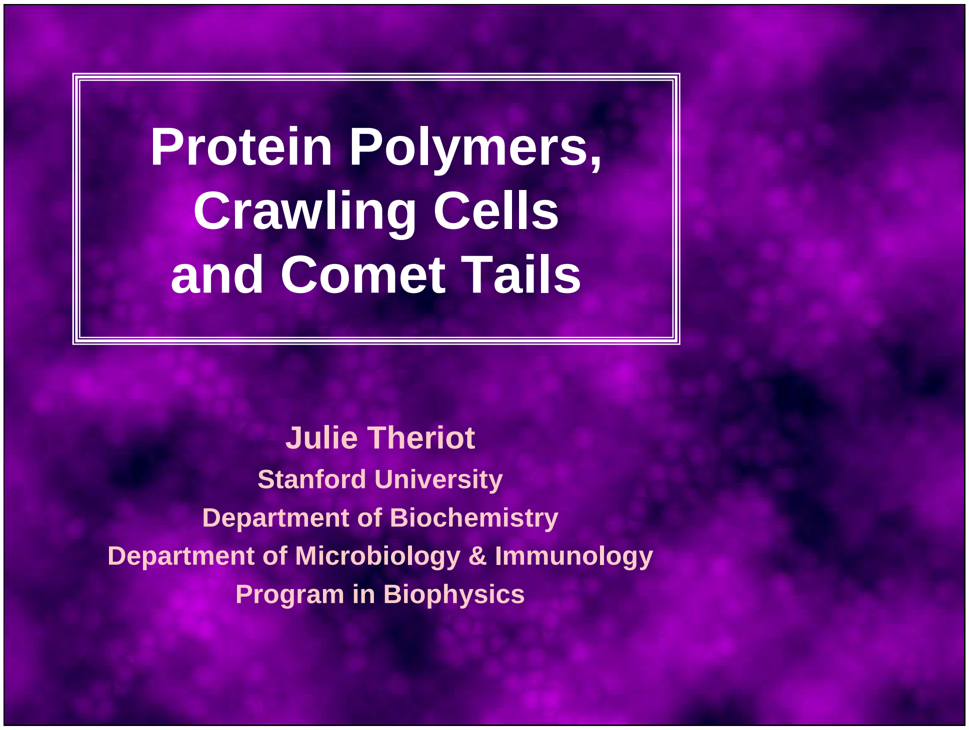
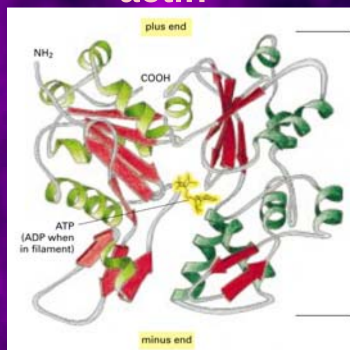
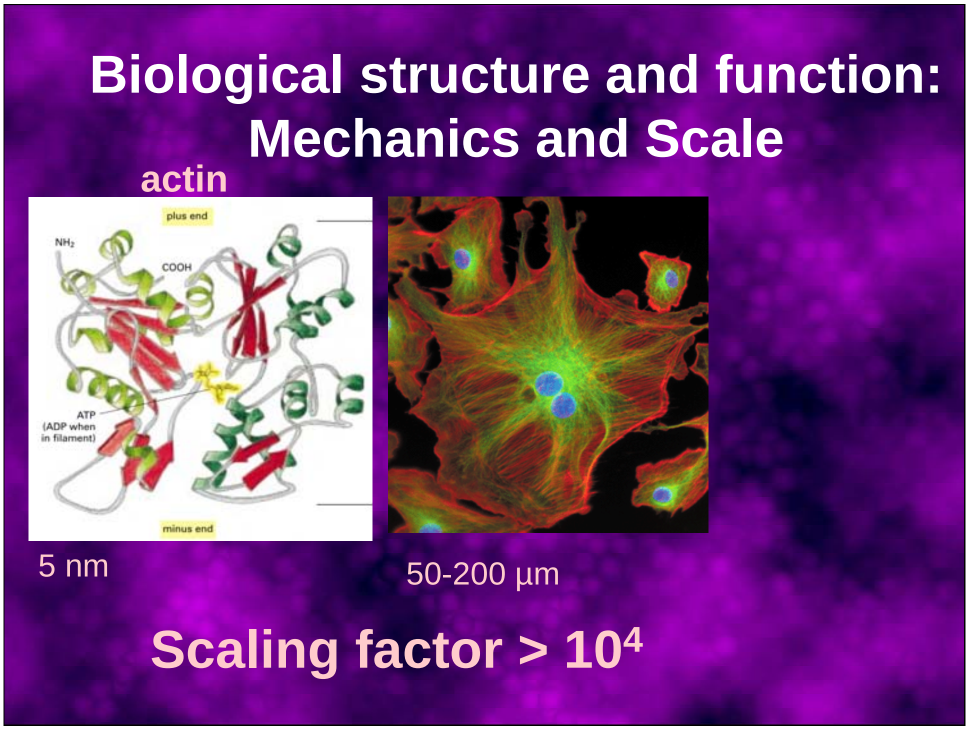

Protein Polymers, Crawling Cells and Comet Tails
Julie Theriot
Stanford University
Department of Biochemistry
Department of Microbiology & Immunology
Program in Biophysics

Biological structure and function: Mechanics and Scale

actin



5 nm


Biological structure and function:
Mechanics and Scale
actin
plus end
NH2
COOH
ATP
(ADP when
in filament)
minus end
5 nm
50-200 µm
Scaling factor > 10^4


Cell organization is DYNAMIC
David
Rogers
1950s
QuickTime™ and a
MPEG-4 Video decompressor
are needed to see this picture.
A neutrophil gives chase...
(real time)

Cell organization is DYNAMIC

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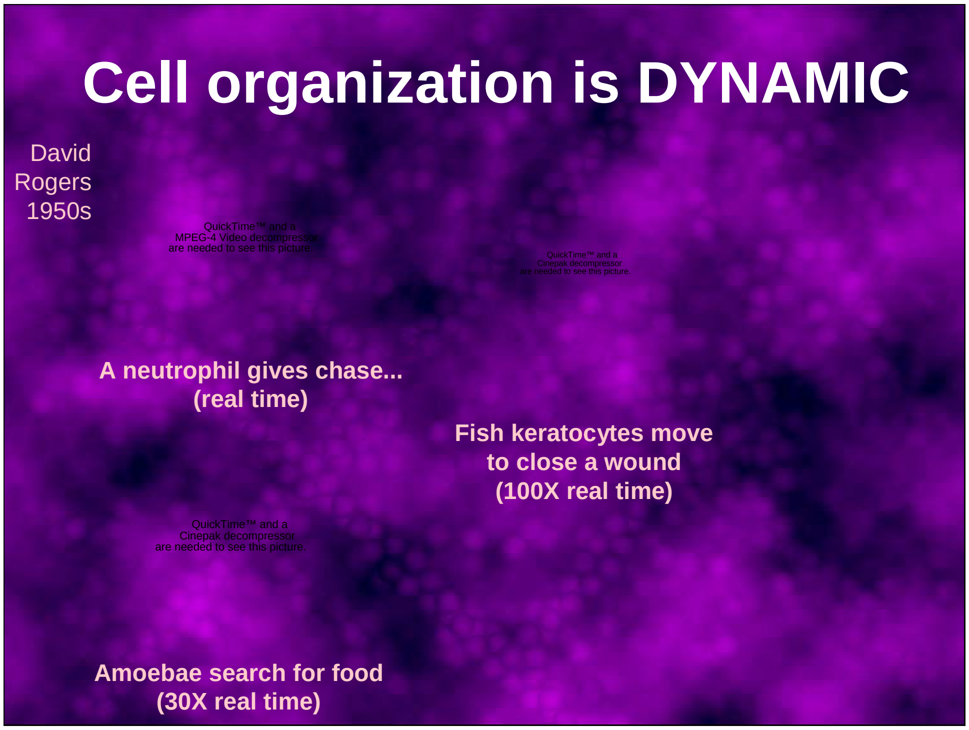
Amoebae search for food
(30X real time)


