Production of Force and Movement by Polymerization of Actin: Mechanism and Reconstitution *In Vitro*

M.-F. CARLIER*, S. WIESNER and D. PANTALONI

*Dynamique du Cytosquelette, Laboratoire d'Enzymologie et Biochimie Structurales C.N.R.S.,
91198 Gif-sur-Yvette, France*

(*Author for correspondence, e-mail: carlier@lebs.cnrs-gif.fr)

1. Introduction

Actin is one of the most abundant and conserved protein in eukaryotes. The main property of actin is the ability to self-assemble in helical, polar, dynamic filaments, one of the main polymers of the cytoskeleton (Oosawa and Asakura, 1975). Actin assembly is the hallmark of the motile response of cells to extracellular signals: Spatially controlled assembly of actin filaments, in response to stimuli, generates cell protrusions called lamellipodia, filopodia and pseudopodia. Motile cells use these extensions to explore the extracellular space and find their way toward their targets, in chemotactic locomotion, embryonic/metastatic cell migration or in morphogenetic processes like neural cone growth (Mitchison and Cramer, 1996; Miki et al., 1998).

The extension of lamellipodia is considered as the paradigm of so-called 'actin-based motile processes'. This thin cellular extension was early recognized as a self-organized system, which continues to display motile behavior once cleaved off the cell body by microsurgery (Euteneur and Schliwa, 1984). The collective action of a population of filaments growing in a well organized polarized array, was a complex cell biology problem. Observations of the forward movement of the leading edge of motile cells and of the associated dynamics of actin filaments in the extending lamellipodia clearly pointed out to two striking features. Filaments with their barbed ends pointing toward the leading edge were constantly generated and growing at the plasma membrane during movement, while depolymerizing at the rear of the lamellipodium (Wang, 1985; Small, 1995). Lamellipodium extension therefore results from the steady state cycle of actin assembly, the flux of depolymerized actin subunits being used to feed the growth of barbed ends formed at specific sites at the leading edge, in a rapid treadmilling process (Wegner, 1976, Figure 1). To challenge this view of the thermodynamic basis for actin-

Figure 1. Actin polymerization and actin-based motility. A: A migrating melanocyte transfected with GFP-tagged actin displays a branched filament array in the extending lamellipodium (from J. V. Small). B: *In vitro* reconstitution of branched filaments with actin polymerized in the presence of activated Arp2/3 complex. Filaments are stained with rhodamine-labeled phalloidin and observed in the light microscope. C: ActA-coated beads induce the formation of branched filaments when placed in *Xenopus* egg extracts (Cameron et al., 2000); D, E, F: actin-based movement of *Listeria* (D), N-WASP coated beads of 2 μm (E) or 10 μm (F) diameter in the reconstituted motility medium. (Figure adapted from Pantaloni et al., 2001).

based motility, it was necessary to reconstitute actin-based movement from pure components.

Paradoxically, bacterial pathogens have been instrumental in helping to understand a process that takes place in eukaryotic cells. A small number of intracellular pathogens, such as *Listeria monocytogenes*, *Shigella flexneri* or *Rickettsia* (as well as a few viruses like vaccinia virus), induce actin polymerization at their surface by harnessing the host actin machinery (Higley and Way, 1997; Beckerle, 1998; Dramsi and Cossart, 1998, Cameron et al., 2000, for reviews). The bacteria continuously generate an actin meshwork or 'tail' that promotes their propulsion in the host cytoplasm. A single bacterial factor, the *Listeria* protein ActA or the unrelated *Shigella* protein IcsA (also called virG) is required and sufficient for actin assembly and bacterial movement (Kocks et al., 1992; Domann et al., 1992; Bernardini et al., 1989). Therefore these pathogens have been rapidly considered as powerful tools to approach the mechanism of actin-based motility. Actin-based motility of *Listeria* could first be monitored *in vitro* in *Xenopus* egg extracts (Theriot et al., 1994), then in platelet extracts, brain and Hela cell extracts (Welch et al., 1997; Laurent and Carlier, 1998; Theriot and Fung, 1998; Laurent et al., 1999). Fractionation of platelet extracts led to the identification of Arp2/3 complex as the cellular factor responsible for local stimulation of actin assembly at the surface of *Listeria* (Welch et

