Actin-based Motility

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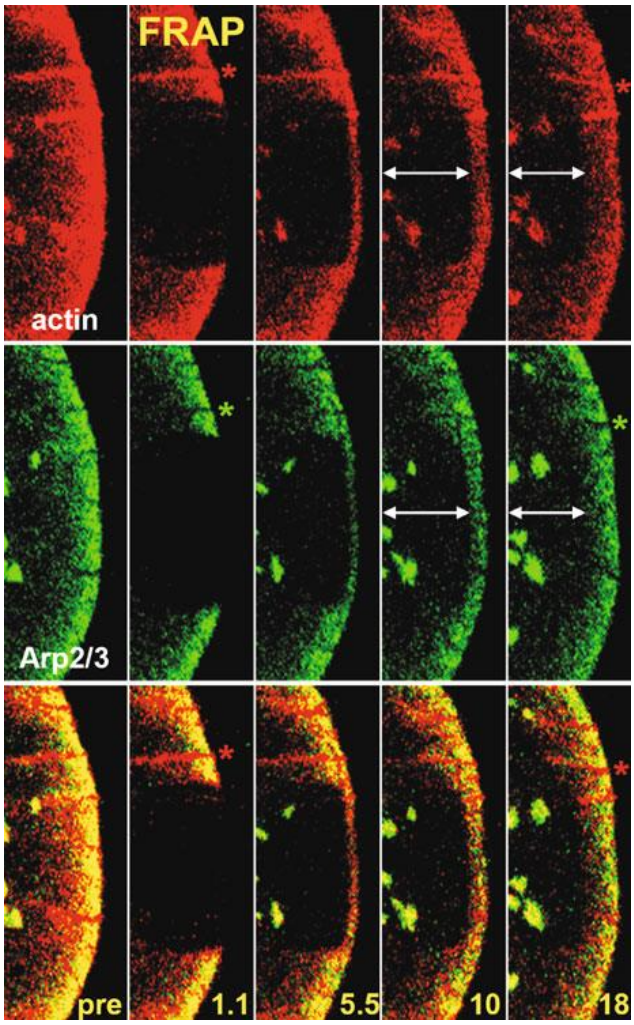
Definitions

Filopodia are rod-like, protrusive and membrane-enclosed bundles of actin filaments formed at the cell periphery, frequently in combination with lamellipodia

Lamellipodium is a sheet-like segment of protruding cytoplasm composed of an actin network devoid of tightly bundled actin.

Turnover: The lamellipodial actin network is not static, but continuously turned over, which occurs through highly coordinated actin filament assembly and disassembly mechanisms.

Treadmilling: Net assembly of ATP-G-actin at one end balances net disassembly of ADP-F-actin at the other end



Rearflow rate ?? (What is this)

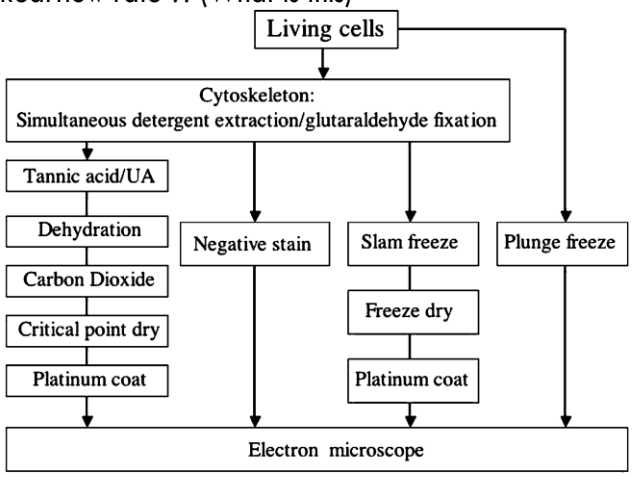
Highlights

Net translocation is achieved by protrusion at the cell front, followed by retraction at the rear.

Protrusion relies on the extension of pseudopodia, in one of three forms: finger like “filopodia”, sheet-like “lamellipodia” or bulbous “blebs”.

