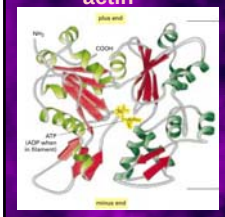
Protein Polymers, Crawling Cells and Comet Tails

Julie Theriot
Stanford University
Department of Biochemistry
Department of Microbiology & Immunology
Program in Biophysics

Biological structure and function: Mechanics and Scale

actin



5 nm

Biological structure and function: Mechanics and Scale

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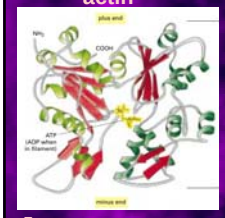

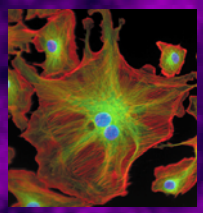

5 nm

50-200 µm

Scaling factor > 10^4

Cell organization is DYNAMIC

David Rogers 1950s

A neutrophil gives chase... (real time)

Cell organization is DYNAMIC

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A neutrophil gives chase... (real time)

Amoebae search for food (30X real time)

Cell organization is DYNAMIC

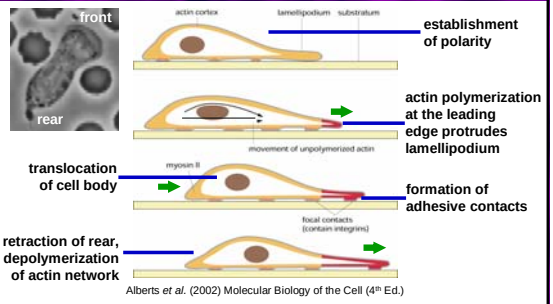
David Rogers 1950s

A neutrophil gives chase... (real time)

Fish keratocytes move to close a wound (100X real time)

Amoebae search for food (30X real time)

Cycle of actin-based cell motility



Alberts *et al.* (2002) Molecular Biology of the Cell (4th Ed.)

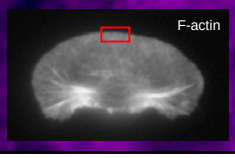
See Lauffenburger and Horwitz, 1996

Dynamics and force generation in the eukaryotic cytoskeleton

- Filaments constructed of many small subunits assemble and disassemble rapidly (actin, microtubules, intermediate filaments)
- Actin filaments and microtubules serve as directional tracks for large families of molecular motor proteins (myosins, kinesins, dyneins)
- Both motors and filament assembly/disassembly can directly generate force
- Intracellular and whole-cell movements almost always require COOPERATION among large numbers of individual force-generating elements

Cell protrusion

- Bootstrapping into unexplored space
- Cannot rely on sliding along preassembled biological elements
- Often uses spatially regulated assembly of actin filament networks : parallel bundles or branched meshes



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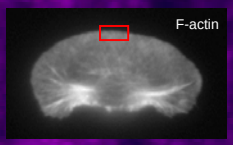
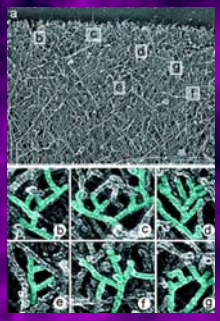
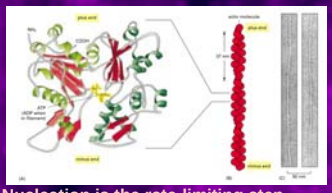




Svitkina and Borisy, 1999
JCB 145:1009-1026

Each actin filament is assembled from soluble monomeric subunits



- Nucleation is the rate-limiting step
- In a living cell, equilibrium is never reached
- Filaments are dynamic (average half-life ~1 min)
- Hundreds of actin-binding proteins modify dynamics and organization of filaments
- Polymerization can generate force, like a molecular motor

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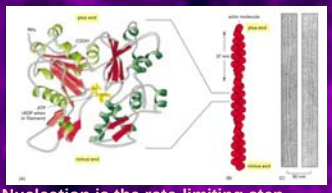

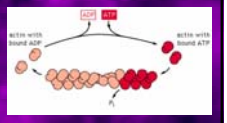

DYNAMICS

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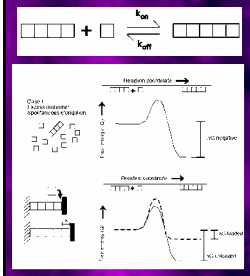
Force generation by protein polymerization



Adapted from:
Hill & Kirschner, 1982
Int. Rev. Cytol. 78: 1-125

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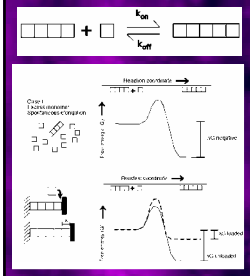

