

BE/APH161 – PHYSICAL BIOLOGY OF THE CELL

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CELLS DECIDE: WHERE TO GO

The Hunters of the Immune Response



- ♦ ***There is another kind of rapid response to environmental cues that is much faster than gene regulation.***
- ♦ ***The “decision” about where to go next is highly regulated and results in the synthesis of new cytoskeletal filaments at the leading edge of the cell.***
- ♦ ***Once again, there is an interesting random walk story behind the scenes.***

CELL MOTILITY AND ACTIN POLYMERIZATION

- ◆ **Motility is driven by the protein actin. Actin assembles into long filaments.**
- ◆ **Through hydrolysis of ATP, these filaments can actually do work as a result of this polymerization process by pushing on membranes, for example.**

(Theriot et al.)

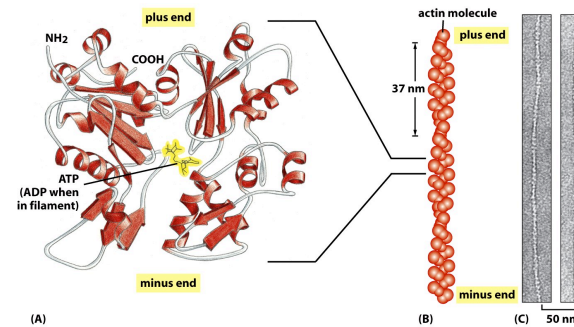
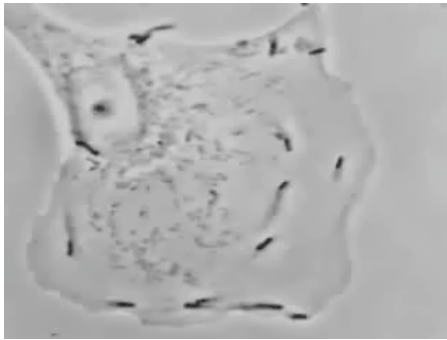


Figure 16-12 Molecular Biology of the Cell 5/e (© Garland Science 2008)