Cell organization is DYNAMIC
David Rogers 1950s
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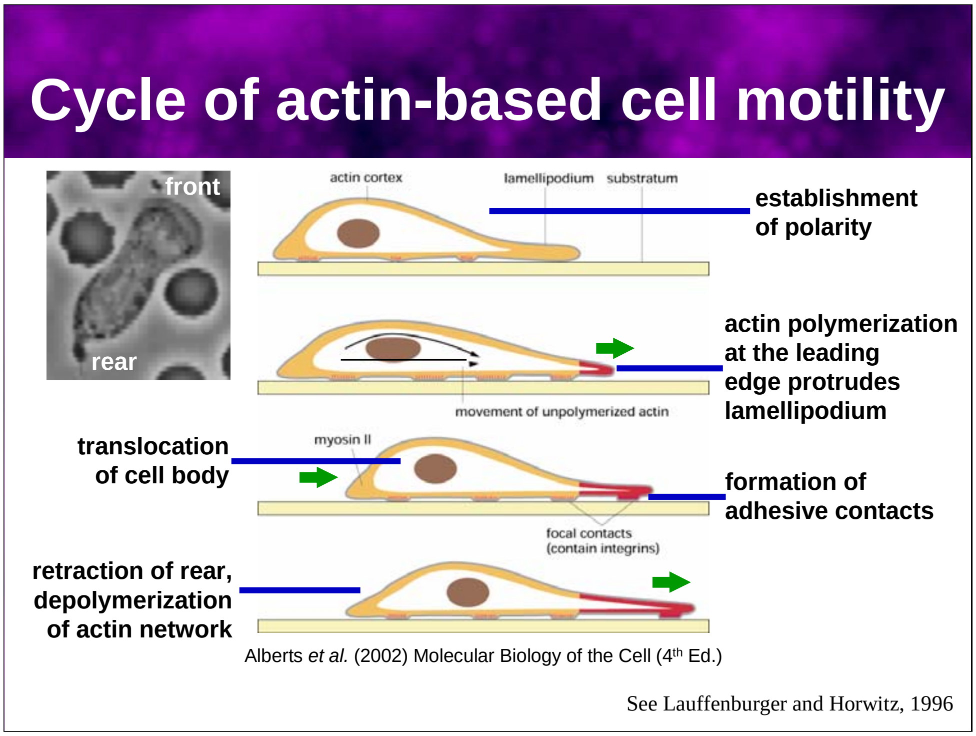
QuickTime™ and a Cinepak decompressor are needed to see this picture.
A neutrophil gives chase... (real time)
Fish keratocytes move to close a wound (100X real time)
QuickTime™ and a Cinepak decompressor are needed to see this picture.
Amoebae search for food (30X real time)



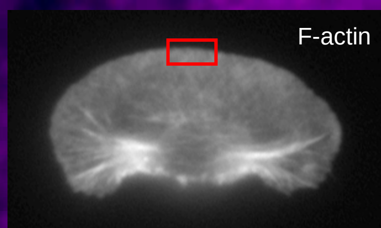
A lot of research has been done on the biochemical components of cell motility. Our interest is on the spatial and temporal organization of the actin cytoskeleton at the level of the entire cell.

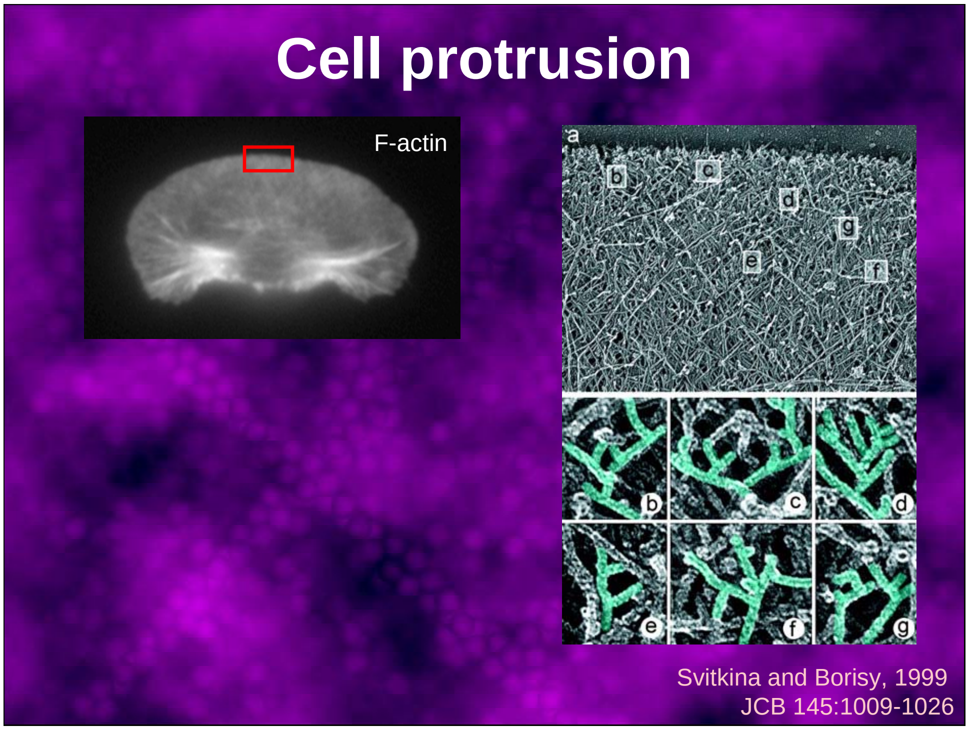
Dynamics and force generation in the eukaryotic cytoskeleton

- 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

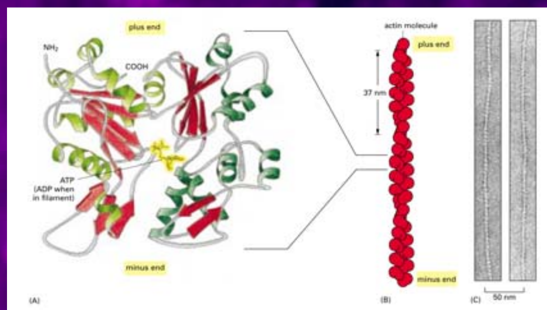
Cell protrusion

- 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




Cell protrusion
F-actin
a
b
c
d
g
e
f
b
c
d
e
f
g
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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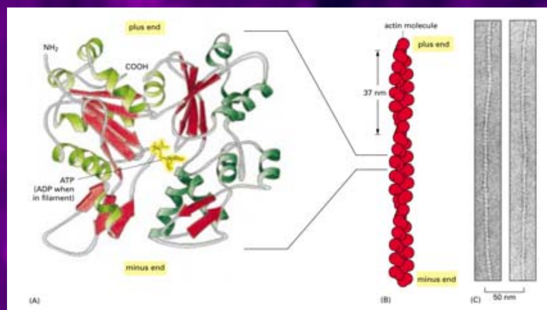