$F_{max} = (kT/\delta) \ln(C/C_{crit})$
$C_{crit} \sim k_{off}/k_{on}$
for actin: $F_{max} \sim$ 5-10 pN
(comparable to myosin or kinesin)

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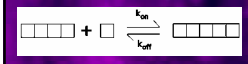

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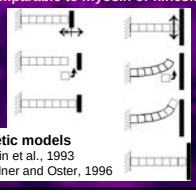


Kinetic models
Peskin et al., 1993
Mogilner and Oster, 1996

Most cells in the human body are bacteria

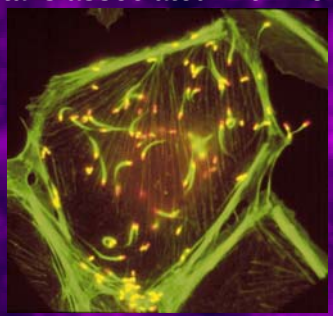
"I am large, I contain multitudes."
Walt Whitman, "Song of Myself"

Actin-based motility of the intracellular bacterial pathogen *Listeria monocytogenes*

Time lapse: 60X

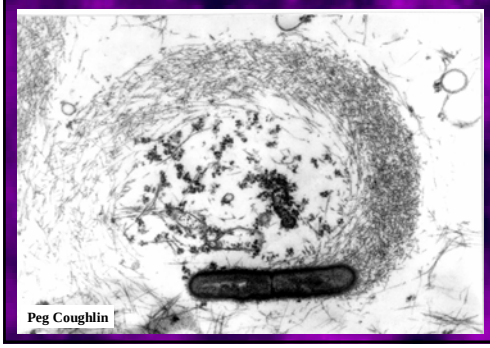
QuickTime™ and a
Cinepak decompressor
are needed to see this picture.

Actin filaments make up the comet tails associated with moving bacteria



Green = actin filaments Red = *L. monocytogenes*

Bacterial surface proteins cause local nucleation of actin filaments



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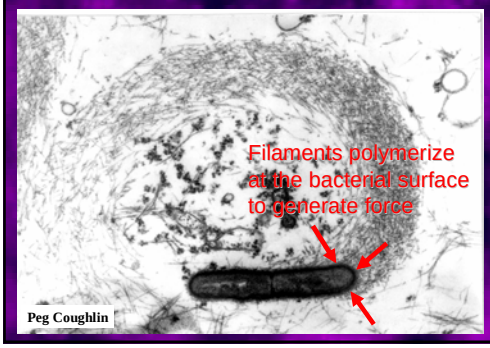

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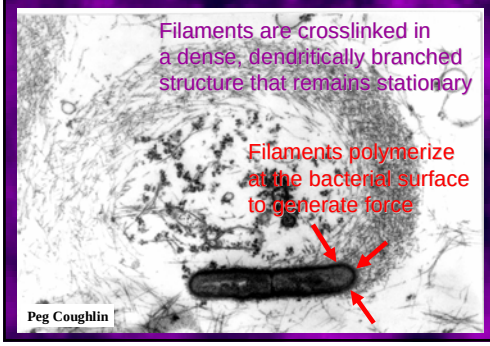

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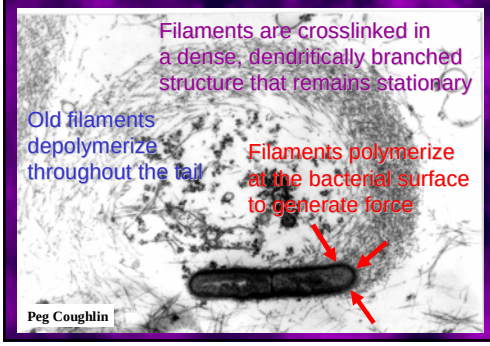
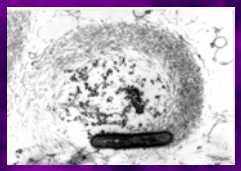
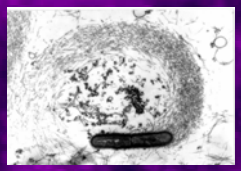




Listeria monocytogenes
Length: 2 µm
Speed: 0.2 µm/sec

Actin monomer size: 4 nm



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Ohio Class SSBN
560 ft
30 ft/sec

