Review

Actin polymerization machinery: the finish line of signaling networks, the starting point of cellular movement

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Abstract. Dynamic assembly of actin filaments generates the forces supporting cell motility. Several recent biochemical and genetic studies have revealed a plethora of different actin binding proteins whose coordinated activity regulates the turnover of actin filaments, thus controlling a variety of actin-based processes, including cell migration. Additionally, emerging evidence is highlighting a scenario whereby the same basic set of actin regulatory proteins is also the convergent node of different signaling pathways emanating from extracellular stimuli, like those from receptor tyrosine kinases. Here, we will focus on the molecular mechanisms of how the machinery of actin polymerization functions and is regulated, in a signaling-dependent mode, to generate site-directed actin assembly leading to cell motility.

Key words. Actin dynamics; signaling complex; lamellipodia; RhoGTPases; actin-binding proteins.

Introduction

The dynamic assembly of actin filaments in response to extracellular signals is at the base of a wide range of fundamental cellular processes through which living cells change shape, extend protrusions like lamellipodia and filopodia, or wrap around a particle, as in a phagocytic cup [1–3]. The bulk turnover of actin subunits is 100–200 times faster in cells than with pure actin, pointing to a complex regulation in vivo. A large repertoire of actin-binding proteins consistently regulates the dynamic assembly and spatial organization of actin filaments, thus orchestrating the motile behavior of cells. Among these are proteins that (i) promote the nucleation of actin, such as the Arp2/3 complex or formins, that (ii) affect the depolymerization of filaments, such as the actin-depolymerizing factor (ADF/cofilin) family and that (iii) associate to monomeric actin, such as profilin and beta-thymosin, and that (iv) cap the ends of filaments. Coordination and integration of the activities of this basic set of proteins is essential to control site-directed actin polymerization in vivo [1–3]. Additionally, complexity is emerging with the discovery that these proteins are, in turn, targets of various signaling pathways emanating from diverse extracellular stimuli, such as those from the receptor tyrosine kinase (RTK) family. Within these pathways, Rho GTPase family members play a key role, acting as molecular switches on which signaling inputs converge and are transduced into a coordinated array of output events regulating site-directed actin dynamics required for cell motility [4–6].
Here, we will examine the most recent evidence highlighting the molecular mechanisms controlling the basic machinery of actin polymerization. Additionally, with the aim of providing examples of how the machinery of actin polymerization is linked and controlled by signaling cascades emanating from Rho GTPases, we will further focus on those pathways and macromolecular complexes permitting a proper temporal and spatial regulation of Rac-mediated actin dynamics leading to membrane protrusion, the first step in the establishment of cell locomotion.

**Actin treadmilling**

Actin, a 43-kDa globular protein, is the most abundant protein in eukaryotes. In physiological medium, actin can undergo polymerization into helical filaments. Actin filaments are polarized, characterized by a fast-growing (plus or barbed) end and a slow-growing or minus end. Actin assembly is coupled with continuous ATP hydrolysis. Actin is an ATPase. Under physiological conditions, MgATP-bound G-actin is incorporated into growing filaments at the barbed end. ATP-actin is then converted in ADP-actin by slow hydrolysis as actin monomers are shifted along the filament toward the pointed ends. Thus, actin assembly at steady state can be described by an ATPase cycle featuring the energetic imbalance between the fast and slow ends of filaments linked to the irreversible hydrolysis of ATP. This cycle comprises three elementary steps: (i) net depolymerization from the pointed end, releasing ADP-G-actin; (ii) exchange of ATP for bound ADP, restoring ATP-G-actin, which (iii) undergoes net assembly at the barbed end. The kinetic constants of all these steps determine both the concentrations of each species at steady state and the turnover rate of actin filaments [7, 8]. It is noteworthy that the concentration of ATP-G-actin, which is kept stationary during this cycle, is intermediate between the critical concentrations (Cc = the minimal G-actin concentration required for assembly at the ends of filaments, resulting from the ratio of rate constants of dissociation and association at actin filament ends) at the barbed and pointed ends. Irreversible hydrolysis of the bound ATP associated with polymerization is at the origin of treadmilling and destabilizes the filaments [3]. Treadmilling is crucial in actin motility. The polymerized array of actin filaments displays a very rapid turnover. Filaments are oriented so that the growing barbed ends face the plasma membrane, while the pointed ends, where depolymerization occurs, are at the rear [9–11] (fig. 1). Notably, a similar architectural and dynamic organization of actin polymers is also used by a number of intracellular pathogens, such as *Listeria monocytogenes*, which hijack components of the actin-treadmilling machinery to generate comet tails for their propulsion. Indeed, characterization of the mechanisms driving *Listeria* or *Shigella* propulsion has been instrumental in uncovering key molecules regulating actin dynamics [12–14]. This arrangement suggests also that an insertional mechanism is at the base of force production needed for locomotion. Finally, the observation that the rate of barbed end growth equals the speed at which lamellipodia extend and *L. monocytogenes* moves provided the first direct support for actin polymerization as the driving force in motility [15, 16].

**The rate of actin treadmilling is controlled by a diversified array of actin-binding proteins**

In vitro studies using purified actin showed that depolymerization from the pointed end is slow and represents the limiting step in the treadmilling rate. As a result, the steady-state concentration of ATP-G-actin is very close to the critical concentration of barbed ends, so that barbed end growth balances pointed end depolymerization [17, 18]. Notably, the growth of barbed ends in in vivo processes, such as during formation of lamellipodia, which are extending plasma membrane protrusions in the direction of migration, is two orders of magnitude faster. This implies that the concentration of monomeric actin is maintained at higher values by factors controlling the dynamics of assembly at the two ends of the actin filament [3]. These factors are proteins that generally bind either to monomeric G-actin or to the filament, thereby regulating the rate of turnover and imposing spatial restrictions required for site-directed actin dynamics. These actin ‘regulatory proteins’ affect the kinetic/thermodynamic parameters of actin assembly at one or the other end of the filament in various fashions (figs 1, 2).

Instrumental in understanding the biochemical contributions of various actin regulatory proteins has been the in vitro reconstitution of actin-based motility of either bacteria or functionalized beads [19, 20]. These approaches led to the identification of the essential proteins for actin-based motility: actin, the actin nucleator complex Arp2/3, an Arp2/3 activator, an actin depolymerizing factor (ADF/cofilin) and a capping protein [21]. The movement is more effective when profilin is also included [21]. Interestingly, the same proteins are required for lamellipodia formation in *Drosophila S2* cells [22]. The fact that five essential proteins are sufficient to reconstitute actin-based propulsion of a particle strongly supports the notion that the regulated treadmilling of actin filaments, coupled to site-directed catalytic generation of new filaments, is at the origin of actin-based movements [23] (see also table 1).

**Severing and depolymerization**

ADF/cofilin are ubiquitous, conserved actin-binding proteins [24]. Their role is to enhance the treadmilling rate of