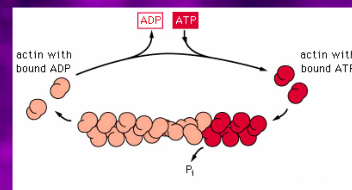

DYNAMICS

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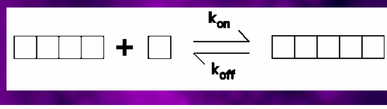
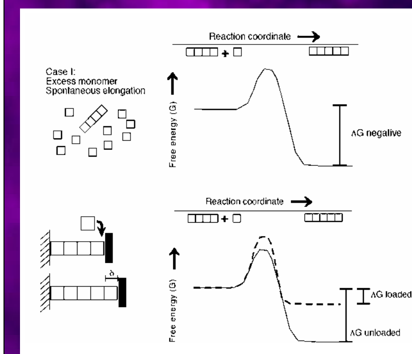
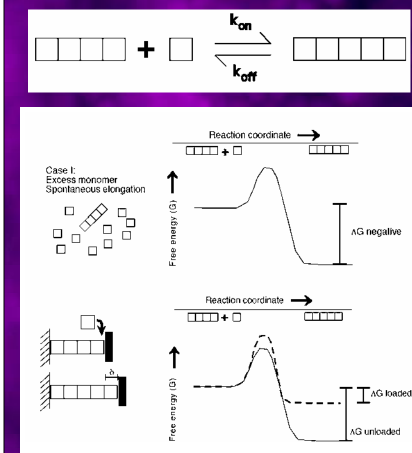
Force generation by protein polymerization





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

Force generation by protein polymerization



$F_{max} = (kT/\delta)\ ln(C/C_{crit})$

$C_{crit} \sim k_{off}/k_{on}$

for actin: $F_{max} \sim$ 5-10 pN

(comparable to myosin or kinesin)

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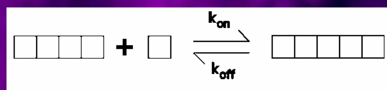

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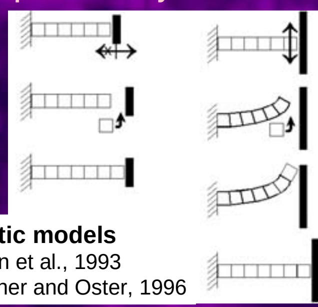
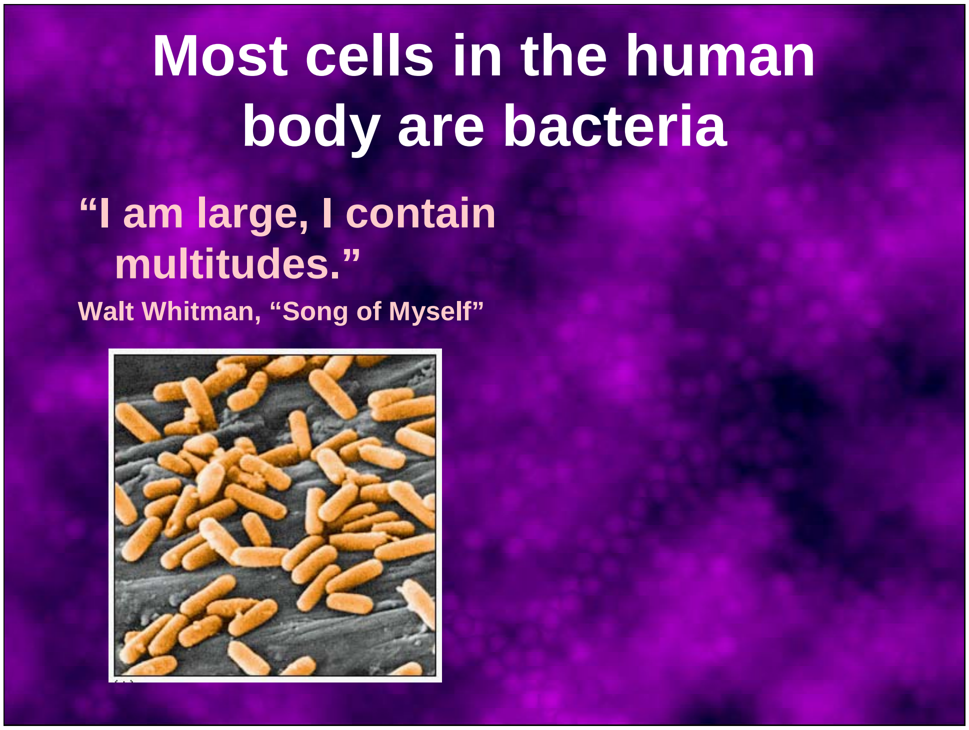
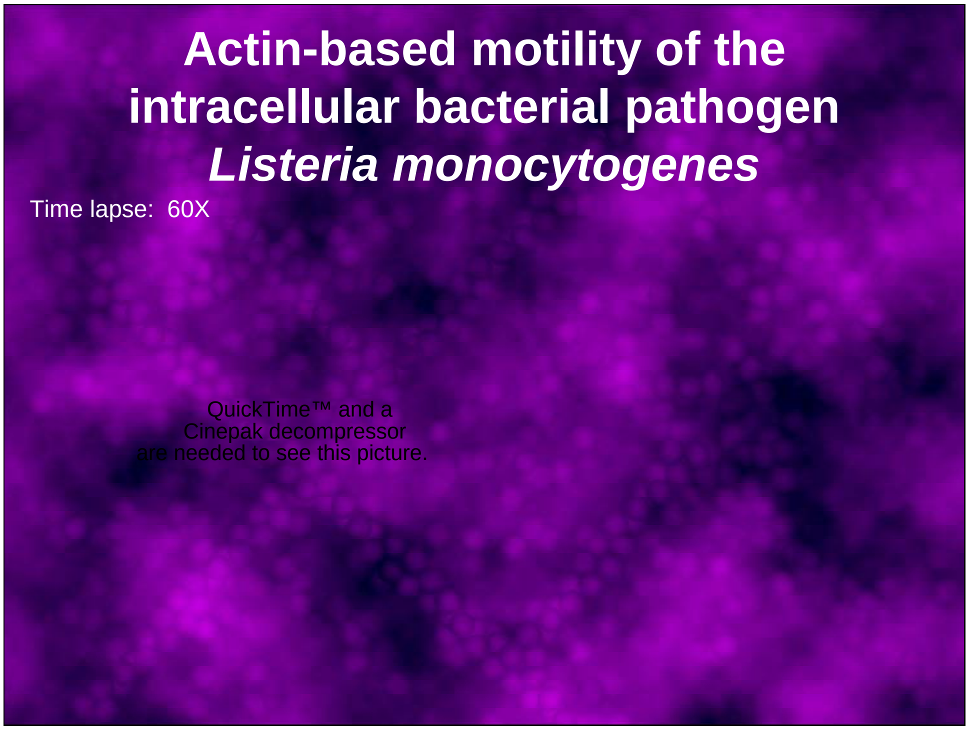


Kinetic models

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”



Speeded up ?X. PtK2 cells. Note the beautiful lamella, by which the cell undergoes it's own actin-based motility.

