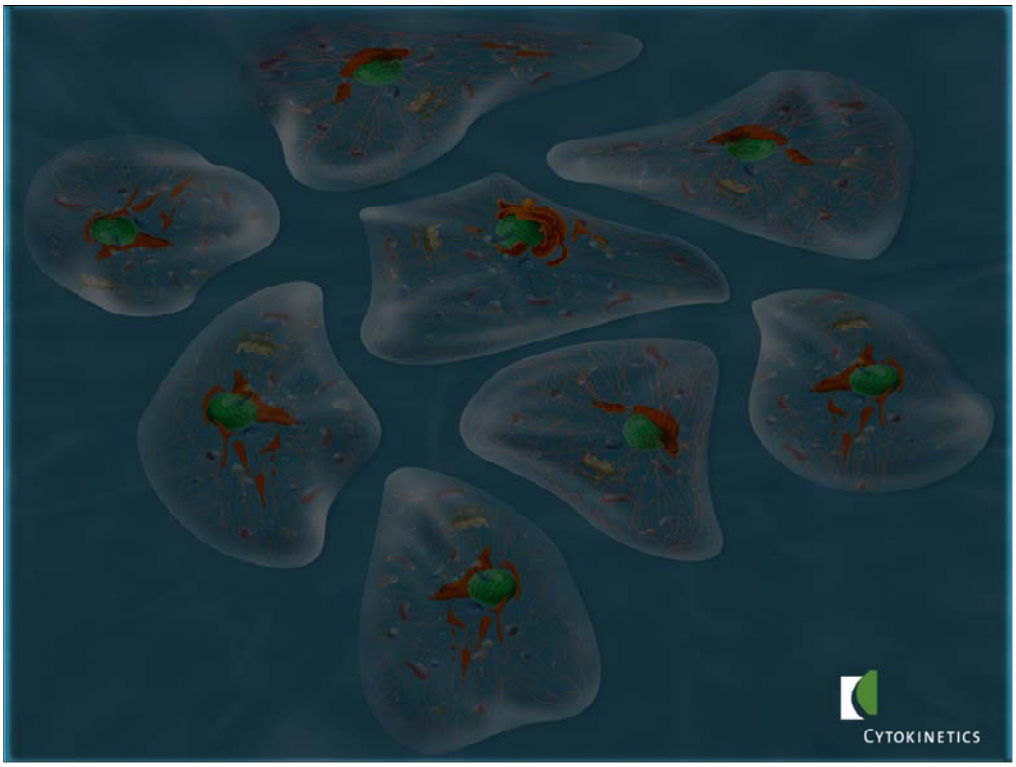


Battery of assays include more than I have had time to present- certainly dissection of the enzymatic by stop flow kinetics

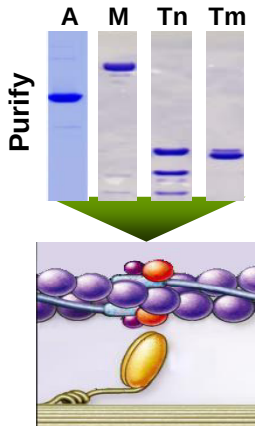
Apply our knowledge of motor proteins to practical outcomes?

Small molecule drugs with therapeutic benefit?

Engineer motors for cells or nanotechnology?