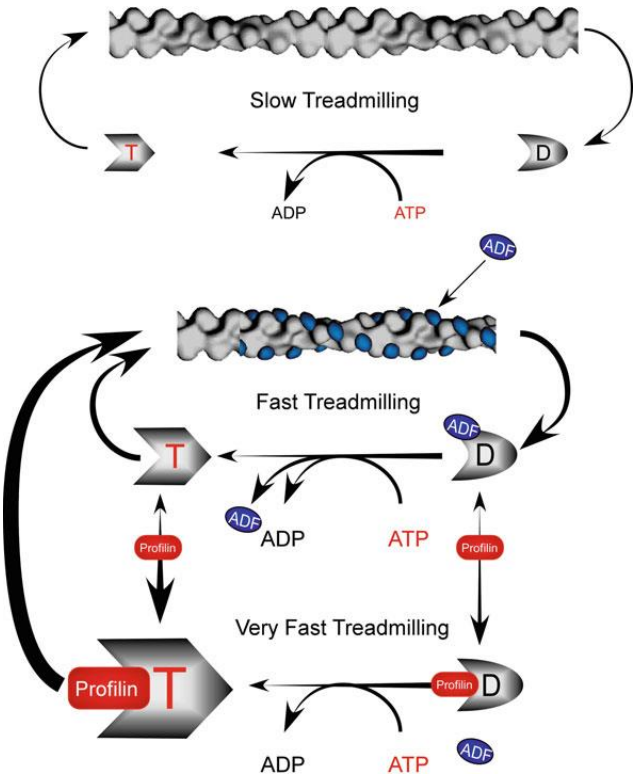
Protrusion of lamellipodia and filopodia is driven by actin polymerization

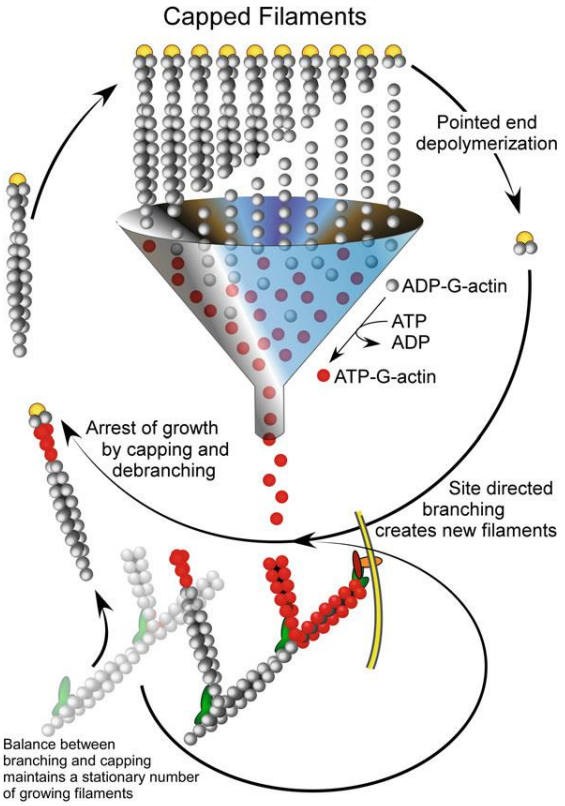
Electron microscopy is the only method currently available to resolve lamellipodia ultrastructure.

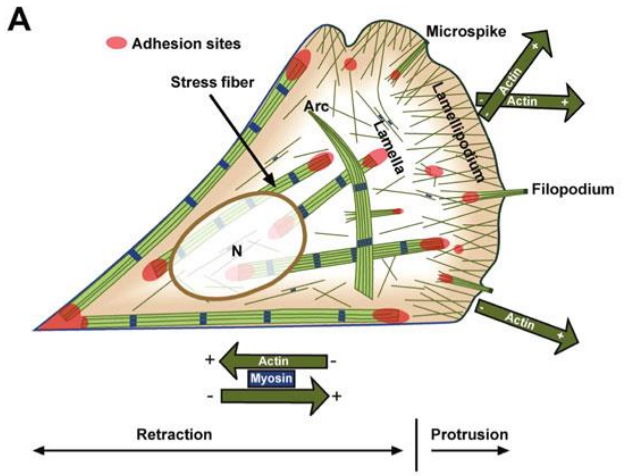


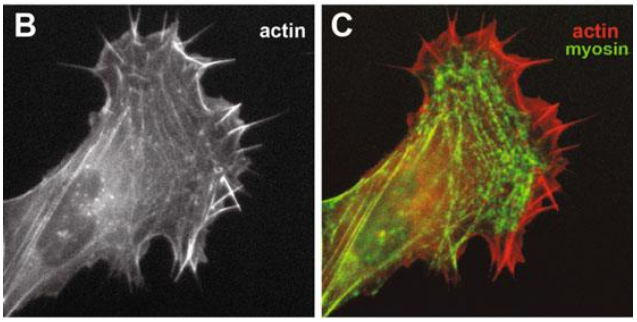
When fluorescent actin is injected into fibroblasts it is rapidly incorporated, within a few minutes into two structures, lamellipodia/filopodia and focal adhesions;

