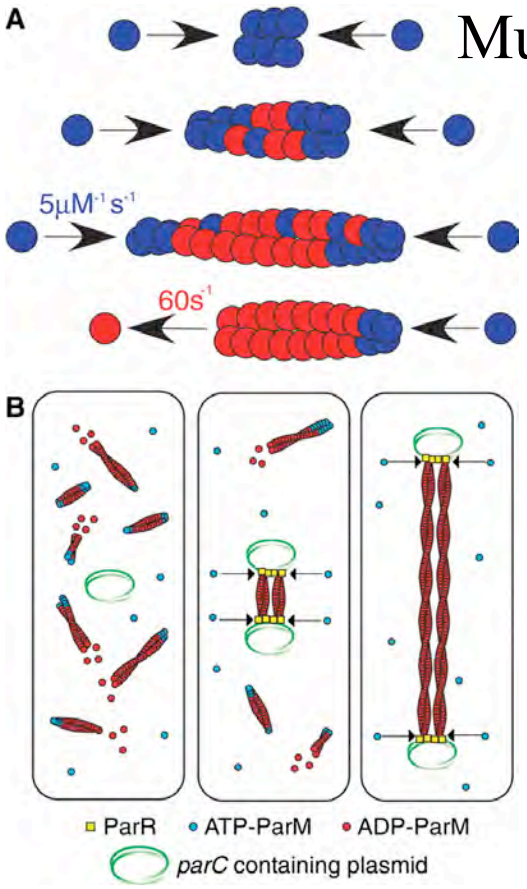
APh161 - Lecture 11: The Cytoskeleton is Always Under Construction