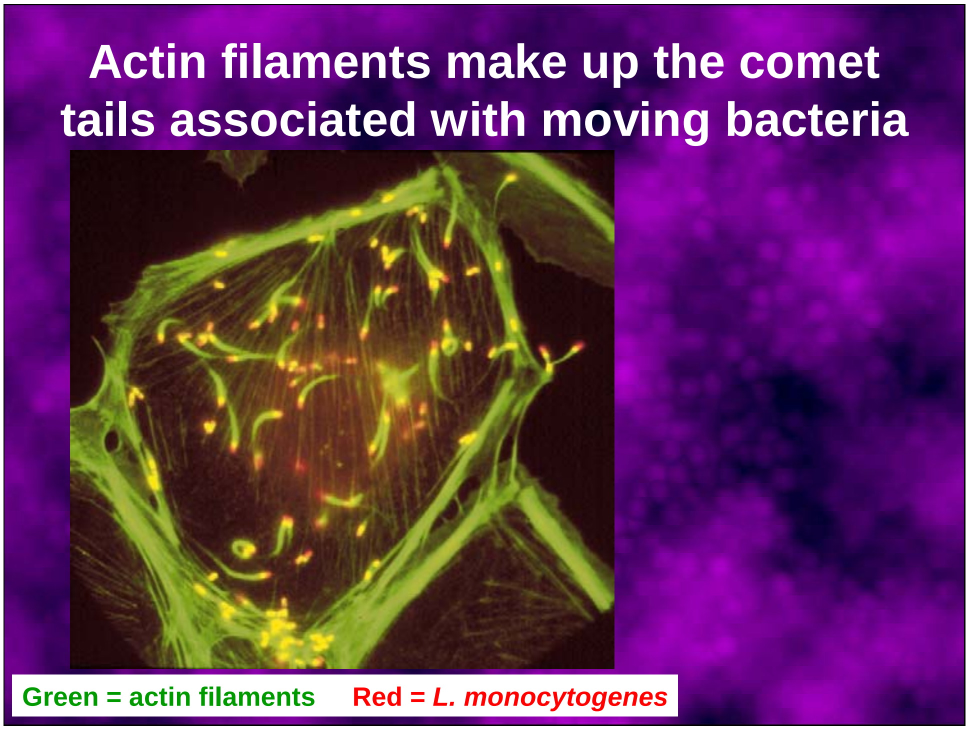
Gram positive bacillus

Propelled through the cytoplasm via actin polymerization

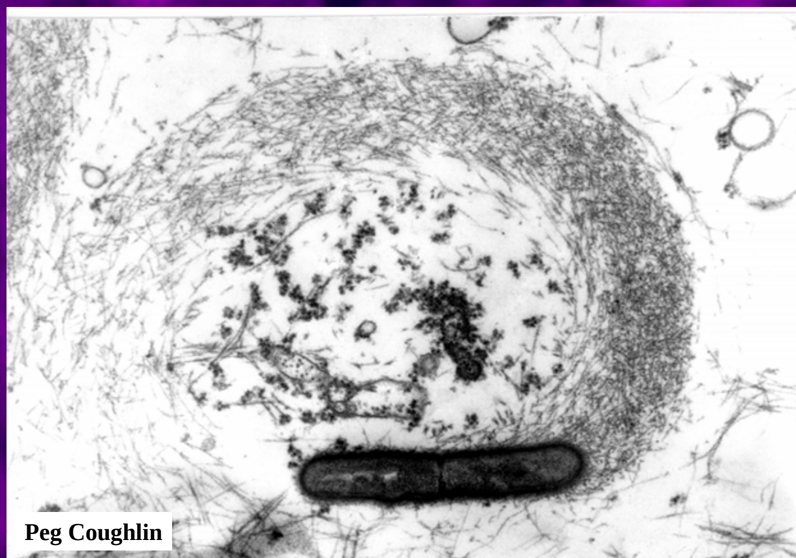
10 different labs working this out I do next step

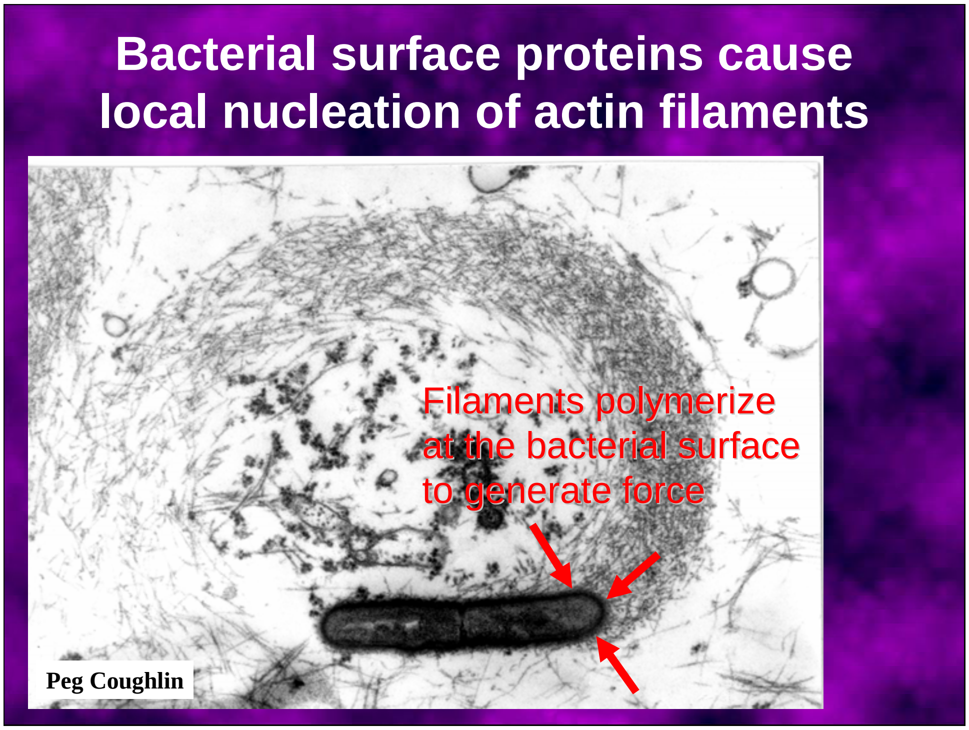
Other organisms which use this form of motility, beautiful example of coevolution. Shigella, Rickettsia, Vaccinia

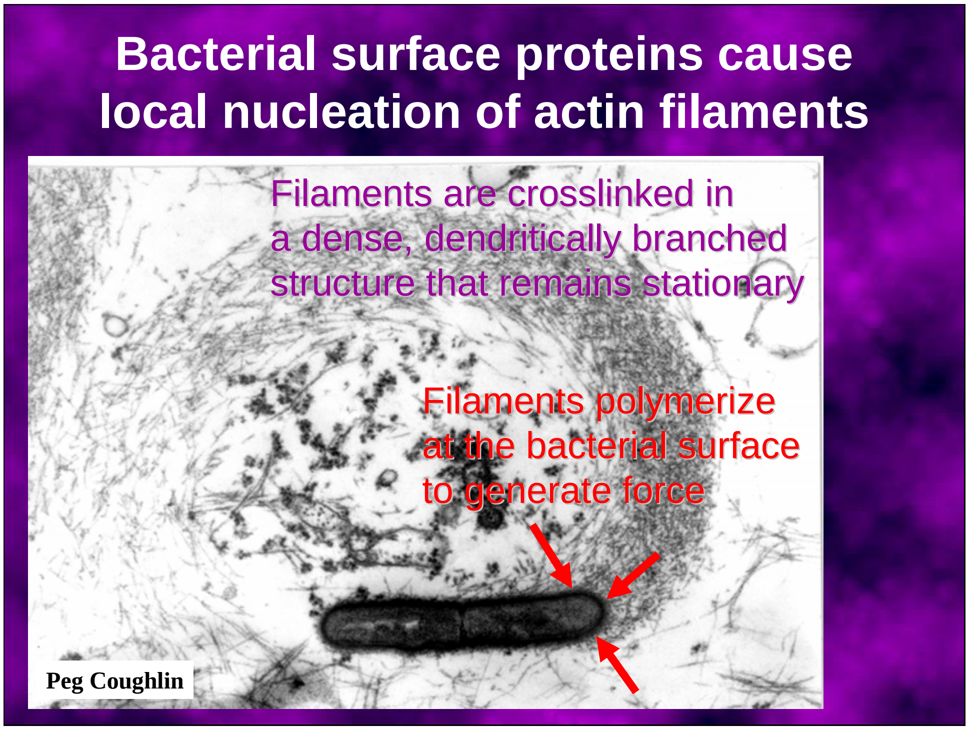
Why evolve this motile mechanism? To run from the immune system.