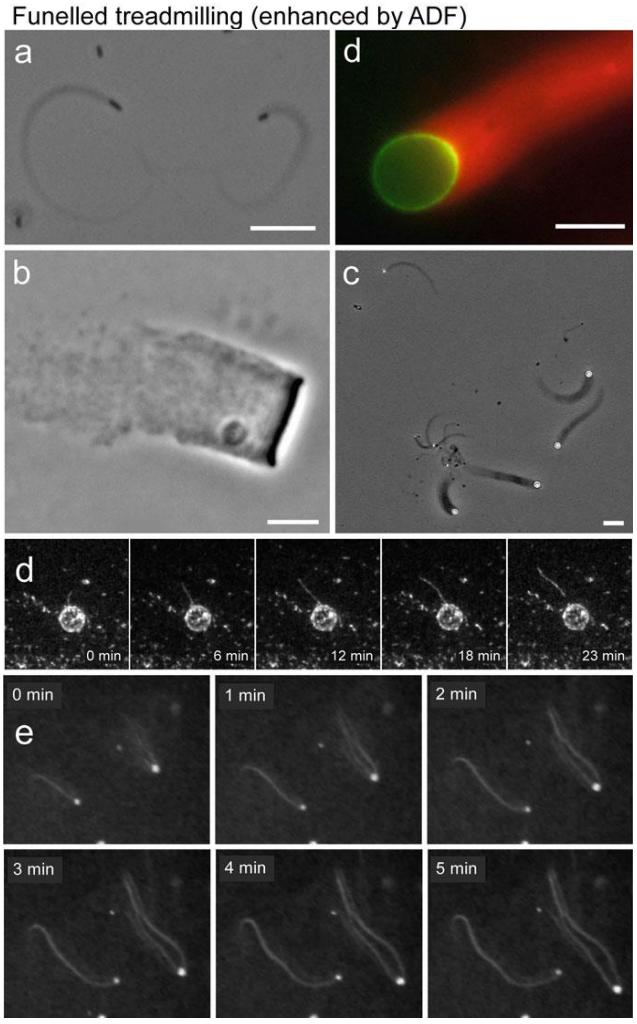
Chapter 1: Filopodia and lamellipodium
Chapter 10: In vitro motile process


Slow Treadmilling
T
D
ADP
ATP
ADF
Fast Treadmilling
T
D
ADF
ADP
ATP
Profilin
Profilin
Very Fast Treadmilling
Profilin
T
Profilin
D
ADF
ADP
ATP


Capped Filaments
Pointed end depolymerization
ADP-G-actin
ATP
ADP
ATP-G-actin
Arrest of growth by capping and debranching
Site directed branching creates new filaments
Balance between branching and capping maintains a stationary number of growing filaments


A
Adhesion sites
Stress fiber
Microspike
Actin
Actin
Arc
Lamellipodium
Lamella
Filopodium
N
Actin
Actin
Myosin
Retraction
Protrusion


B
actin
C
actin
myosin


Funelled treadmilling (enhanced by ADF)
a
d
b
c
d
0 min
6 min
12 min
18 min
23 min
0 min
e
1 min
2 min
3 min
4 min
5 min

Chapters

1.2 Choosing Protrusion: Nerve growth cones are enriched in filopodia, although not devoid of lamellipodia.

1.3 Signaling: Similiarly, ***Rho-GTPases*** and their direct activators (such as guanine-nucleotide exchange factors, GEFs) have emerged as central regulators of protrusion, with ***Cdc42 and Rac1*** inducing filopodia and lamellipodia, respectively.

1.4 Pushing Michanery: More recent studies using this approach have directly demonstrated that actin filaments do not branch in lamellipodia.

Actin networks are structured by the Arp2/3-complex and its associated proteins.