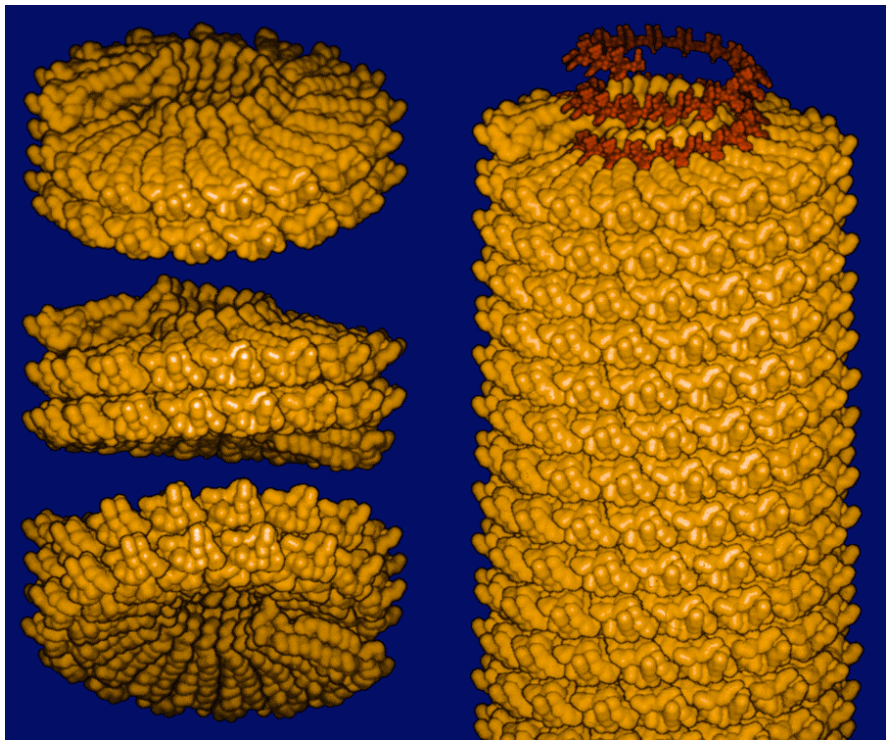
Mullins *et al.*



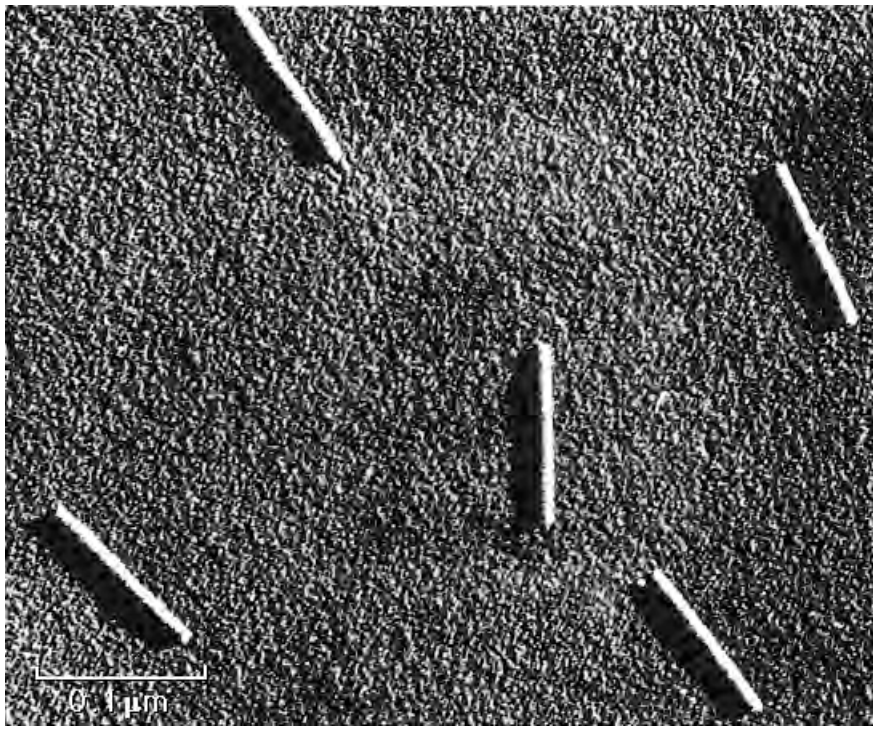
Rob Phillips

California Institute of Technology

Polymerization Processes 1: Tobacco Mosaic Virus



Caspar



A reminder on the cytoskeleton

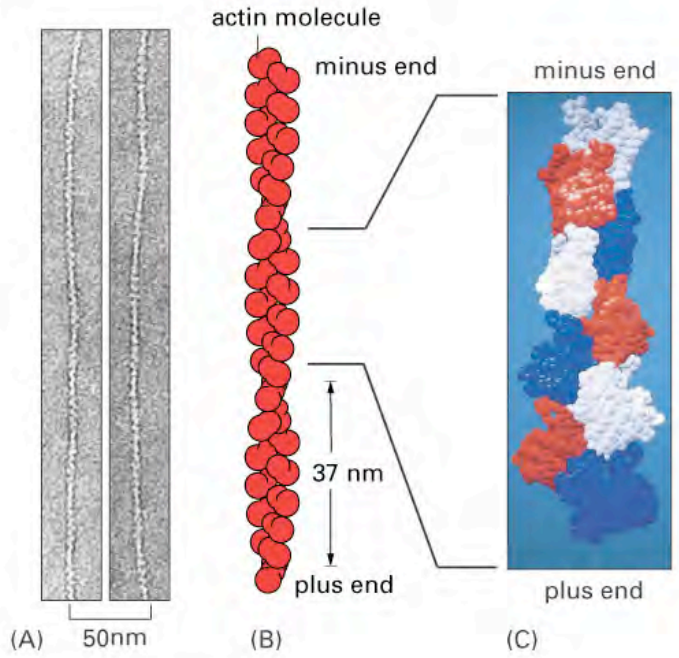


Figure 17-30 Essential Cell Biology, 2/e. (© 2004 Garland Science)

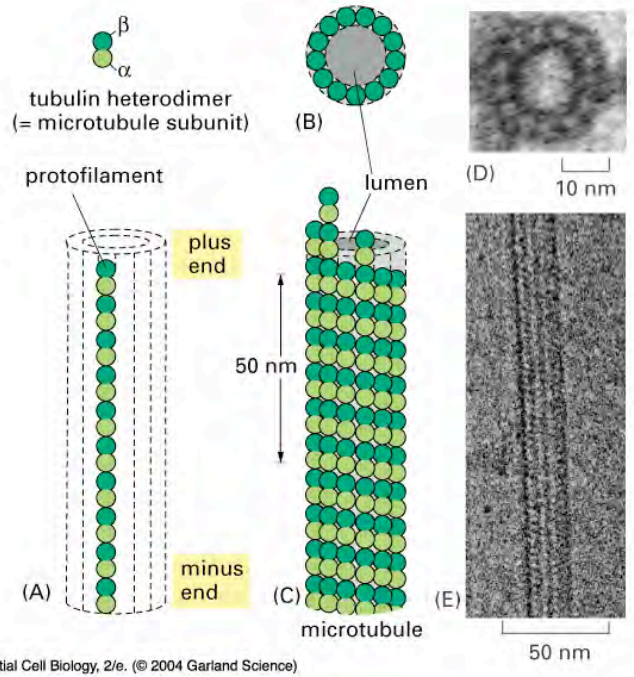


Figure 17-10 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Cytoskeletal Action During Cell Division