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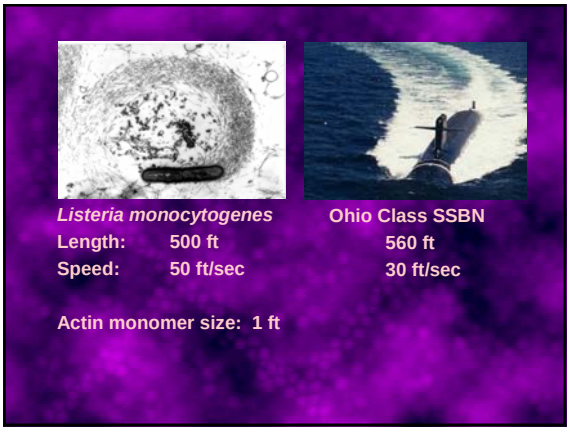
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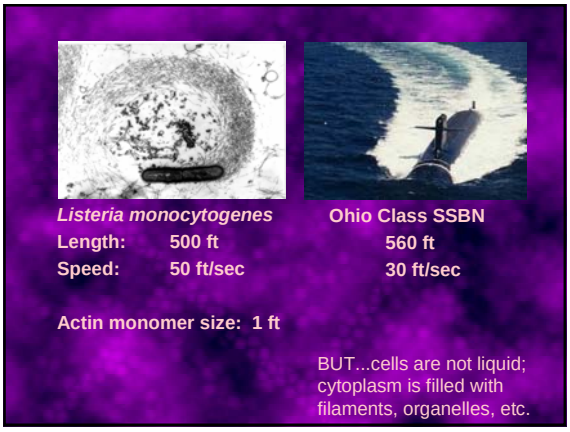
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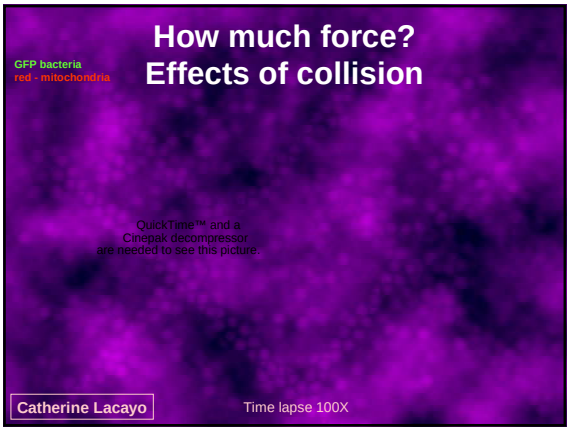
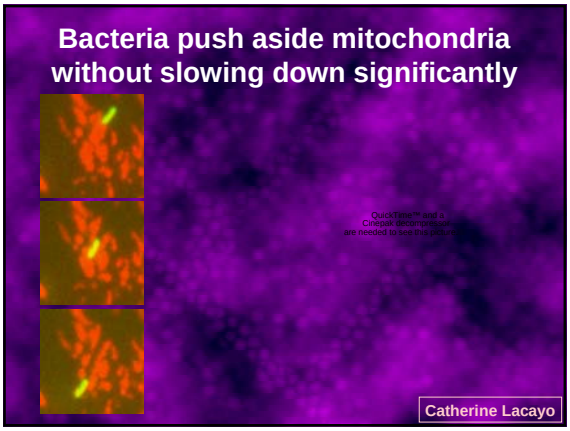
BUT...cells are not liquid; cytoplasm is filled with filaments, organelles, etc.


How much force?
Effects of collision
GFP bacteria
red - mitochondria
QuickTime™ and a
Cinepak decompressor
are needed to see this picture.
Catherine Lacayo
Time lapse 100X


Bacteria push aside mitochondria without slowing down significantly
QuickTime™ and a
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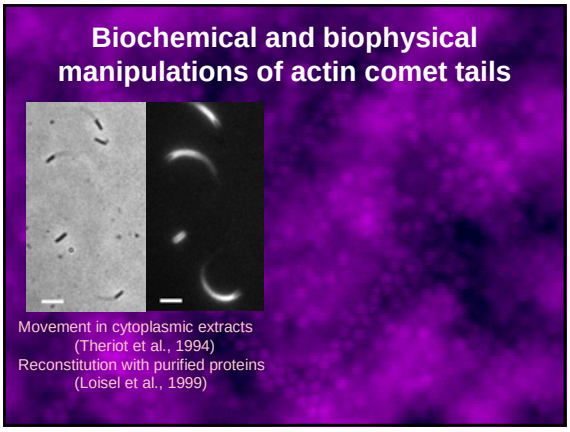
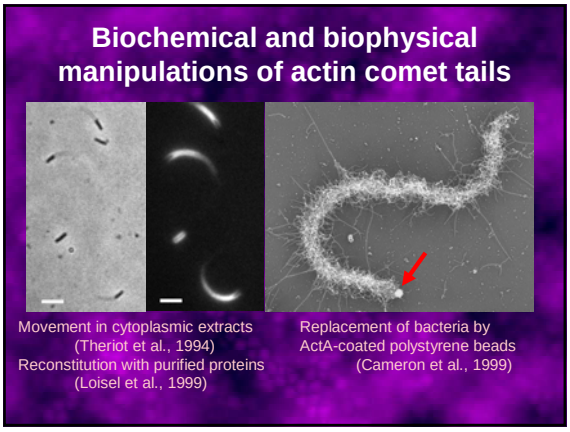
Catherine Lacayo