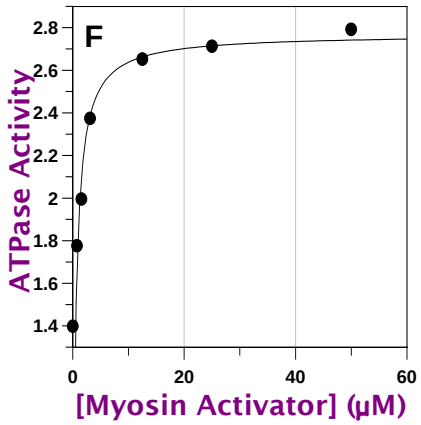

CYTOKINETICS

Reconstituting the Sarcomere for Drug Discovery



CYTOKINETICS

Reconstituting the Sarcomere for Drug Discovery



High Throughput Screen
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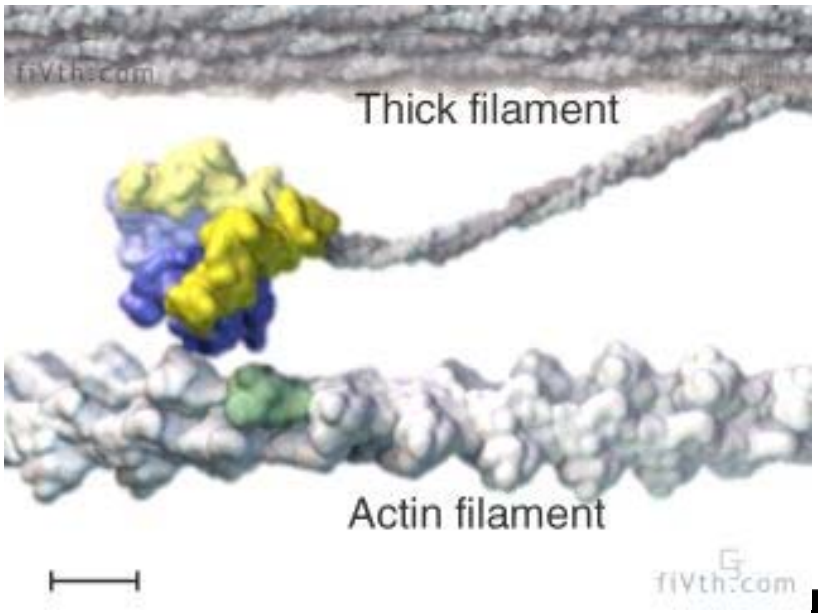
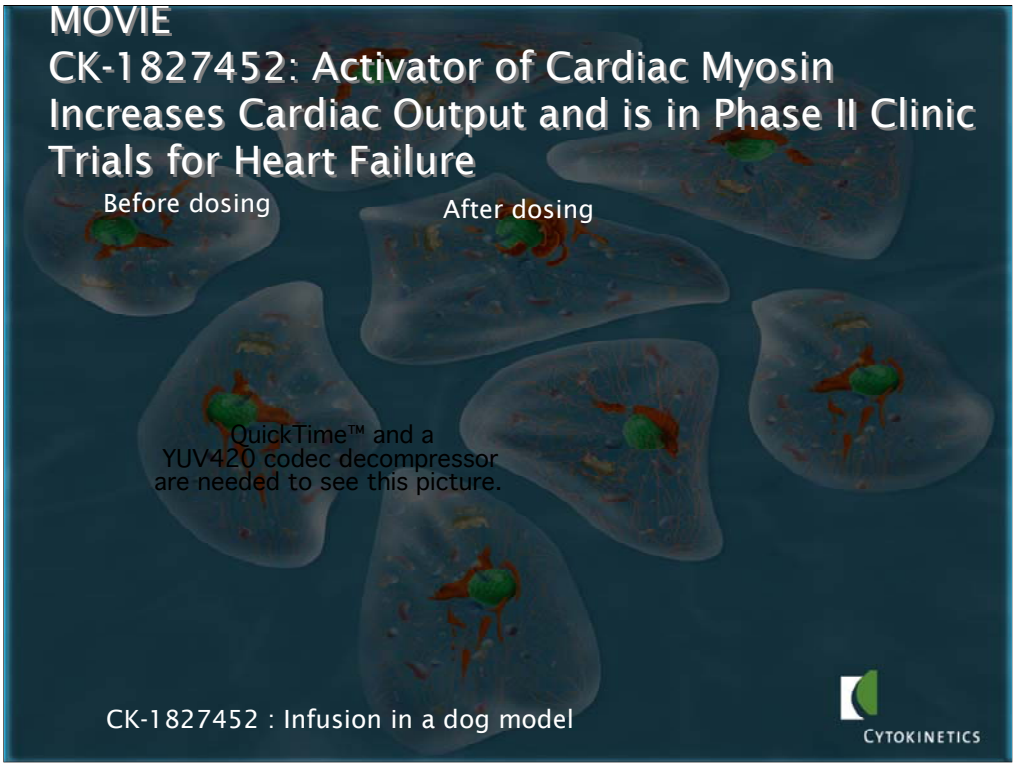
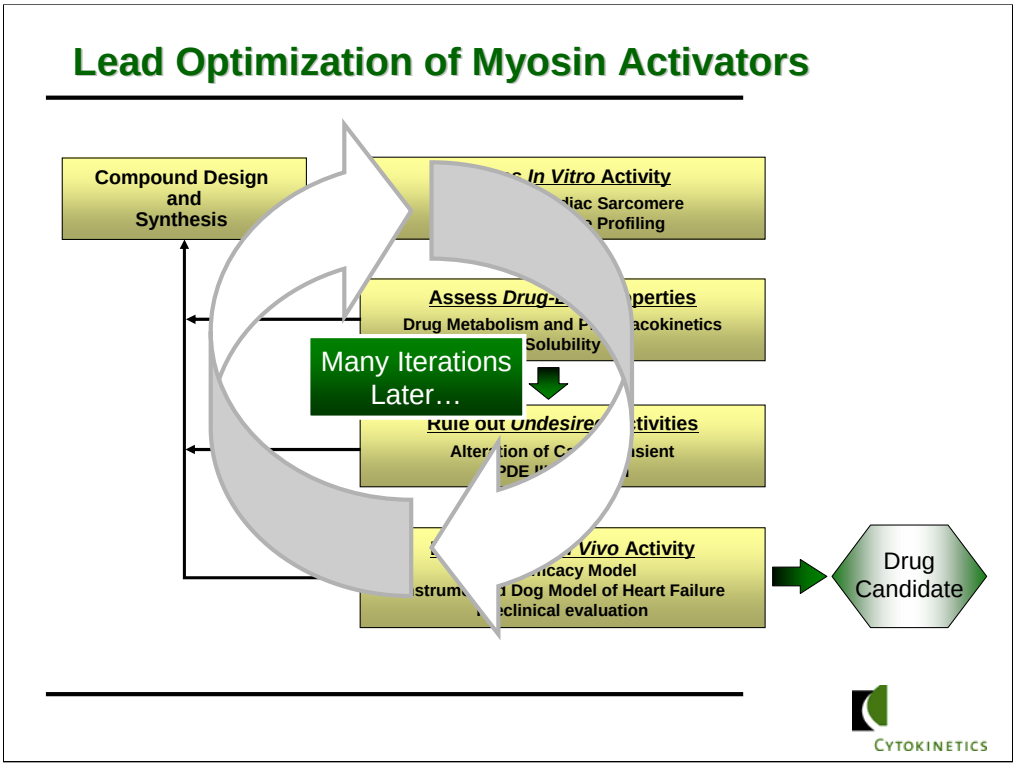
CYTOKINETICS