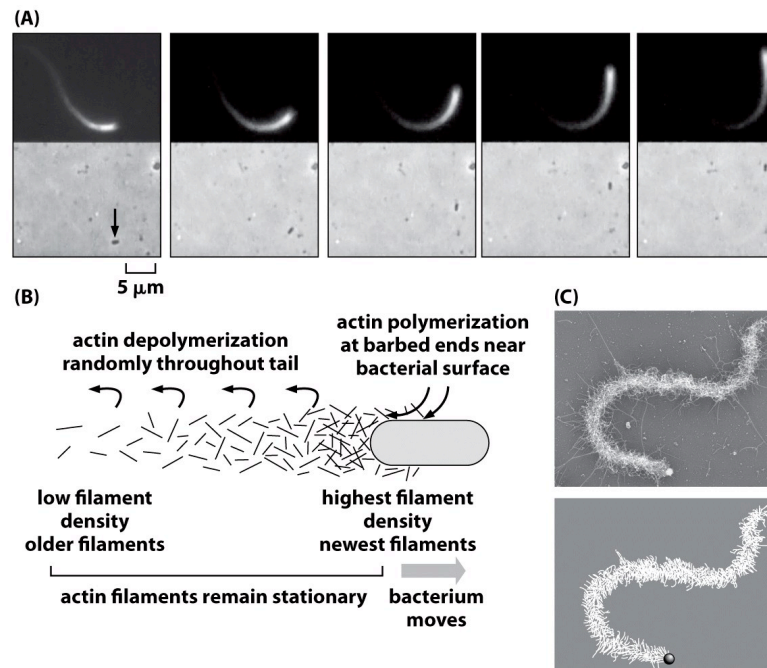
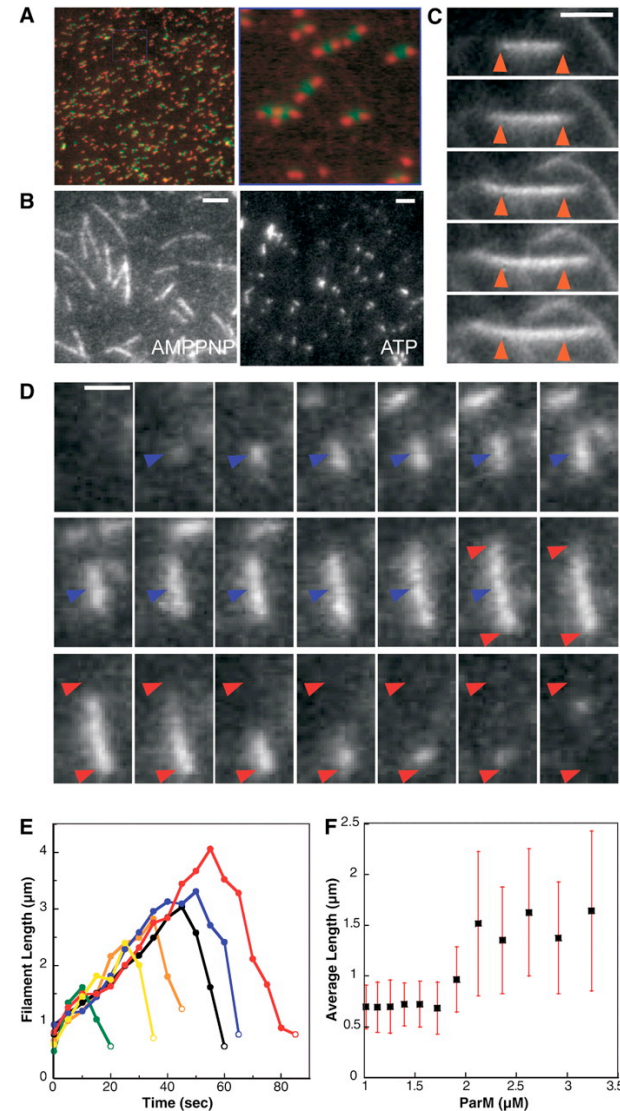
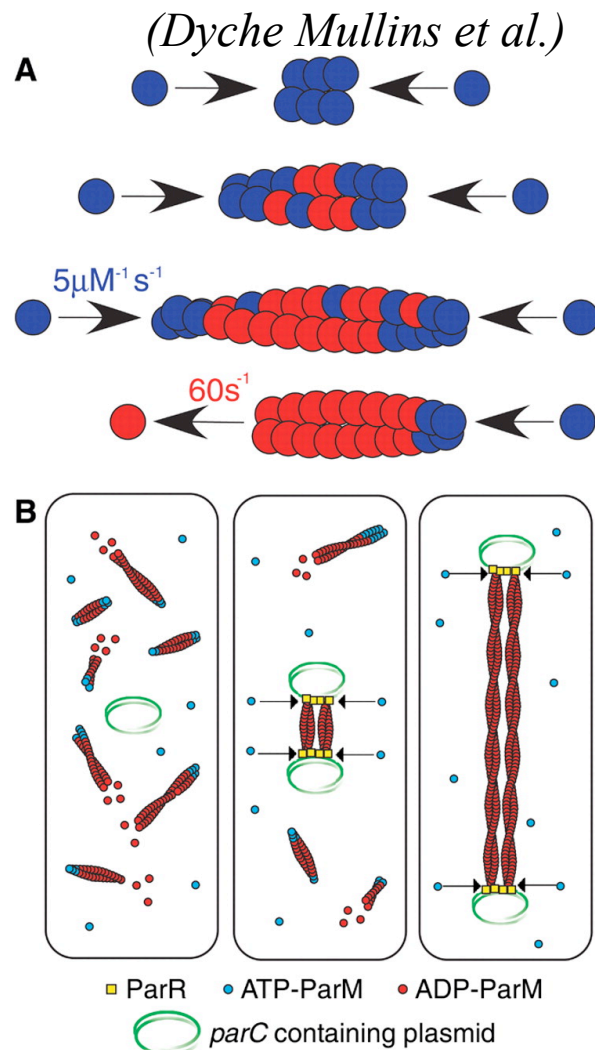


Figure 15.3 Physical Biology of the Cell (© Garland Science 2009)