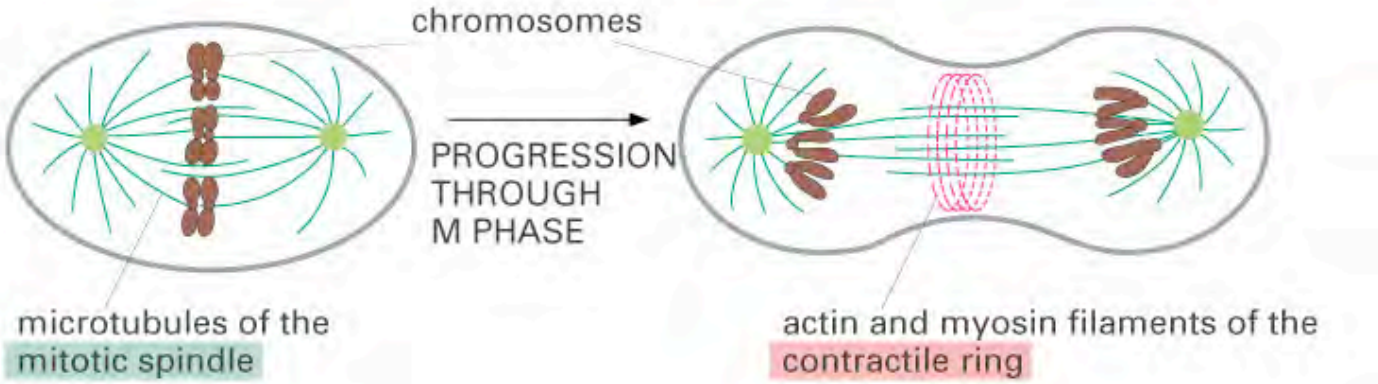
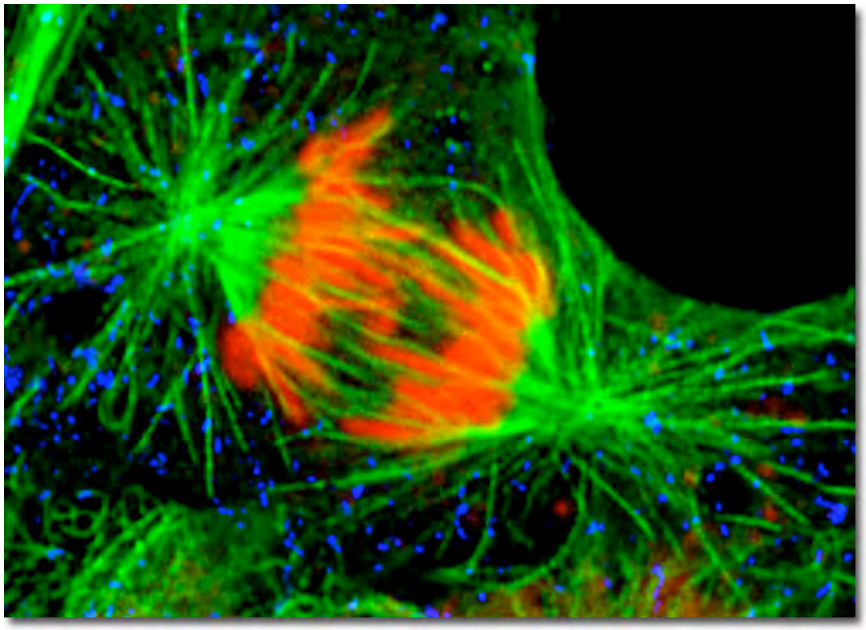
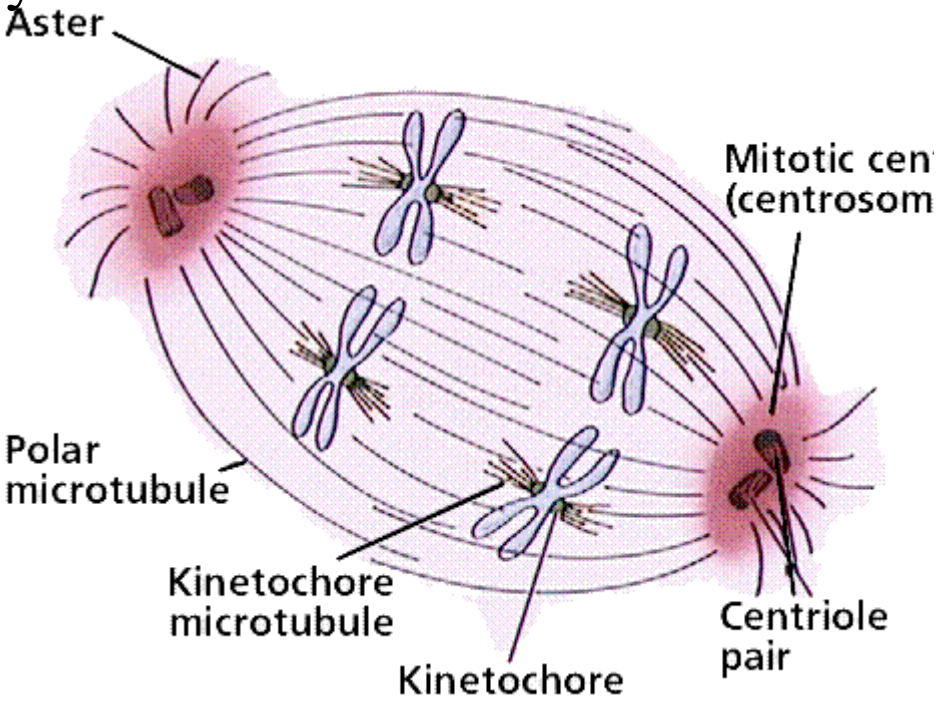


Figure 19-4 Essential Cell Biology, 2/e. (© 2004 Garland Science)

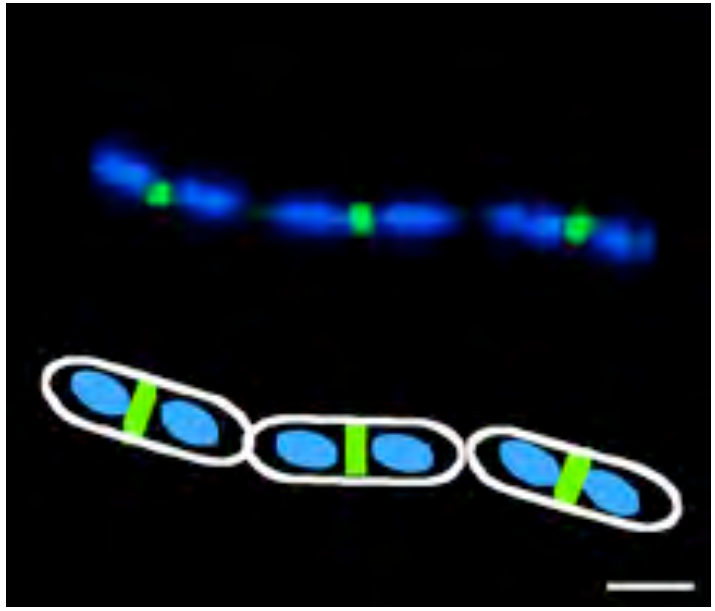
Cytoskeleton Mediated Mitosis

Show the movies in the Lecture 11 directory – see http://www.bio.unc.edu/faculty/salmon/lab/mitosis/mitosismovies.