Myosin Activating Drugs Accelerate Phosphate Release

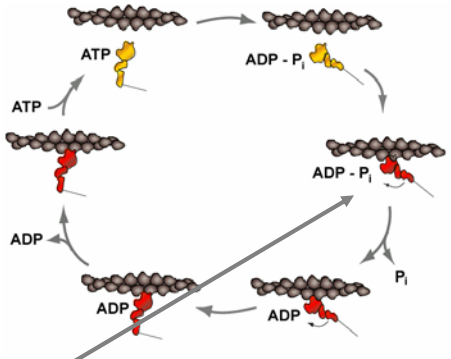



MOVIE
CK-1827452: Activator of Cardiac Myosin Increases Cardiac Output and is in Phase II Clinic Trials for Heart Failure
Before dosing
After dosing
QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.
CK-1827452 : Infusion in a dog model
CYTOKINETICS


Lead Optimization of Myosin Activators
Compound Design and Synthesis
In Vitro Activity
Profiling
Many Iterations Later…
Drug Candidate
CYTOKINETICS

How might a myosin activator work?

The Chemical and Mechanical Cycles are Linked



Chemical Cycle
Accelerate the rate limiting step in ATP hydrolysis cycle

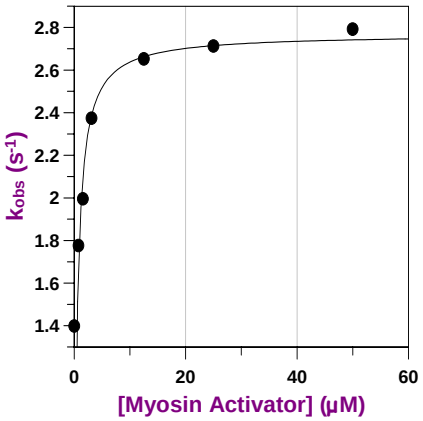
Mechanical Cycle
Increase the number of myosin heads interacting with the actin filament during the cardiac cycle

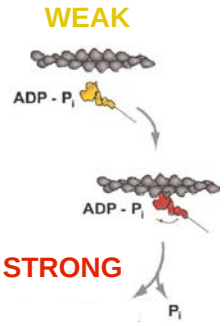
= strongly bound state

CYTOKINETICS

How do cytoskeletal motors produce motion?