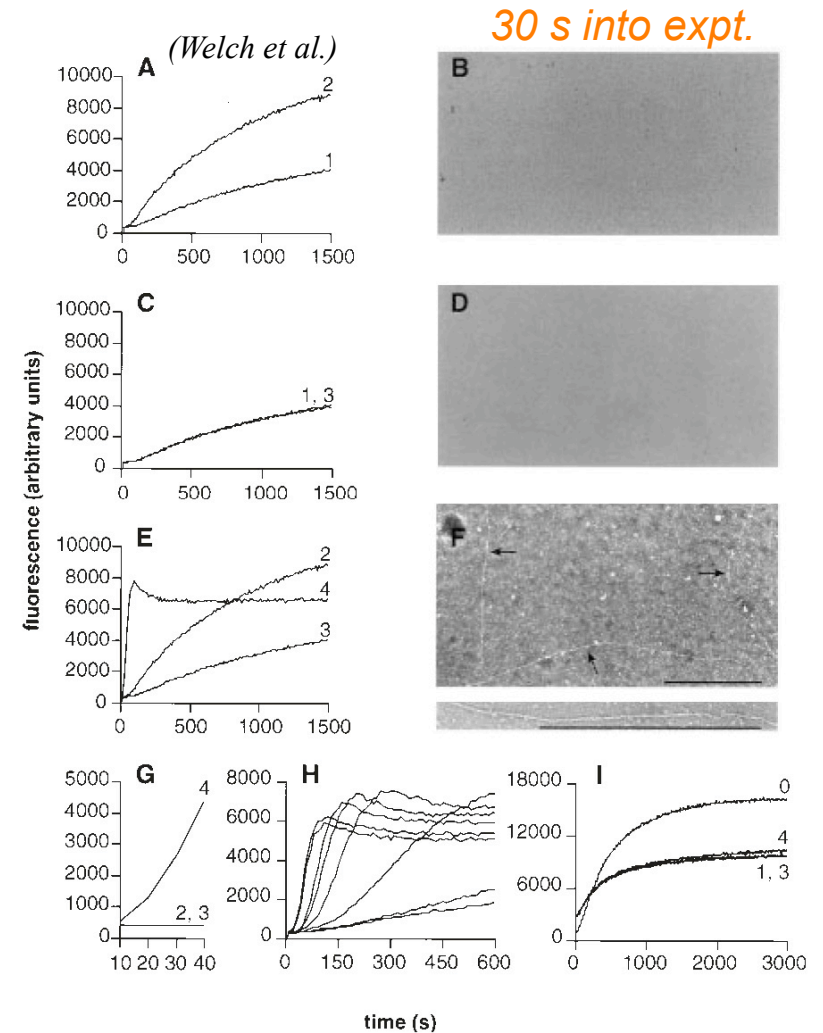
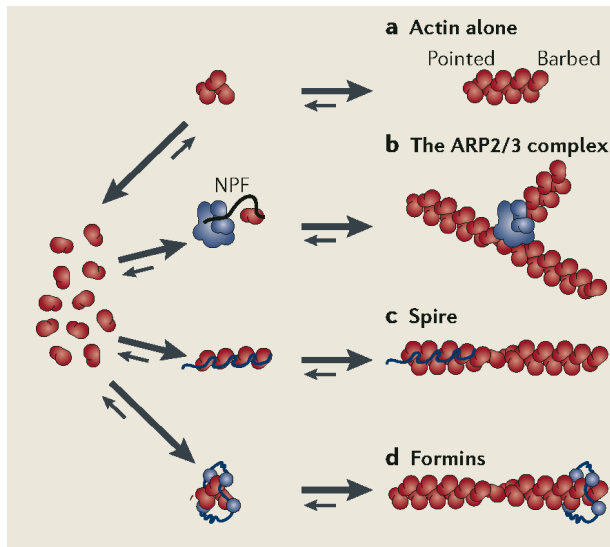
THE STORY OF PAR-M

- One of the most interesting stories for me is that of plasmid segregation in bacteria.
- This process is mediated by the bacterial cytoskeleton protein ParM.



HOW IS POLYMERIZATION CONTROLLED IN SPACE AND TIME?

- Just as with the measurement of cell growth described earlier, bulk growth assays using light absorption (or fluorescence) as a readout have spoken volumes on mechanism.
- One of the outcomes of such experiments is the discovery of molecules that nucleate polymerization of new filaments (or branches off of existing filaments).
- Using these experiments, they proved the existence of protein nucleating factors.



ACTIN POLYMERIZATION DYNAMICS

- ◆ *In vitro* assay for measuring the amount of actin filaments as a function of time.
- ◆ The nucleation process leads to a lag phase.