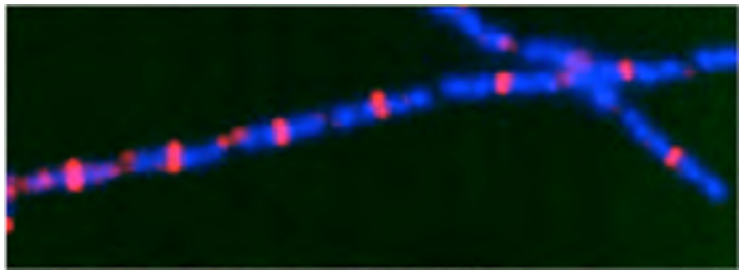




Cell Division in Bacteria

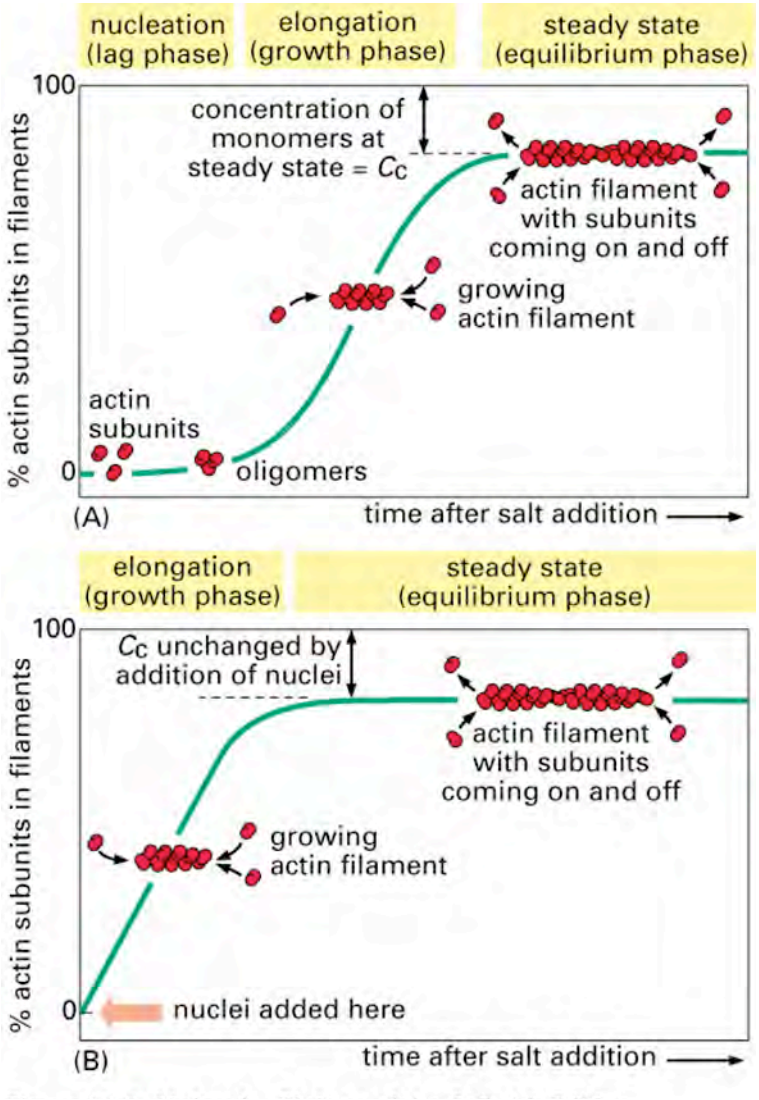


Petra Levin



FtsZ rings during division

Actin Polymerization Rate in vitro