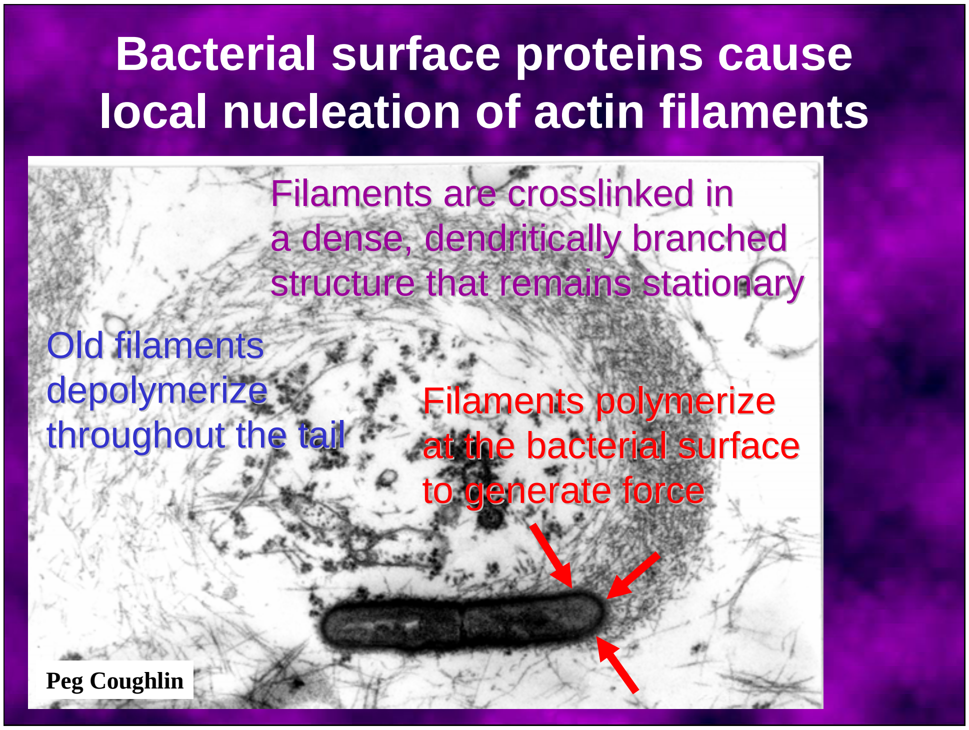

Actin filaments make up the comet tails associated with moving bacteria
Green = actin filaments
Red = L. monocytogenes

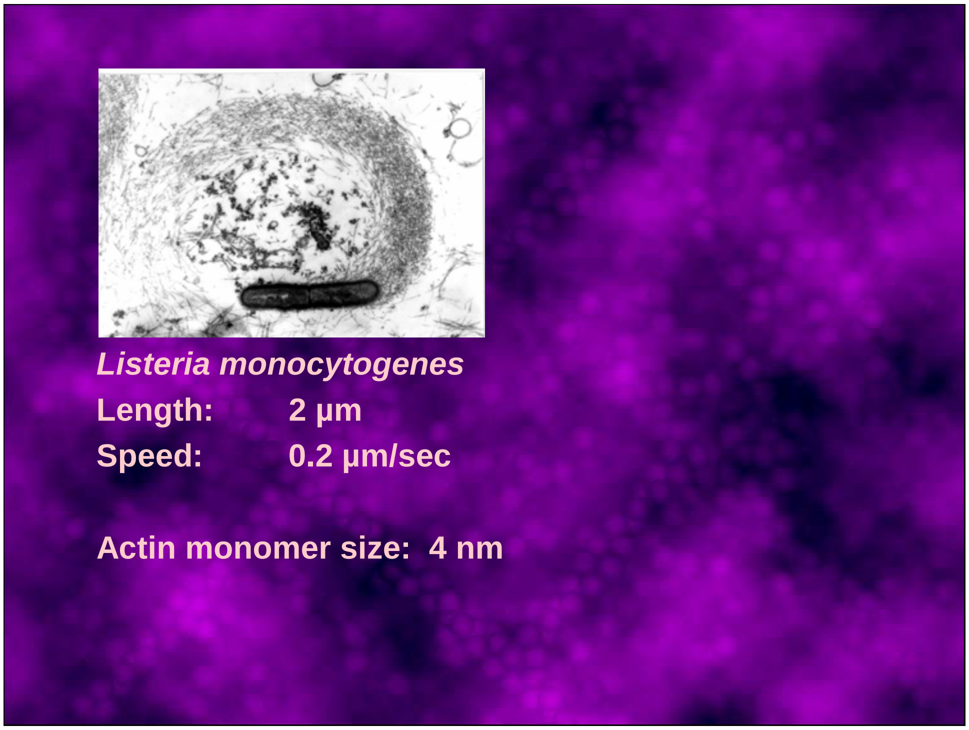
Bacterial surface proteins cause local nucleation of actin filaments

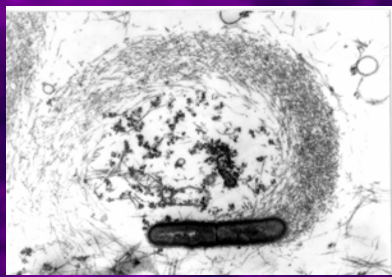



Bacterial surface proteins cause local nucleation of actin filaments
Filaments polymerize at the bacterial surface to generate force
Peg Coughlin


Bacterial surface proteins cause local nucleation of actin filaments
Filaments are crosslinked in a dense, dendritically branched structure that remains stationary
Filaments polymerize at the bacterial surface to generate force
Peg Coughlin