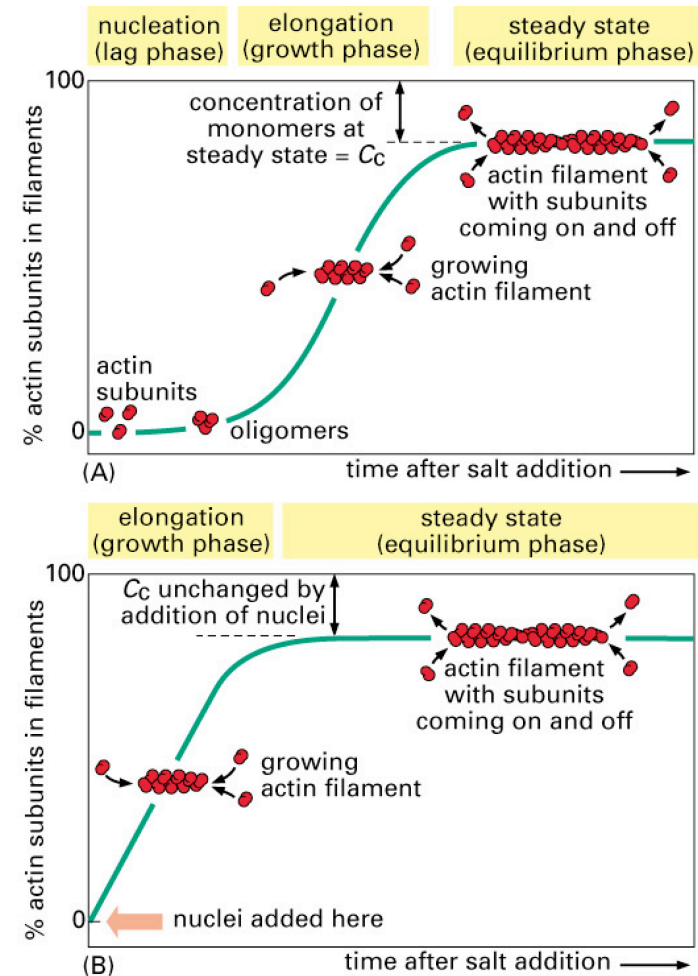


Figure 16-5. Molecular Biology of the Cell, 4th Edition.

HOW IS POLYMERIZATION CONTROLLED IN SPACE AND TIME?

- There are a host of “accessory” proteins that participate in the **REGULATION** of the actin polymerization process.