Actin and its Partners

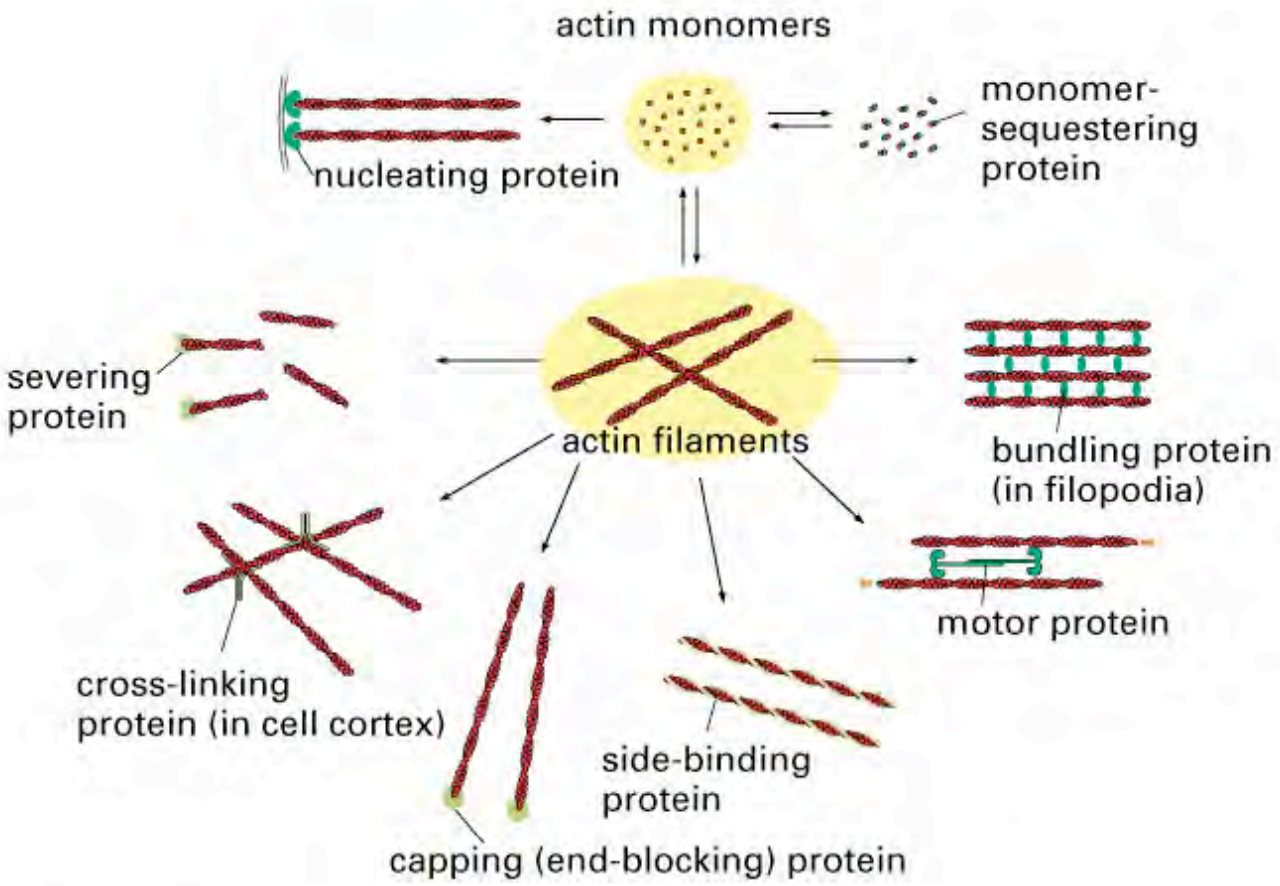
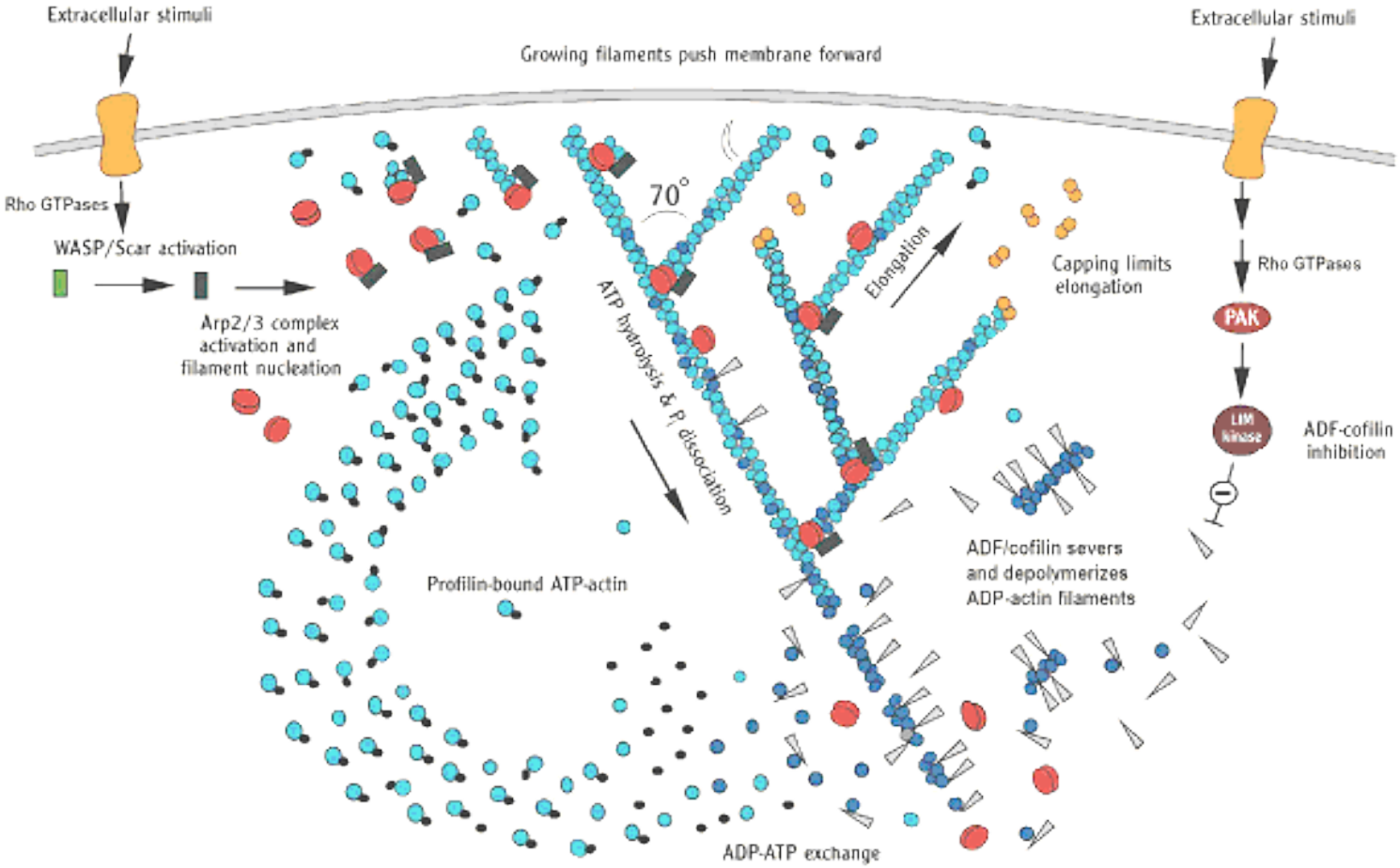


Figure 17-32 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Life at the Leading Edge