Biochemical and biophysical manipulations of actin comet tails
Movement in cytoplasmic extracts
(Theriot et al., 1994)
Reconstitution with purified proteins
(Loisel et al., 1999)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Replacement of bacteria by
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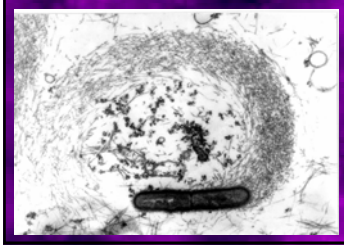
(Cameron et al., 1999)

Biochemical events in comet tail growth (10 years, 20 labs)



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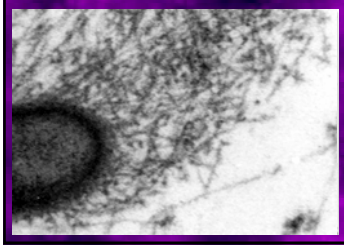

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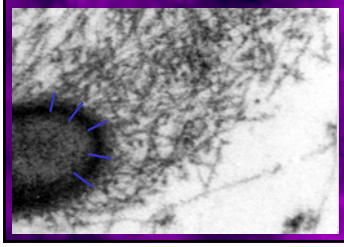

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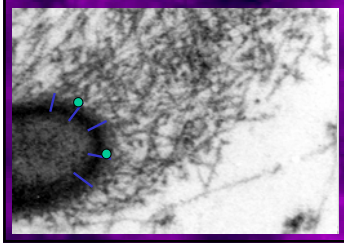

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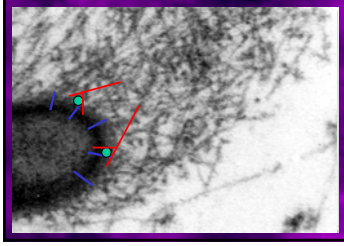

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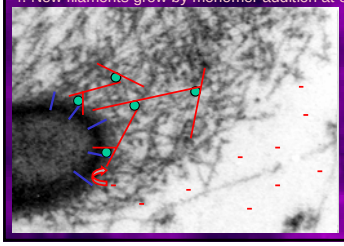

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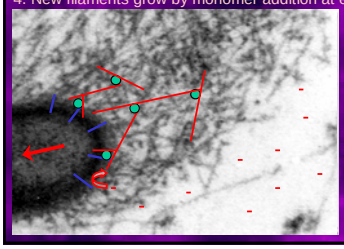

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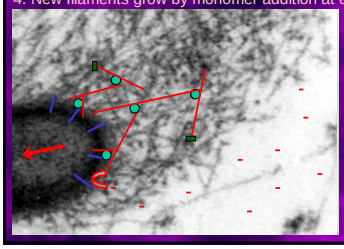

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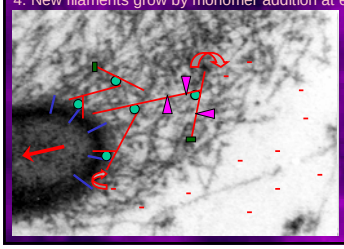

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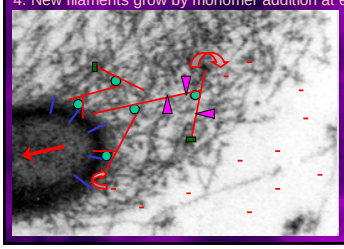

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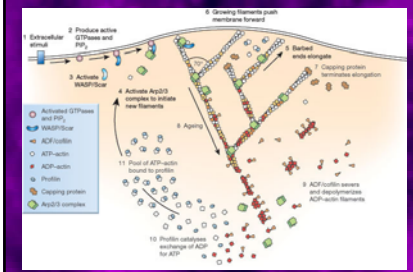
8. Return to step 2 and repeat ad infinitum

A similar actin nanomachine at the leading edge of crawling cells



Pollard and Borisy, 2003

Current research questions

How much force is generated by actin polymerization?

What happens when multiple filaments need to work together?

How are actin forces coordinated with other cellular forces over long distances and long times?

How does a cell regulate its spatial organization and movement as its environment changes?