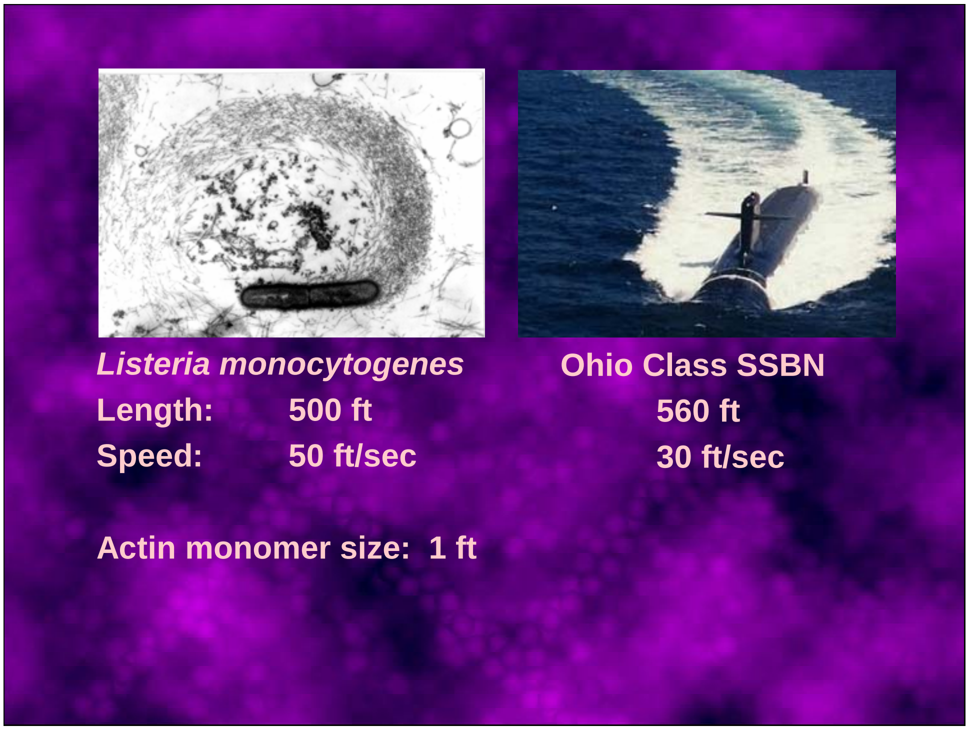

Bacterial surface proteins cause local nucleation of actin filaments
Filaments are crosslinked in a dense, dendritically branched structure that remains stationary
Old filaments depolymerize throughout the tail
Filaments polymerize at the bacterial surface to generate force
Peg Coughlin


Listeria monocytogenes
Length: 2 µm
Speed: 0.2 µm/sec
Actin monomer size: 4 nm



Listeria monocytogenes		Ohio Class SSBN
Length:	2 μm	560 ft
Speed:	0.2 μm/sec	30 ft/sec

Actin monomer size: 4 nm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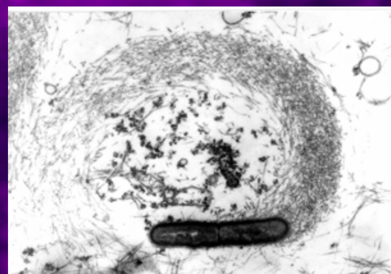

	Listeria monocytogenes	Ohio Class SSBN
Length:	500 ft	560 ft
Speed:	50 ft/sec	30 ft/sec

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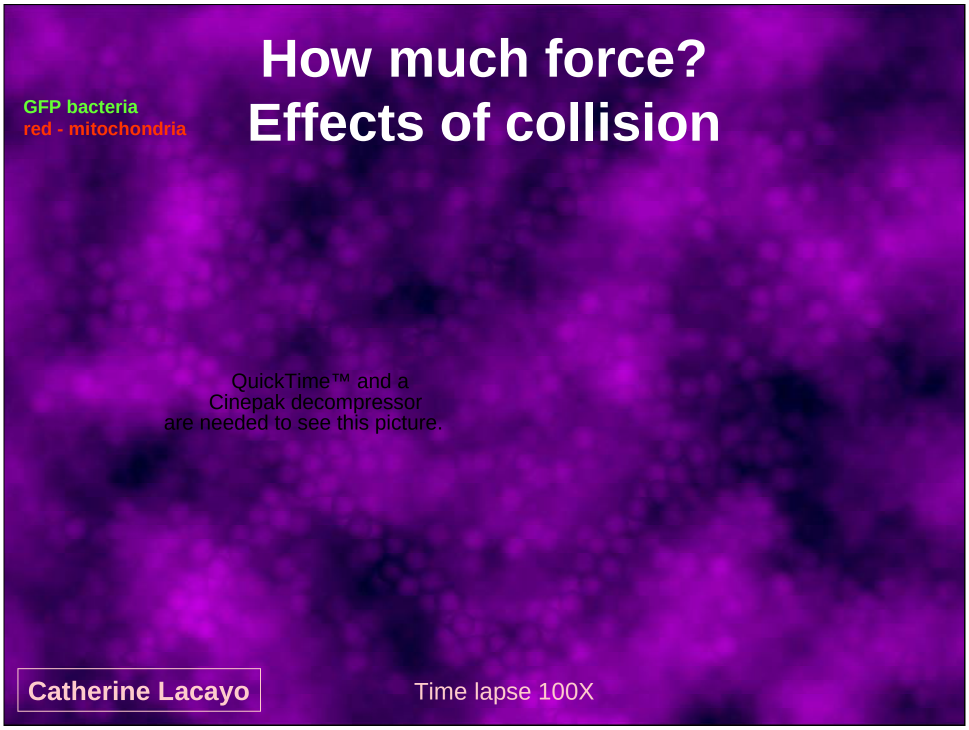
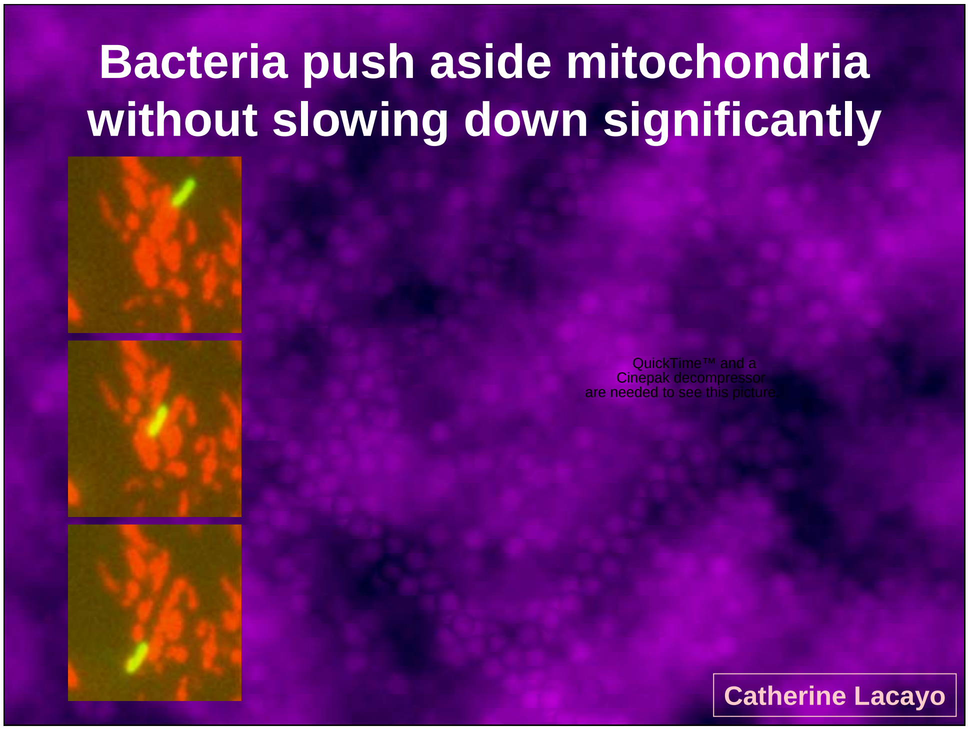
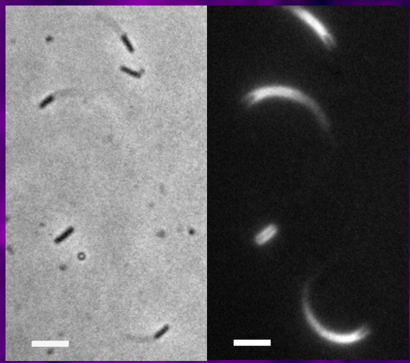
BUT...cells are not liquid;
cytoplasm is filled with
filaments, organelles, etc.