Microtubule Dynamics: Part of the Polymerization Puzzle

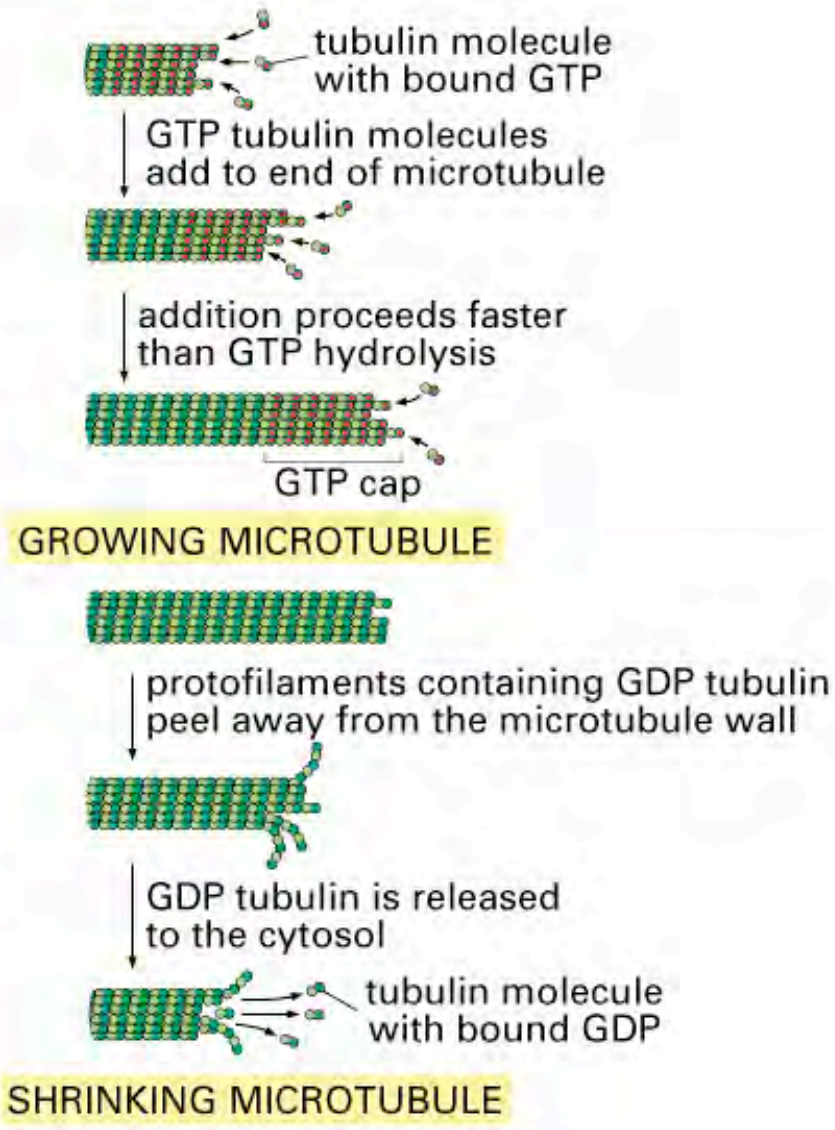
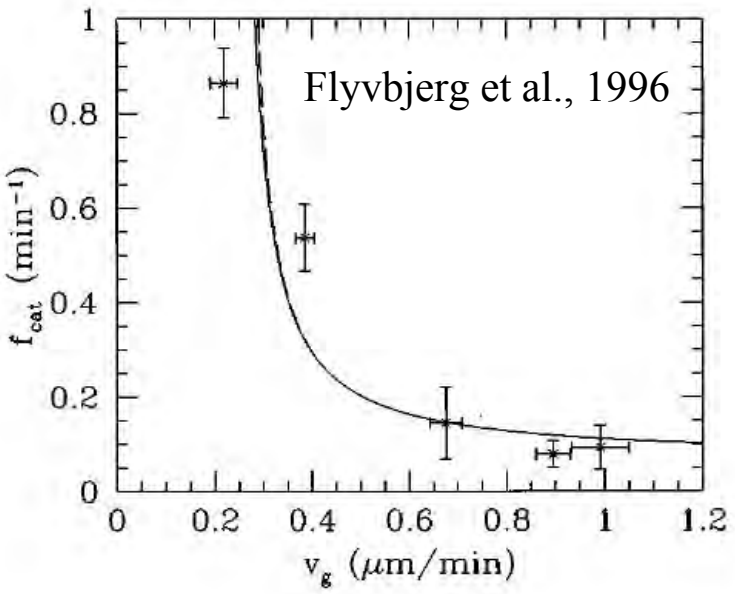


Figure 17-13 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Microtubule Dynamics In Vitro

Show Borisy movies,
http://www.borisylab.nwu.edu/pages/supplemental/treadmill.html

Catastrophe rate vs. growth rate



PHYSICAL REVIEW E VOLUME 50, NUMBER 2 AUGUST 19

Phase diagram of microtubules

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Physics Department, Princeton University, Princeton, New Jersey 08544
(Received 4 April 1994)

We map the phase diagram of microtubules as a function of temperature and tubulin concentration. We observe spontaneous and site-nucleated microtubule assembly. At temperatures and concentrations below the onset of spontaneous nucleation, we measure the steady-state proportion of occupied nucleation sites and the distribution of lengths of site-nucleated microtubules. Our observations reveal a transition in the length dynamics of microtubules from bounded to unbounded growth. This transition is also evident in the length dynamics of individual site-nucleated microtubules. The transition to spontaneous microtubule assembly completes the phase diagram. We measure the temperature and concentration dependence of the latent time for spontaneous nucleation of microtubules in the bulk.

PACS number(s): 87.15.−v, 64.60.−i

I. INTRODUCTION

The protein *tubulin* is found in every cell of every living organism (with the exception of bacteria) [1]. In the presence of guanosine-tri-phosphate (GTP) and magnesium ions (Mg^{2+}), tubulin-GTP complexes form [2] and aggregate [3] into long, hollow cylinders called *microtubules.* Microtubules are about 25 nm in outer diameter, incorporate 1625 tubulin dimers per micrometer of their length [4] and easily grow long enough to span a cell (10–100 μm). In the cell, microtubules form a network that supports the overall structure and guides internal transport [5]. Fundamental cellular processes, like locomotion, morphogenesis, and reproduction, rely on the ability of microtubules to change their organization. This ability derives from a unique feature of microtubule assembly called *dynamic instability* [6].

The term dynamic instability describes the fact that individual microtubules are dynamic structures that fluctuate erratically between assembling and disassembling. These fluctuations are not microscopic; they often involve most or all of the microtubule. A typical plot of the length of a single microtubule over time is shown in Fig. 1. This so-called dynamic instability depends on the hydrolysis reaction which turns the GTP of the tubulin-GTP complex into guanosine-di-phosphate (GDP) and releases energy ($\sim 8kT$/reaction) [7].