Myosin activators increase productive ATP hydrolysis





In the <u>presence</u> of actin, the myosin activator *accelerates* phosphate release and thus productive ATP hydrolysis

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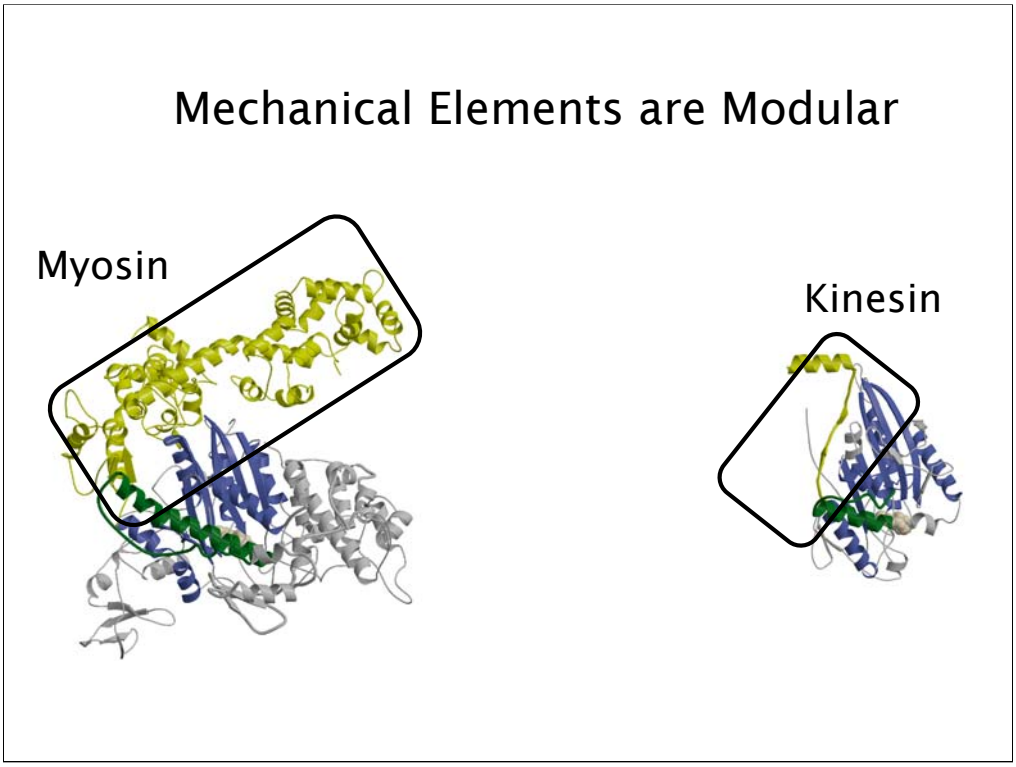
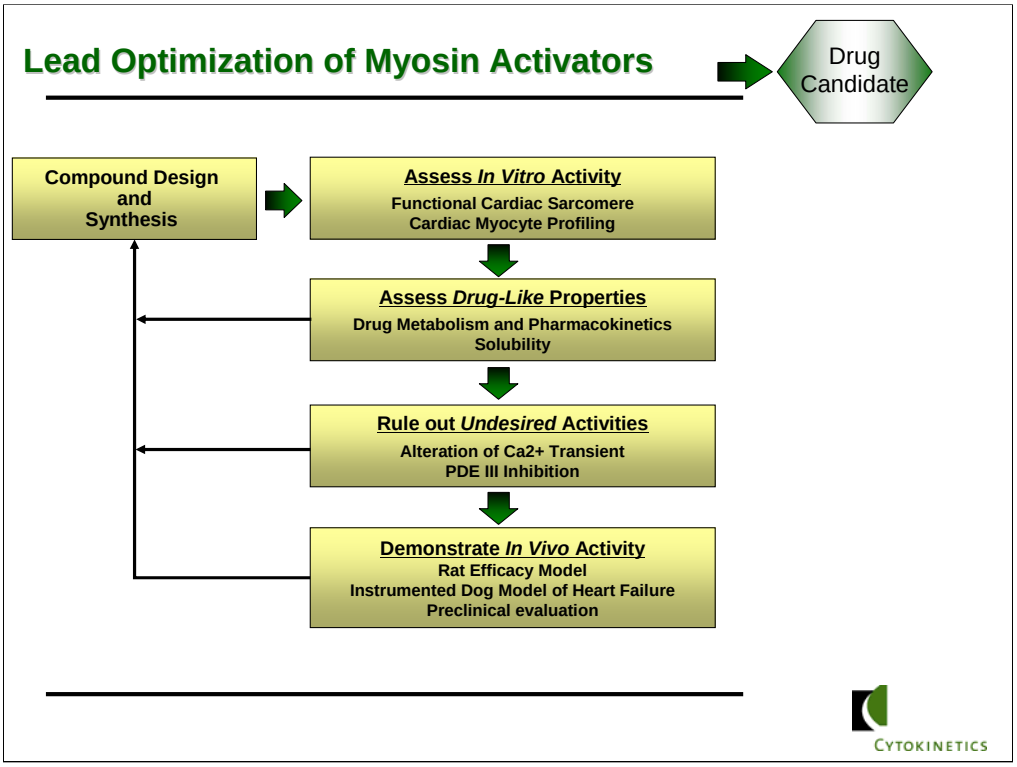
What are we trying to learn?