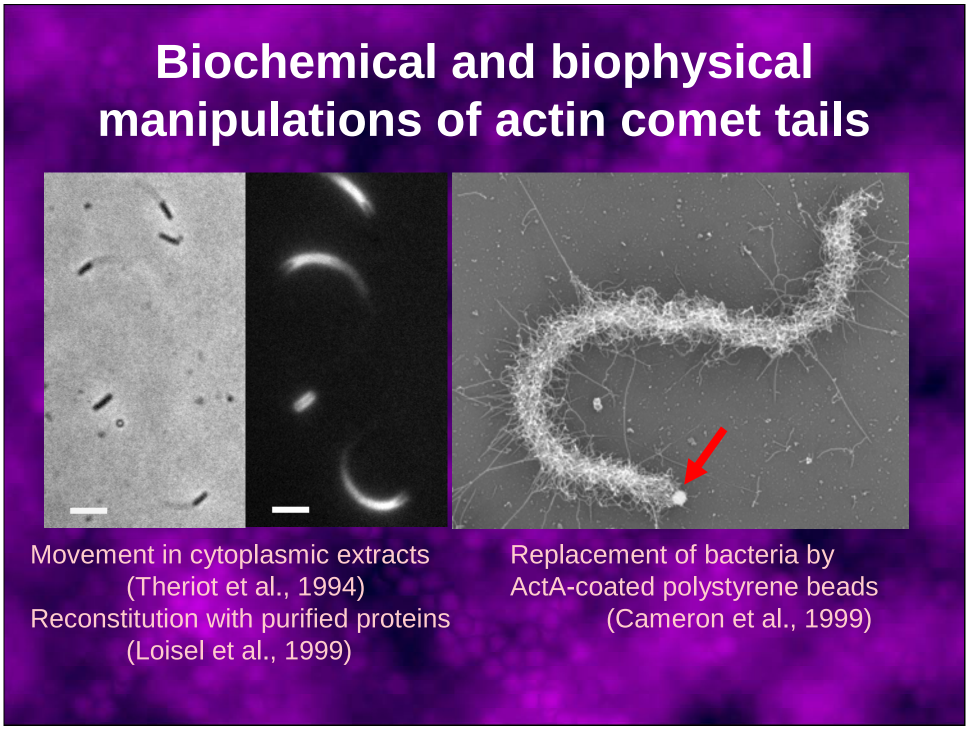

How much force?
Effects of collision
GFP bacteria
red - mitochondria
QuickTime™ and a
Cinepak decompressor
are needed to see this picture.
Catherine Lacayo
Time lapse 100X


Bacteria push aside mitochondria without slowing down significantly
QuickTime™ and a
Cinepak decompressor
are needed to see this picture.
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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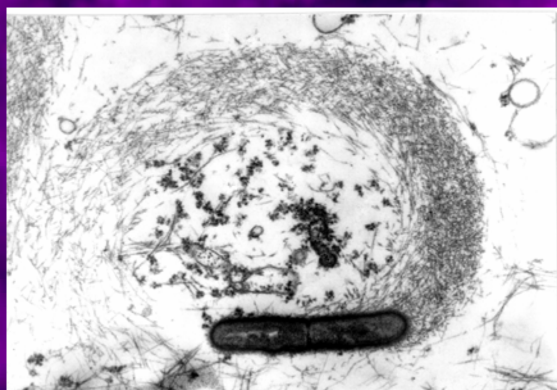
Replacement of bacteria by
ActA-coated polystyrene beads
(Cameron et al., 1999)

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



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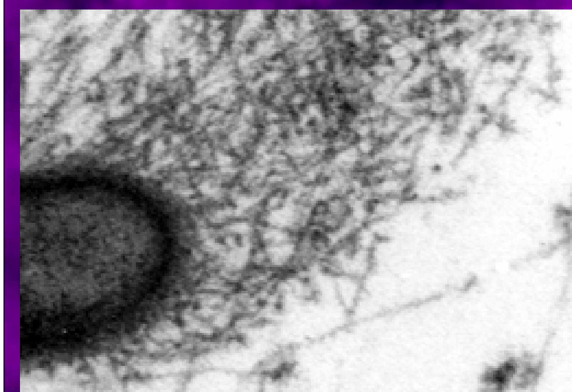

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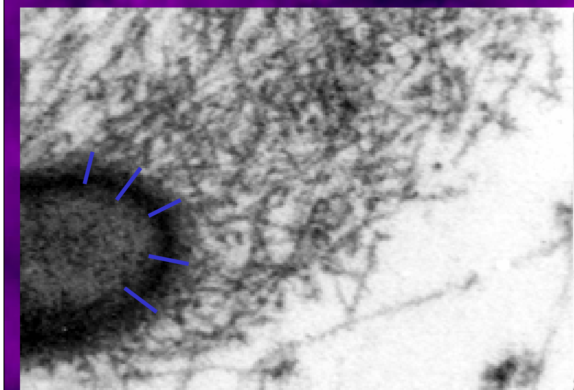
1. Bacterium presents one surface protein (*Listeria* ActA, *Shigella* IcsA(VirG))



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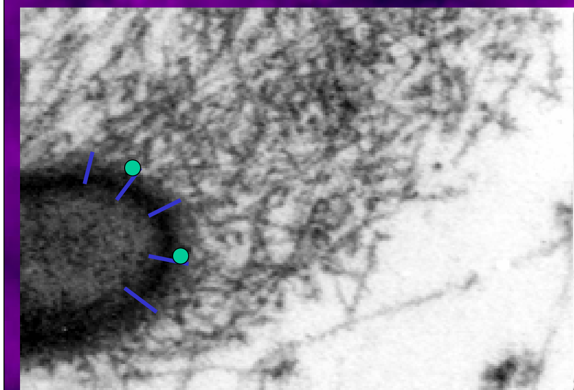

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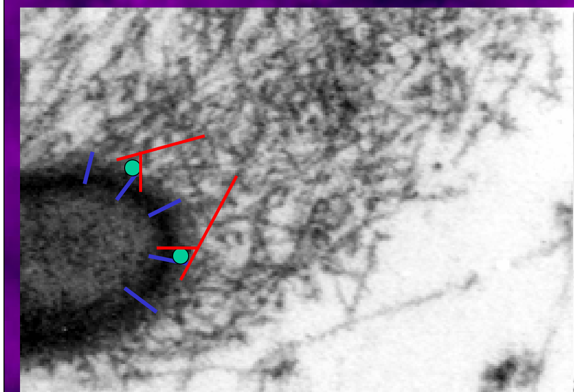

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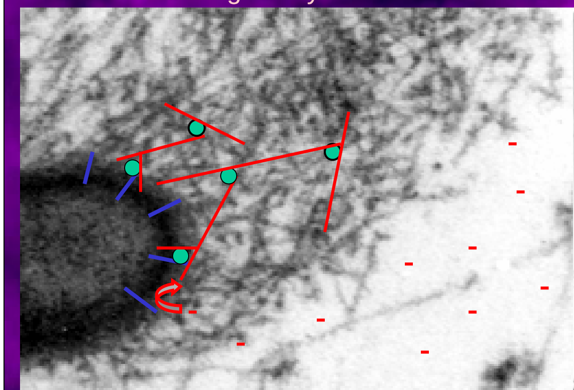