Dynamic instability has been the subject of research for the past decade. The key element of all models is a competition between growth and the process of hydrolysis, often considered in terms of a growth front and a possible hydrolysis front. Crudely, when the hydrolysis front overtakes the growth front, the microtubule structure becomes unstable. The question of exactly how the hydrolysis and growth reactions are coupled continues to draw attention [8]. Experimental control parameters i clude temperature and tubulin concentration, as well the *p*H of the solution, the presence of certain other pr teins [microtubule-associated proteins (MAP's)] [9], a the concentrations of GTP and Mg^{2+} ions [10]. Of thes the most biologically relevant parameter is perhaps t concentration MAP's. However, to understand dynam instability the concentration of tubulin has been the pr ferred variable, since it affects the growth velocity but n the hydrolysis of GTP.

In the work described here, we vary both temperatu and tubulin concentration and map the phase diagram microtubule growth. We study both spontaneously n cleated assembly and growth from nucleation sites [1 The resulting phase diagram is shown in Fig. 2.

At low temperatures, microtubules only grow from n cleating sites. For a range of temperatures, a steady sta is reached in which only a fraction of the sites have n

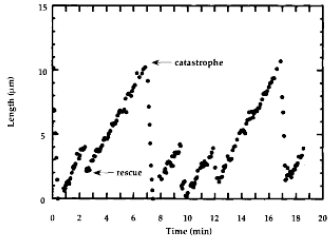

FIG. 1. A typical time series of the length of a single micr tubule. The velocities of growth range between 1.6 and μm/min (40–65 dimers/sec), while the velocities of shorteni are 10 times faster. The transitions from growth to shorteni are called *catastrophes;* transitions from shortening to grow are called *rescues.*

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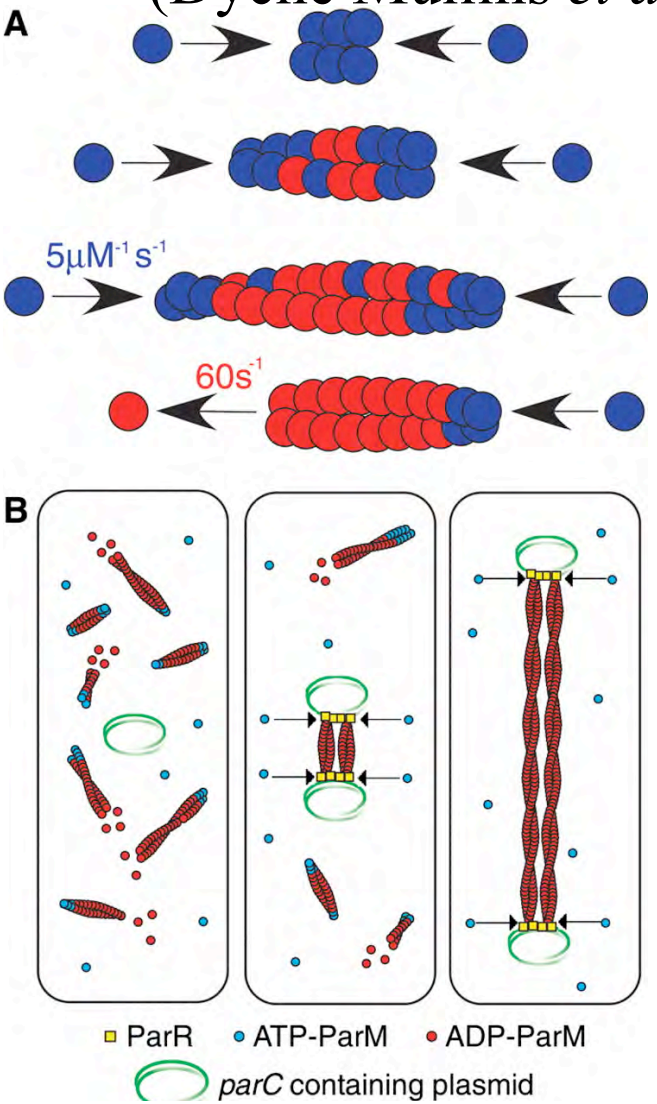
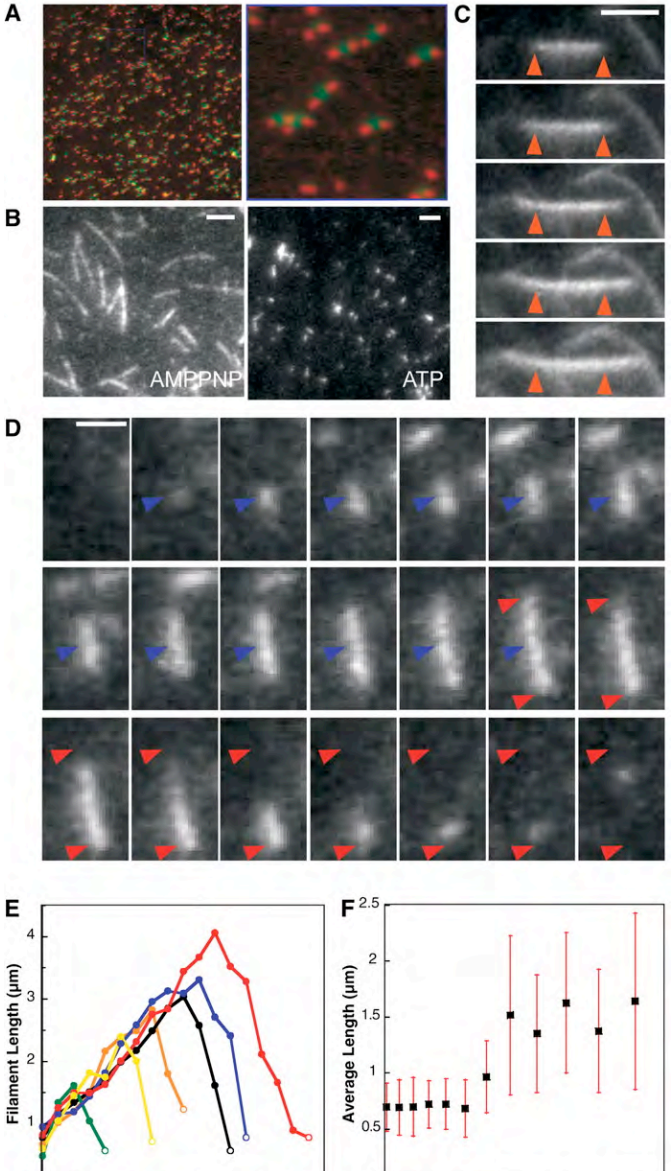
The Story of ParM