1.5 Nucleators and Elongators:

Nucleators: Arp2/3-complex + three WAVE isoforms (1, 2 and 3), haematopoietic WASP, N-WASP, WASH, WHAMM and JMY proteins and etc (interactions depend on WA-domain)

Elongators: Ena/VASP protein and other factors

1.6 Turnover: The rate of net protrusion of the cell depends on the efficiency with which the retrograde flow is coupled to the substrate

Efficient substrate coupling of the network allows a more or less direct conversion of actin polymerization into protrusion.

The Arp2/3 complex likely resides at the actin filament pointed ends, acting as a pointed end capper after serving its nucleation role at the lamellipodium tip.

The accumulation of VASP in Cytochalasin B-treated B16-F1 cells shown here is caused by the selective block of its release from the lamellipodium tip, which might be the decisive parameter for turnover.

1.7 Filopodia formation: Filopodia formation presumably involves the clustering of filament plus ends, the zipping up of filaments into a bundle and filament elongation at the tip.

1.8 Protruision to Retraction: Re-organisations of bundles serve to generate anti-parallel arrangements of actin filaments in the lamella that can form contractile assemblies with myosin.

The contractile assemblies of actin at the immediate rear of a cell are seeded by previous regional and transient lamellipodia and filopodia activity.

10.2.1 Actin self-assembly: G (globular) actin when low ionic strength and F (filamentous) actin when increasing ionic strength

Actin is an ATPase.

10.2.2 Treadmilling Regulation: Like in most regulated biological systems, the enhancement in treadmilling provided by ADF and profilin is balanced by antagonists such as tropomyosin, which stabilize filaments in a conformation that is incompatible with ADF binding.

Accelerating the rate limiting step in the ATPase cycle of actin and improving the efficiency (or vectoriality) of treadmilling are the key targets.

10.2.3 Global inhibition: Capping proteins enhance the rate of barbed end growth and control the length or life-time of newly created filaments

SUMMARY: concept of treadmilling and its regulation by proteins known to play an important role in a large number of motile processes guided the general strategy for reconstitution of actin-based propulsion of a functionalized particle. Directional movement resulted from local stimulation of actin assembly at the surface of a particle. Constant creation (“birth”) of filaments is balanced by capping (“death”) thatarrests their growth. Newly created filaments grow transiently in a medium that acts as a chemostat by maintaining a stationary supply of ATP-G-actin at a concentration above the critical concentration of barbed ends. In this medium all pointed ends depolymerize, thus maintaining a treadmilling cycle that supports a stationary perpetuated meshwork. Treadmilling organizes the polarity of movement. ATP is the source of energy. The chemostat consists of a solution of F-actin in ATP in the presence of profilin, ADF/cofilin and capping proteins. Force is produced by site-directed polarized growth of filaments. Because the reactions of actin assembly develop in a medium of low Reynolds number, movement stops as soon as force – i.e. filament growth - is arrested.

10.2.4 Listeria & Shigella (Dendritic branched filament arrray): Pathogens turned out to initiate the cascade of reactions leading to actin assembly either by expressing at their surface a protein that behaves as a functional homolog of WASP family proteins (ActA for Listeria or that mimics Cdc42 to activate N-WASP (IcsA protein for Shigella).

Propulsive movement of Listeria and Shigella thus could be observed by placing the bacteria in a reconstituted motility assay containing F-actin assembled in the presence of Arp2/3, ADF/cofilin, profilin, a capping protein and a large enough supply of ATP.

10.2.5 Chemically and Geometrically Controlled Functionalized Particles:

Solid (polystyrene microbeads) or soft (giant unilamellar vesicles) were functionalized with N-WASP

Force production or velocity; trajectory analysis; Actin Arp 2/3, N-WASP distribution during movement.

10.2.6 Formin-Coated Particles (Actin parallel bundles):

To challenge - Formin-based motile processes in vivo were also mediated by regulated teadmilling.

The major difference between the motility mediated by formation of a dendritic array and by processive assembly of linear actin bundles resides in the different regulatory effect of capping proteins: capping proteins are required for the formation and maintenance of dendritic actin arrays whereas they accelerate forminbased propulsive movement for a transient period of time, but eventually compete with formins for barbed end binding, causing arrest of movement.

10.3 Perspectives of motility assays:

Higher complexity: additional ingredients, genetic interactions, more actin-assembly promoting machineries

Structural analysis and dynamic imaging

Coordinated dynamics