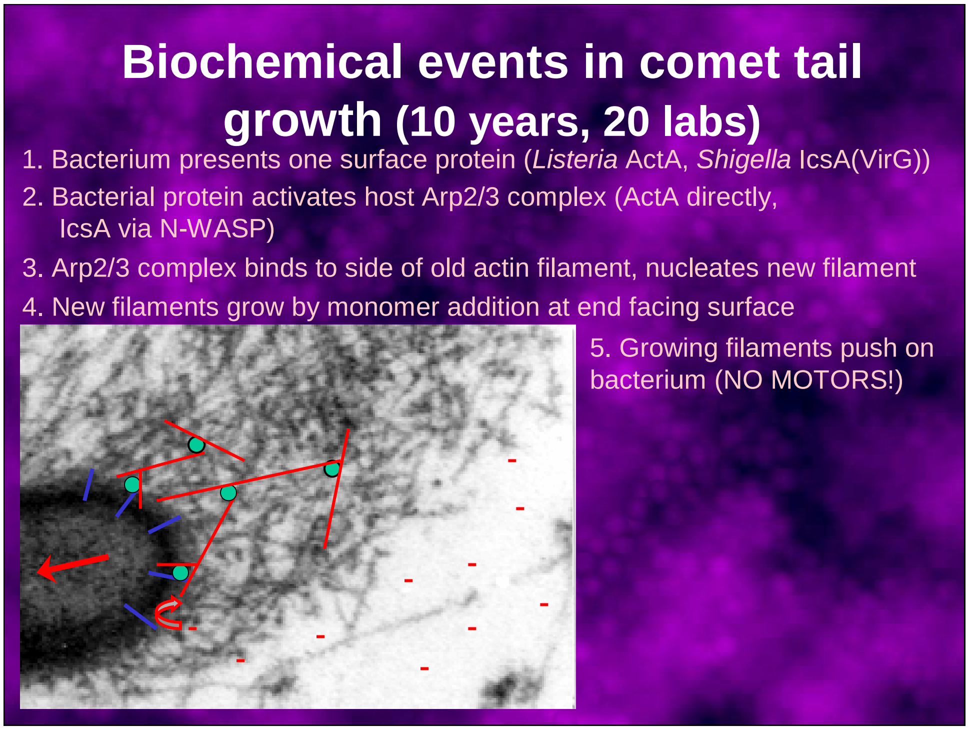
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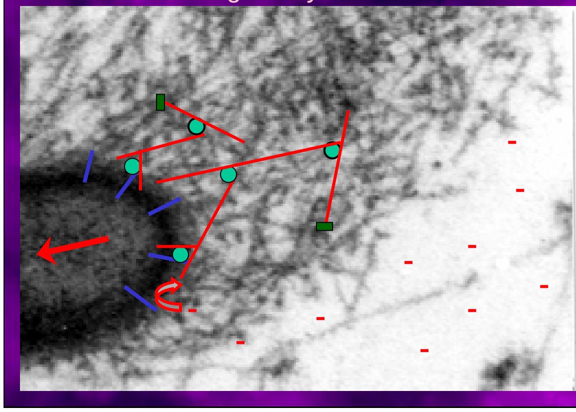

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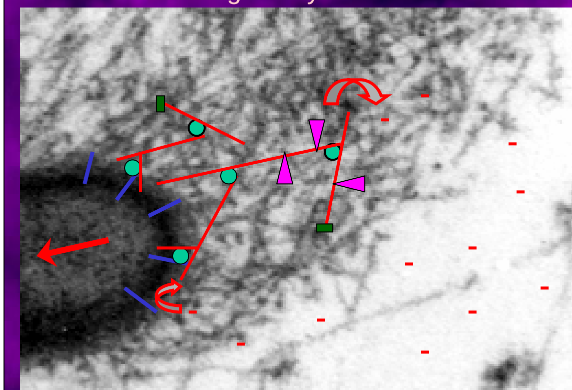

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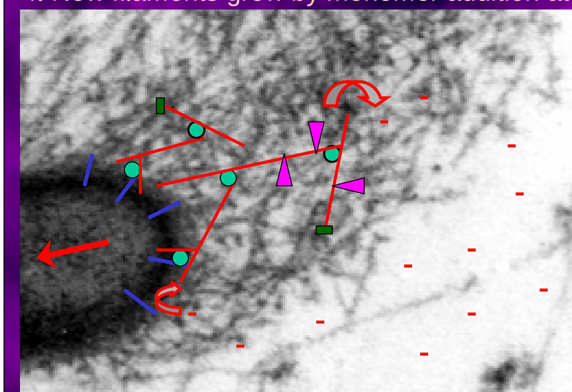

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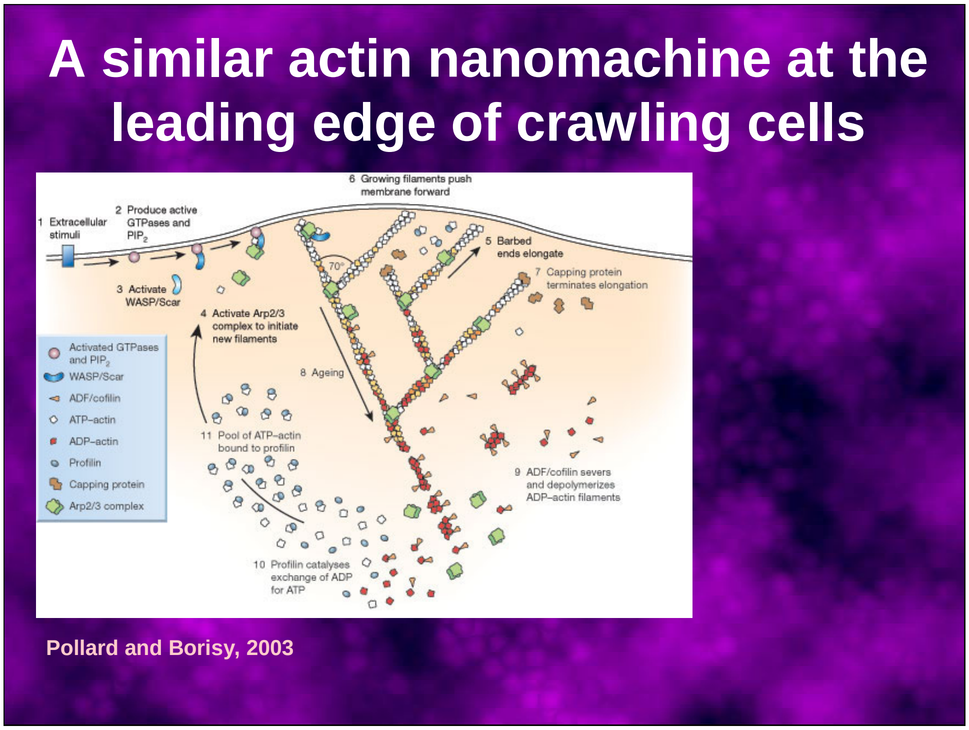
8. Return to step 2 and repeat ad infinitum


A similar actin nanomachine at the leading edge of crawling cells
6 Growing filaments push membrane forward
1 Extracellular stimuli
2 Produce active GTPases and PIP2
3 Activate WASP/Scar
4 Activate Arp2/3 complex to initiate new filaments
5 Barbed ends elongate
7 Capping protein terminates elongation
70°
8 Ageing
9 ADF/cofilin severs and depolymerizes ADP–actin filaments
10 Profilin catalyses exchange of ADP for ATP
11 Pool of ATP–actin bound to profilin
Activated GTPases and PIP2
WASP/Scar
ADF/cofilin
ATP–actin
ADP–actin
Profilin
Capping protein
Arp2/3 complex
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?