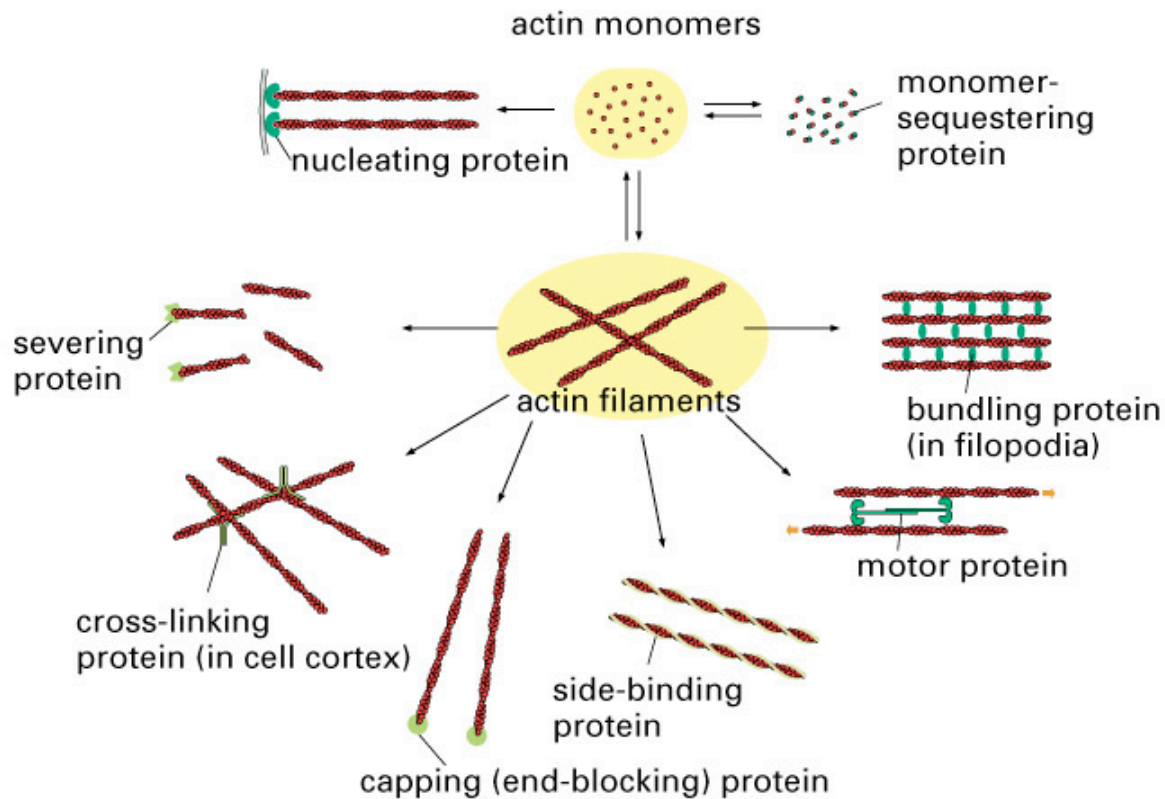
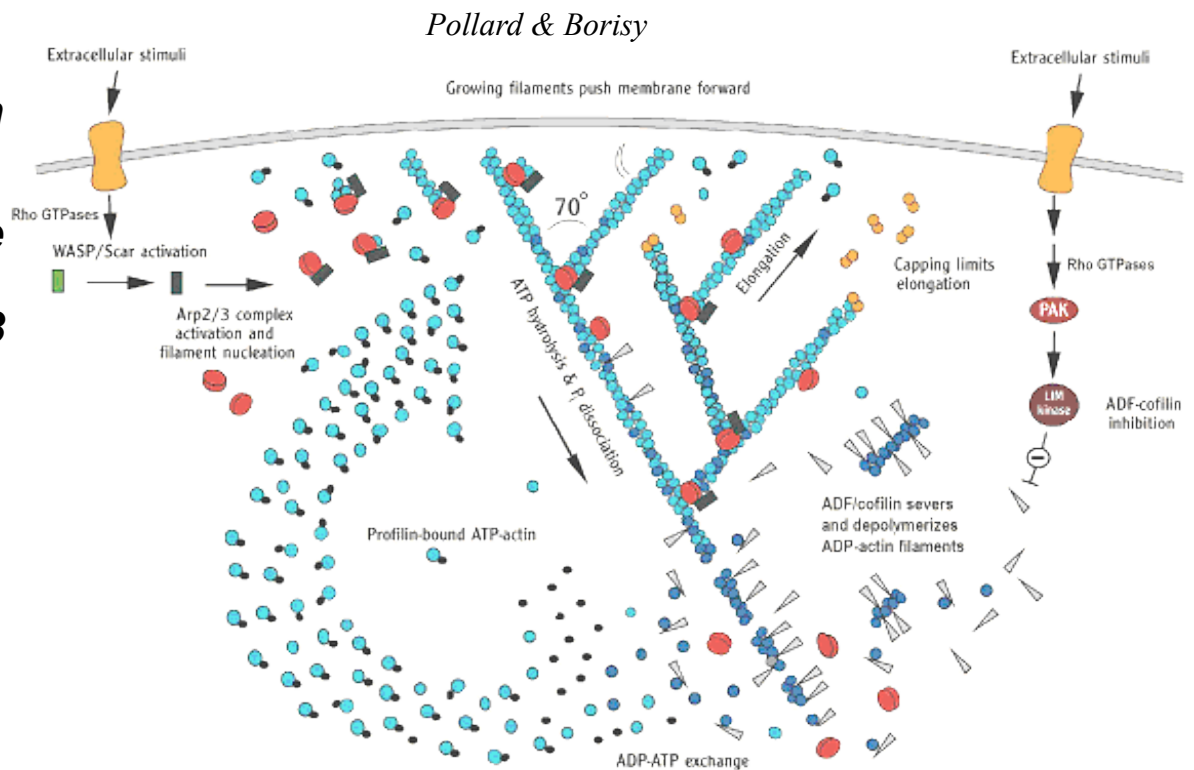


Figure 17-32 Essential Cell Biology, 2/e. (© 2004 Garland Science)

THE CONTROL OF ACTIN POLYMERIZATION AT THE LEADING EDGE

- ◆ **Spacetime control of polymerization is mediated by a host of different proteins that do stuff such as: cap, nucleate, branch, sequester, etc. the actin itself.**
- ◆ **Our story will focus on one little piece of this complex system, namely, the way in which Arp2/3 leads to the synthesis of new filaments.**
- ◆ **Key point: signal integration – how do cells know when and where to put in new actin filaments?**



ARP2/3 AND ACTIN NUCLEATION

- ◆ **Arp2/3 needs to be activated itself as a result of signal integration.**
- ◆ **Once activated Arp2/3 promotes actin filament nucleation and growth.**