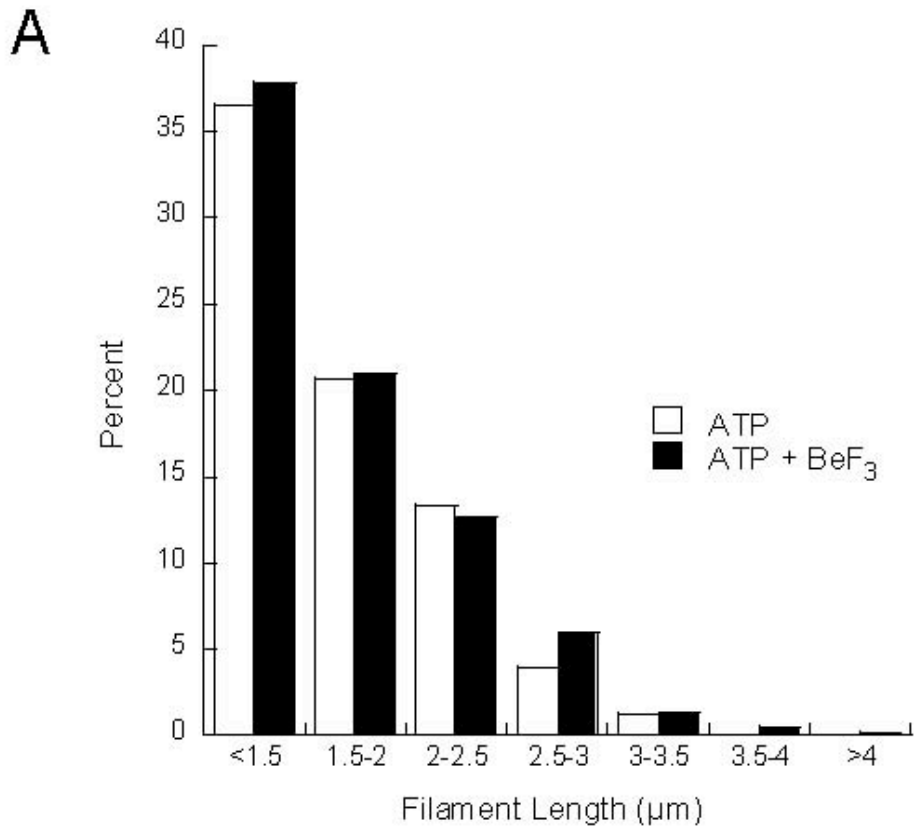
(Dyche Mullins *et al.*)





ParM Length Distribution

Mullins et al., Science 2004



Polymerization of Pyrene Labeled Actin

Lim et al., Science 2004

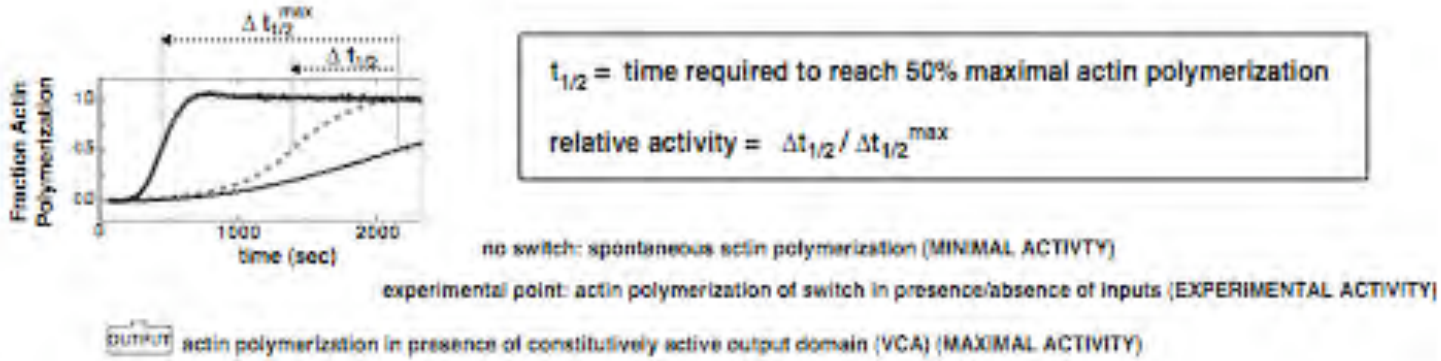


Fig. S1. Metric for relative activity of N-WASP switches based on half-time of actin polymerization. Activity of switch proteins was determined using a fluorescence-based actin polymerization assay (20, and "Methods"). Time required to reach 50% polymerization ($t_{1/2}$) was used as a metric for activity. Minimal activity was defined as the $t_{1/2}$ observed with spontaneous actin polymerization under these conditions in the presence of Arp2/3 but no nucleation promoting factors. Maximal activity was defined as the $t_{1/2}$ in the presence of the isolated output domain. Relative activities of individual constructs were scored by measuring the change in $t_{1/2}$ relative to the difference between maximum and minimum activities.