al., 1997), and the important discovery that its biological activity required interaction with the *Listeria* protein ActA (Welch et al., 1998). Recruitment and activation of Arp2/3 complex by *Shigella* was also demonstrated using cell extracts (Egile et al., 1999). Following these achievements, the Arp2/3 complex was recognized to play an important role in lamellipodium extension, as well as in a variety of motile processes. Electron microscopy of the actin filament array in the lamellipodium showed the filaments were branched at 70° angle, the barbed ends of the mother and daughter filaments facing the leading edge of the cell, and Arp2/3 complex was localized at the branches (Svitkina and Borisy, 1999).

The full reconstitution, from a minimum set of pure components, of actin-based motility of *Listeria* or *Shigella* or of a particle able to initiate the cascade of events leading to local activation of polarized actin assembly, was a challenging issue. Its achievement, two years ago (Loisel et al., 1999; Pantaloni et al., 2001 for review), has opened new perspectives in the experimental approach of the mechanism of production of force by vectorial assembly of a polymer.

2. Regulated Treadmilling of Actin is the Thermodynamic Basis of Actin-Based Motility

• Actin Treadmilling

Two essential biochemical functions appear required to maintain a stationary movement: 1) Filaments exposing free barbed ends able to grow actively must be created and maintained at specific sites; 2) Growth of these filaments at the specific sites must be made possible by the maintenance of a concentration of monomeric actin well above the critical concentration for barbed end assembly. These two functions are mediated by different proteins: 1) The Arp2/3 complex is responsible for the constant generation of new filaments at a surface; 2) proteins which regulate the treadmilling of actin filaments maintain the flux of subunits i.e. provide the 'fuel' feeding this engine.

Genetic identification of proteins that are involved in cell motility and biochemical analysis of proteins that control the dynamics of actin filaments (Carlier, 1998; Carlier et al., 1999) have helped to elaborate a minimum reconstituted medium that would be made of pure proteins and would support sustained actin-based movement of *Listeria* and *Shigella* (Loisel et al., 1999). Three essential components in the motility medium and their properties are listed below.

• Actin Depolymerizing Factor

Actin Depolymerizing Factor (ADF)/cofilin is responsible for the rapid turnover of actin filaments in motile regions of the cell. Upon binding to F-ADP-actin, ADF-bound filaments become more dynamic due to a large increase in the rate of depolymerization from the pointed end, which is the rate-limiting step in the treadmilling cycle. At the new steady state of actin assembly a high concentration of monomeric ATP-G-actin supports fast barbed end growth, to balance ADF-induced fast pointed end depolymerization (Carlier et al., 1997).

- *Capping proteins*

Capping proteins block actin assembly-disassembly at the barbed ends. If a large proportion of filaments are capped at their barbed ends, the concentration of monomeric actin settles at a value very close to the critical concentration at the pointed end. The few non-capped barbed ends grow at a high rate so as to balance depolymerization from all the pointed ends. Treadmilling therefore is funnelled (Carrier and Pantaloni, 1997).

- *Accessory proteins: profilin, VASP, alpha-actinin*

The profilin-actin complex productively associates with barbed ends exclusively, hence profilin cooperates with ADF to enhance the processivity of the treadmilling of actin filaments (Didry et al., 1998). Interestingly, this synergy between ADF and profilin is an example of biological complexity, in which the individual properties of two proteins combine to generate new behavior (Ricard, J., 1999).

VASP is a focal adhesion protein, also present at the leading edge of motile cells (Smith et al., 1996; Gertler et al., 1996; Rottner et al., 1999), which binds to a central proline rich region of ActA and is almost essential in movement of *Listeria* (Loisel et al., 1999).

Alpha-actinin is not required for bacterial actin-based motility, but its ability to crosslink the filaments is important to anchor the actin tail of the bacteria in the background meshwork that forms the medium. By preventing the drift of the bacterium + tail, alpha-actinin makes the measurements of bacterial speed easier and more accurate.

