Drunken sailors and sober cells, the ways they move

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Cell motility is conserved across billions of years of evolution.

- White blood cells (Slightly speeded up)
  David Rogers, 1950
  Blood smear
  100,000 years ago

- Amoeba (Real time)
  Feeds on bacteria in soil
  2 billion years ago

- Fish scale cell (30 X speeded up)
  Theriot lab
  Wound healing
  500 million years ago
"Run and Tumble" motility of Listeria Monocytogenes

Listeria’s “Run and Tumble”

< 30°C

~ 37°C

Gründling et al, 2004
Intracellular pathogens also show similar behavior.
Cellular and pathogenic motility have the same molecular basis

1. Similar rates of propulsion (~µm/min)
2. Suggests identical molecular basis.
3. Evolutionary conserved
Bacterial and cellular motility have the same molecular basis

Svitkina et al 1997; Gary Borisy

Actin filament

Cameron et al, 2001
Actin filaments are formed from monomers

How do such small molecules self-assemble to form structures orders of magnitude bigger

Pollard and Cooper, 2001
Fluorescence microscopy enables direct visualization of actin filaments.
Fluorescence microscopy enables direct visualization of actin filaments

- Filament growth
- Thermal fluctuations

Flow

Flow
Actin polymerization can generate force

Short filament (<10 µm)

Long filament (>10 µm)

Displacement

5 pN

Buckling
Filament networks enable overcoming buckling

Productive

Non Productive

Listeria

{Productive

Non Productive}
Requirements for actin propulsion

Polymerization = force

Filament mesh prevents buckling

Elongate productive Filaments

Regeneration of monomers
Approach: What I can not create, I don’t understand.

1. Identify essential components
2. Characterize the component’s function
3. Reconstitute function i.e. motility
Requirements for actin propulsion

Polymerization = force

Filament mesh prevents buckling
“Branchers” branch actin filaments to form a dense mesh

Svitkina & Borisy, 1999

Mullins et al., 1999

Actin

Brancher (Arp2/3)

Smith et al, 2013; Gelles and Goode lab

Filament binding Arp2/3 elongation

binding dissociation
Requirement 1: Generating a dense mesh of filaments

Dense branching forms a mesh

Listeria mimicking bead

Actin

Brancher

Listeria placed in mix of Actin + Arp

Gel formed

Wiesner et al, 2003
Requirement 1: Branching generates a dense mesh of filaments.
Requirements for actin propulsion

Polymerization = force

Filament mesh prevents buckling

Elongate productive Filaments
Requirement 2: Blocking unproductive growth
Requirement 2: Absence of capping leads to a “fish bone” comet.
Requirements for actin propulsion

Polymerization = force

Filament mesh prevents buckling

Elongate productive Filaments

Regeneration of monomers
Requirement 3: Disassembly of the network & monomer regeneration
Requirement 3: Disassembly of the network & monomer regeneration

- **Fast** (10 monomers/µM/s)
- **Slow** (0.2 monomers/s)

Debrancher – GMF
**Actin Depolymerizing Factor (ADF)**

5 Plus ends
1 minus ends
Requirement 3: Debrancher increases the number of minus ends

Debrancher

Gandhi et al, 2010; Sean Guo; Goode and Gelles labs
Requirement 3: Disassembly of the network & monomer regeneration

Debrancher

Plus ends

Minus ends
Requirement 3: Depolymerizer fragments actin filaments
Requirement 3: Depolymerizer also depolymerizes filaments

Depolymerization increases 20x

Shekhar et al, 2017
Requirement 3: Depolymerizer fragments and depolymerizes filaments

Fast Depolymerization

Shekhar et al, 2017
Requirement 3: Disassembly of the network & monomer regeneration
1. Actin monomers polymerize to form filaments.

2. Branchers branch filaments.

3. Cappers block filament growth.

4. Depolymerizers depolymerize.

5. Bacteria (Listeria)
Reconstitution of Actin based motility

Loisel et al, 1999
Reconstitution of Actin Based Motility

Branching activator (ActA)

Listeria
Truly an international and interdisciplinary effort

Bruce Goode

Jeff Gelles

Jané Kondev