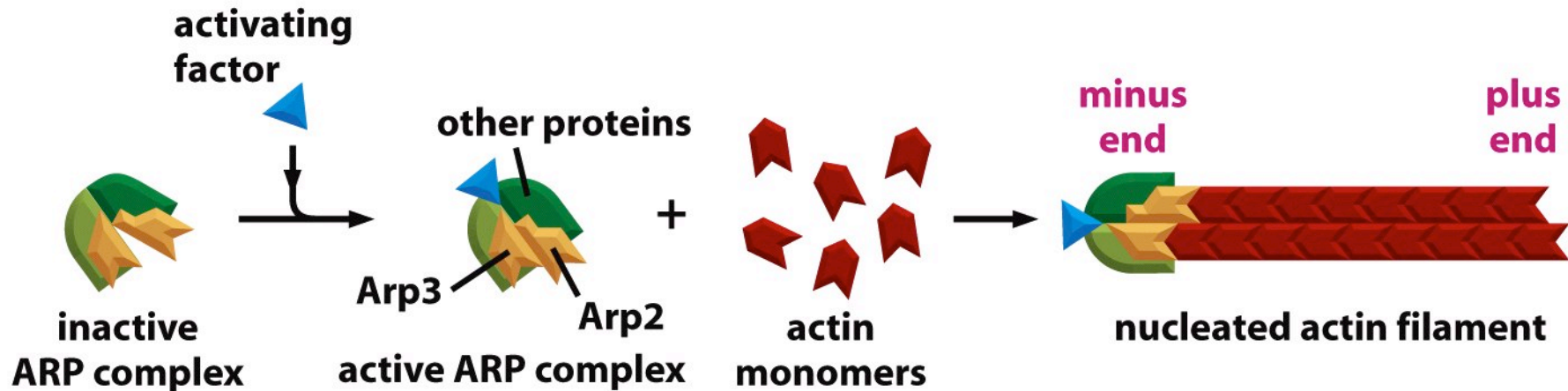


Figure 16-34b Molecular Biology of the Cell 5/e (© Garland Science 2008)

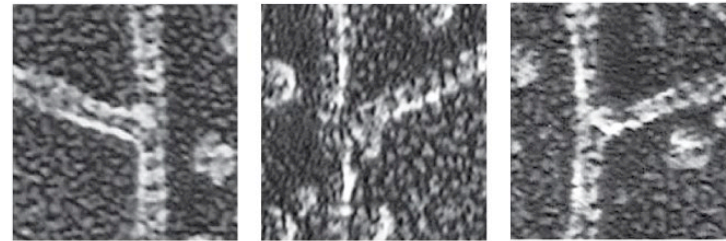
. (B) A model for actin filament nucleation by the ARP complex. In the absence of an activating factor, Arp2 and Arp3 are held by their accessory proteins in an orientation that prevents them from nucleating a new actin filament. When an activating factor indicated by the blue triangle binds the complex, Arp2 and Arp3 are brought together into a new configuration that resembles the plus end of an actin filament. Actin subunits can then assemble onto this structure, bypassing the rate-limiting step of filament nucleation (see Figure 16-10). (C) The ARP complex nucleates filaments most efficiently when it is bound to the side of a preexisting actin filament. The result is a filament branch that grows at a 70° angle relative to the original filament. Repeated rounds of branching nucleation result in a treelike web of actin filaments. (D) Top, electron micrographs of branched actin filaments formed by mixing purified actin subunits with purified ARP complexes. Bottom, reconstructed image of a branch where the crystal structures of actin and the ARP complex have been fitted to the electron density. The mother filament runs from top to bottom, and the daughter filament branches off to the right where the ARP complex binds to three actin subunits in the mother filament (D, from R.D. Mullins et al., Proc. Natl Acad. Sci. U.S.A. 95:6181-6186, 1998. With permission from National Academy of Sciences, and from N. Volkman et al., Science 293:2456-2459, 2001. With permission from Macmillan Publishers Ltd.)