How do motors contribute to cell biological processes?



Although quite different in structure, you can see that these distinct mechanical elements are found in the exact same position relative to the enzyme core, due to a preserved communication pathway from the y-phosphate sensor.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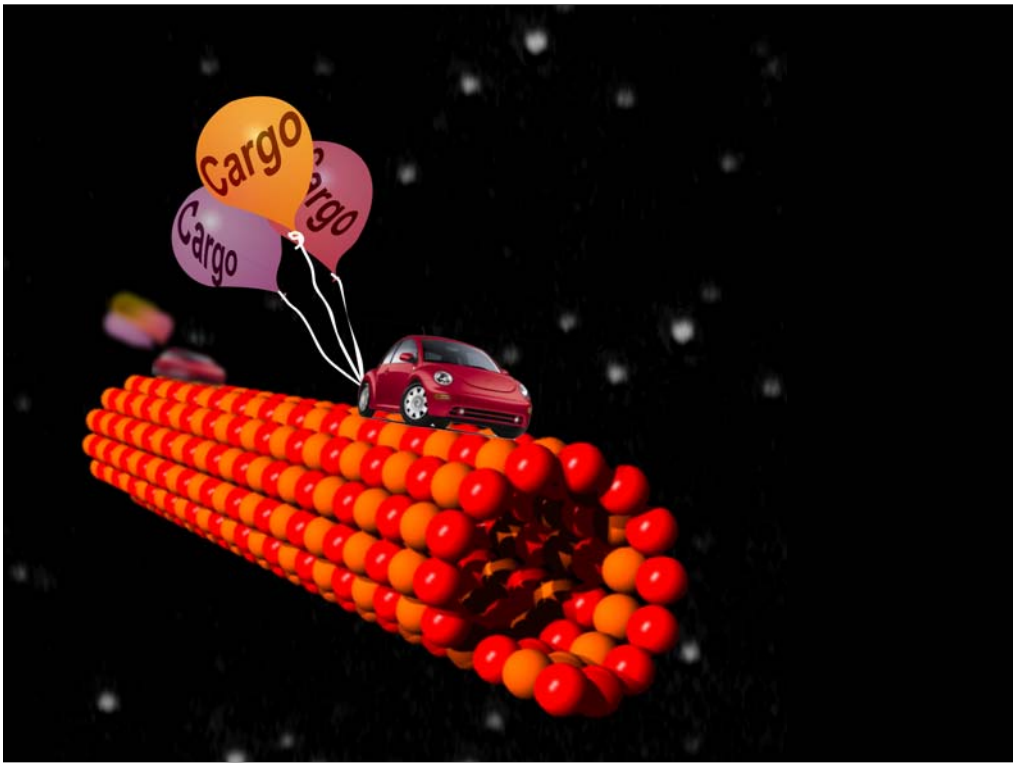
Drug Candidate
Compound Design and Synthesis
Assess In Vitro Activity
Functional Cardiac Sarcomere
Cardiac Myocyte Profiling
Assess Drug-Like Properties
Drug Metabolism and Pharmacokinetics
Solubility
Rule out Undesired Activities
Alteration of Ca2+ Transient
PDE III Inhibition
Demonstrate In Vivo Activity
Rat Efficacy Model
Instrumented Dog Model of Heart Failure
Preclinical evaluation
CYTOKINETICS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