- *Arp2/3 complex*

Arp2/3 complex is a conserved seven subunit complex comprising in particular the Actin related proteins Arp2 and Arp3. The Arp2/3 complex is the downstream target of a variety of signalling pathways leading to actin polymerization (Rohatgi et al., 1998; Higgs and Pollard, 1999, for review). It multiplies actin filaments by branching when it interacts with an activator. The *Listeria* protein ActA directly activates Arp2/3 complex (Welch et al., 1998; Boujemaa et al., 2001). The *Shigella* protein IcsA does not activate Arp2/3 complex, but recruits N-WASP (Suzuki et al., 1998; Egile et al., 1999), the ubiquitous homolog of WASp/Scar family proteins, which are the natural activators of Arp2/3 complex in eukaryotic cells (Takenawa and Miki, 2001, for review). Hence both *Listeria* and *Shigella* use Arp2/3 complex to generate new actin filaments at their surface upon activation.

- *Functionalized polystyrene beads: alternative to bacteria*

Polymer beads are a valuable alternative to microorganisms. Polystyrene-based (PS) beads are most often used. They allow easy immobilization of proteins activating Arp2/3 complex (N-WASP, ActA) under controllable conditions of surface density of immobilized protein. PS beads of sizes between 50 nm and 10 μ m have been successfully used in reconstituted motility assays (Yarar et al., 1999; Cameron et al., 1999; Wiesner et al., manuscript in preparation; Bernheim et al., manuscript in preparation).

3. Reconstitution of Actin-Based Movement: The Motility Medium

The different players outlined above act together to support the formation of a steady branched filament array. Constant filament branching and growth occurs at the surface of bacteria or beads by local activation of Arp2/3 complex. In a medium containing ATP as an energy source, filaments maintained in a dynamic steady state in the presence of ADF/cofilin, profilin and capping proteins act as a chemostat that buffers polymerizable monomeric actin (ATP-G-actin + profilin-ATP-G-actin) at a high concentration, suitable for sustained barbed end growth. The higher the concentration of capping proteins, the shorter the length of filaments in the actin tail (Pantaloni et al., 2000). A steady number of filaments is maintained by the balance between the multiplication of filaments by branching and the cooperative depolymerization of filaments from the pointed ends fiber by fiber, elicited by ADF.

Importantly, no myosin motor is involved in actin-based motility. The concentration dependence of all components shows an optimum for motility, indicating some interplay between them. At optimum concentrations of all components, the particle velocity (several $\mu\text{m}/\text{min}$), the length of the actin tails (a few μm), the trajectories, are similar to those observed *in vivo* or in cell extracts. The formation of a non polarized 'cloud' of F-actin around the particle is followed by breakage of symmetry (van Oudenaarden and Theriot, 2000) in which the cloud becomes a single polarized actin meshwork associated with the establishment of a stationary regime of actin-based movement. Peculiar phenotypes of movement are observed sometimes, like multiple actin tails on a single bead. When two tails are formed, they adopt a helical symmetric disposition. Analysis of the formation of these helical tails is in progress (Wiesner et al., manuscript in preparation).

4. Perspectives: Open Issues in the Mechanism of Actin-Based Motility

Important issues remain to be solved concerning the relationship between the biochemical reactions involved in motility and the resulting production of force.

Theoretical brownian ratchet models of actin-based motility have been proposed. They first relied on the actin polymerization-biased diffusion of *Listeria*, then on the elastic properties of the actin filament (Mogilner and Oster, 1996). Another model at the mesoscopic scale relies on the elastic properties of the actin meshwork and the shear stress due to the growth of the gel at the bacterium surface (Gerbal et al., 1999). A microscopic model for force production requires knowledge of the biochemical properties of the proteins that compose the motile machinery assembled at the surface, and the kinetic analysis of the autocatalytic filament branching process. High resolution particle tracking of a bead or bacterium (Kuo and McGrath, 2000) in a chemically controlled motility assay, or on the rheological properties of the transiently branched filament network will also provide important information on the molecular mechanism of movement.

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