STRUCTURAL STUDIES OF ARP2/3

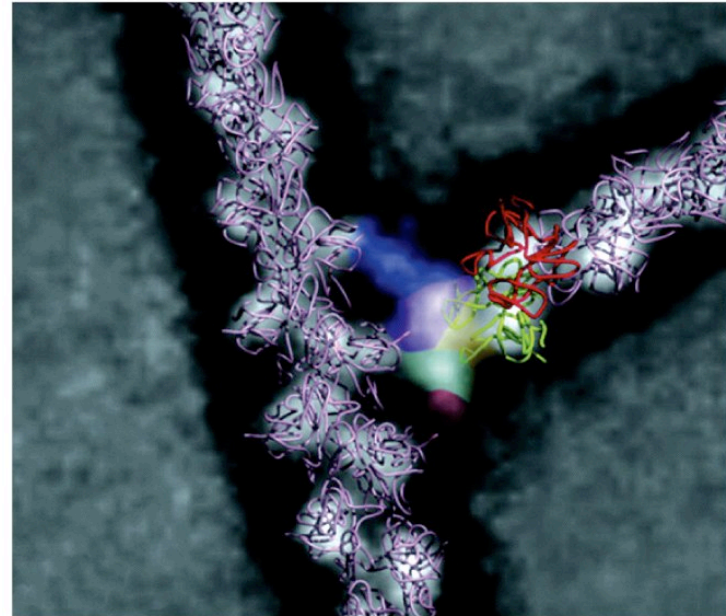
- ◆ **Electron microscopy images reveal the nature of Arp2/3 action on actin filaments.**

(D) Top, electron micrographs of branched actin filaments formed by mixing purified actin subunits with purified ARP complexes. Bottom, reconstructed image of a branch where the crystal structures of actin and the ARP complex have been fitted to the electron density. The mother filament runs from top to bottom, and the daughter filament branches off to the right where the ARP complex binds to three actin subunits in the mother filament (D, from R.D. Mullins et al., *Proc. Natl Acad. Sci. U.S.A.* 95:6181-6186, 1998. With permission from National Academy of Sciences, and from N. Volkmann et al., *Science* 293:2456-2459, 2001. With permission from Macmillan Publishers Ltd.)

Author: Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter



100 nm

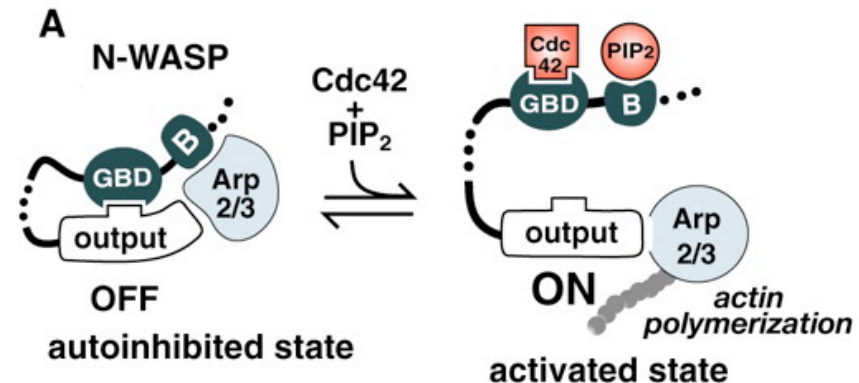


10 nm

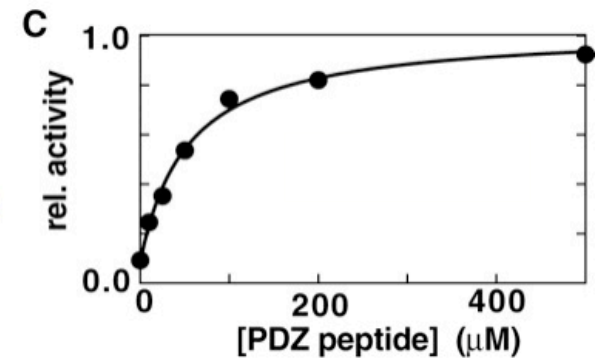
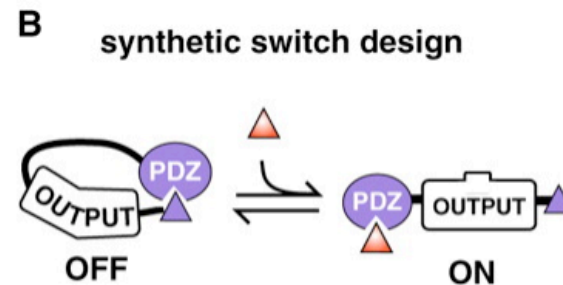
Figure 16-34d Molecular Biology of the Cell 5/e (© Garland Science 2008)

SIGNALING AND POLYMERIZATION

- External signals activate Arp2/3 which in turn nucleates actin polymerization.
- Group of Wendell Lim has used a Lego approach to mix and match components so that signals normally reserved for other circuits can induce polymerization.
- Do we really “understand” how these molecules work? Let theory and predictions be the judge of that!



(Lim et al., Science)





COMPUTING THE ACTIVITY OF THE SWITCH

- ◆ *Competition between tethered ligand and free ligands.*
- ◆ *Use simple ideas from statistical mechanics to reckon the free energy of the various contributions.*

STATE	ENERGY	MULTIPLICITY	BOLTZMANN WEIGHT
	$\epsilon_b + L\epsilon_{sol}$	$\frac{\Omega!}{L!(\Omega-L)!} \left(\frac{(N_R + N_L)!}{\left(\left(\frac{N_R + N_L}{2} \right)! \right)^2} \right)$	$e^{-\beta L\epsilon_{sol}} e^{-\beta\epsilon_b}$
	$L\epsilon_{sol}$	$\frac{\Omega!}{L!(\Omega-L)!} 2^{N_R} 2^{N_L}$	$e^{-\beta L\epsilon_{sol}}$
	$\epsilon_b + (L-1)\epsilon_{sol}$	$\frac{\Omega!}{(L-1)!(\Omega-(L-1))!} 2^{N_R} 2^{N_L}$	$e^{-\beta(L-1)\epsilon_{sol}} e^{-\beta\epsilon_b}$

Figure 19.37 Physical Biology of the Cell (© Garland Science 2009)

TUNING TETHERS TO ALTER SIGNALING

- ◆ We view the tether length as a dial that can be tuned in order to vary the probability that N-Wasp will activate Arp2/3.
- ◆ As with many free energy stories, the point here is a competition between the free and tethered ligands and their entropies (especially).

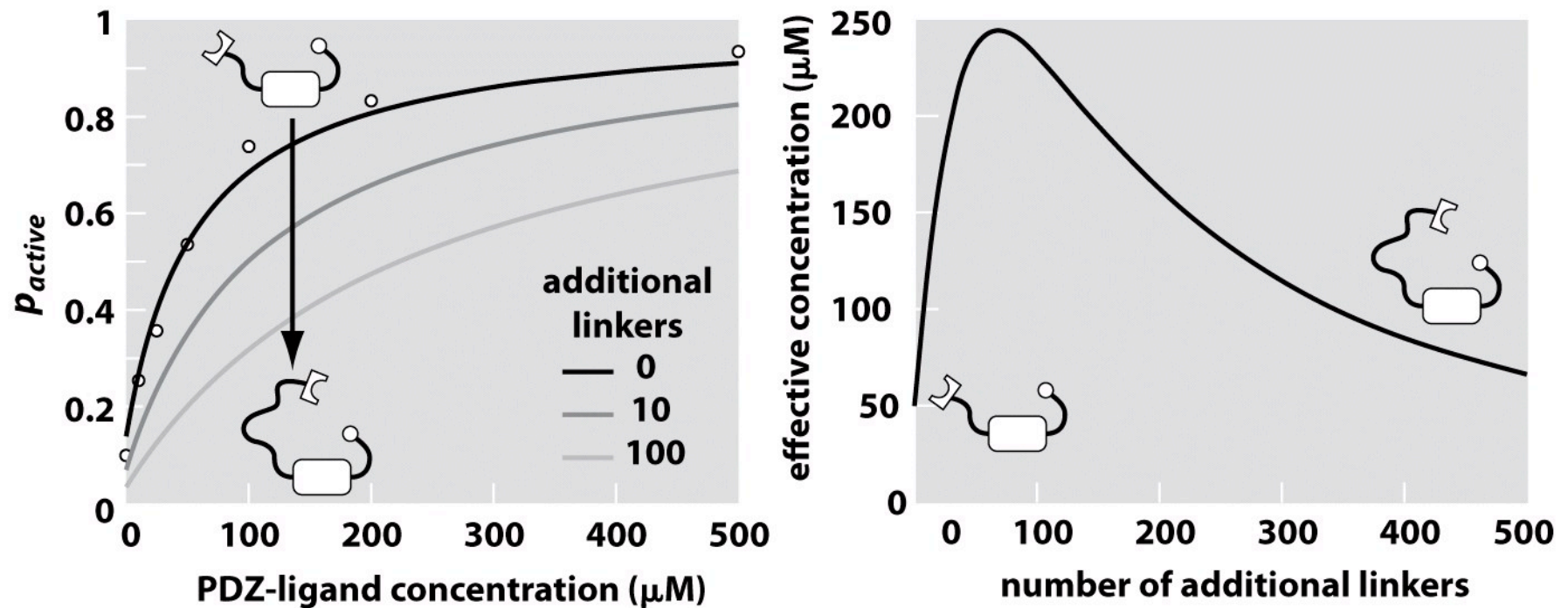


Figure 19.39 Physical Biology of the Cell (© Garland Science 2009)