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REVIEW

SPECIAL ISSUE: RECONSTITUTING CELL BIOLOGY

Dynamic stability of the actin ecosystem

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ABSTRACT

In cells, actin filaments continuously assemble and disassemble while maintaining an apparently constant network structure. This suggests a perfect balance between dynamic processes. Such behavior, operating far out of equilibrium by the hydrolysis of ATP, is called a dynamic steady state. This dynamic steady state confers a high degree of plasticity to cytoskeleton networks that allows them to adapt and optimize their architecture in response to external changes on short time-scales, thus permitting cells to adjust to their environment. In this Review, we summarize what is known about the cellular actin steady state, and what gaps remain in our understanding of this fundamental dynamic process that balances the different forms of actin organization in a cell. We focus on the minimal steps to achieve a steady state, discuss the potential feedback mechanisms at play to balance this steady state and conclude with an outlook on what is needed to fully understand its molecular nature.

KEY WORDS: Actin, Cytoskeleton, Steady state

Introduction

A key property of most living systems is their ability to move and/or change shape according to environmental cues. This is instrumental for the cell or the tissue to carry out its biological program, including cell processes such as division and motility, and developmental processes like morphogenesis. Understanding the dynamic steady state of actin is a major challenge in cell and developmental biology because actin is a key player and driving force in the construction of the complex and dynamic scaffolding that makes up the internal architecture of eukaryotic cells (Blanchoin et al., 2014; Chhabra and Higgs, 2007). Whereas many molecules that are involved in building the actin cytoskeleton are known, the basic rules that control the coordinated dynamics of structures that exist at the same time in the cell, such as branched networks, parallel bundles and antiparallel contractile bundles, are still poorly understood. The dynamic steady state of actin has four facets: (1) nucleation (formation of actin dimers or trimers), (2) elongation and capping (controlled polymer growth), (3) disassembly (breakdown of actin structures) and (4) recycling (replenishment of the pool of actin monomers that are charged with ATP) (Fig. 1). Here, we consider each facet in detail and highlight the potential existence of feedback mechanisms that could balance the dynamics of cellular actin, and the need for reconstitution experiments to fully dissect the balance of the steady state of actin.

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The pool of actin monomers

The elementary building block for actin assembly is the actin monomer. The concentration of actin monomers is quite variable in different living systems, ranging from potentially as low as 0.01 µM in the yeast *Saccharomyces cerevisiae* to 300 µM in unactivated platelets (Karpova et al., 1995; Pollard et al., 2000). Since the rate of actin assembly is directly proportional to the concentration of monomers (Pollard et al., 2000), this variability means that the dynamic steady state of actin is not the same in different cell types; actin filaments could potentially grow orders of magnitude faster in platelets than in yeast.

In the cell, most of the pool of polymerizable actin is bound to profilin (Fig. 1 and Kaiser et al., 1999). However, some actin monomers in a given cell type might not be polymerizable as they can be sequestered by proteins such as thymosin β4 (Tβ4) (Fig. 1 and Pantaloni and Carlier, 1993). In this context, determining the exact concentration of polymerizable actin at a given time is challenging (Raz-Ben Aroush et al., 2017) as the balance between sequestered and polymerizable actin during dynamic actin assembly is not well characterized (Skruber et al., 2018). In addition, Tβ4 has been proposed to play an active role in preventing monomer incorporation into branched networks and in targeting cytosolic actin monomers to formin-mediated assembly at the leading edge of cells (Vitriol et al., 2015). This polymerizable pool of actin monomers needs further characterization at the cellular level, but also at the subcellular level – at sites of active actin assembly – where the pool of polymerizable actin can become depleted (Boujemaa-Paterski et al., 2017). In addition, different actin networks compete for actin monomers, and this is crucial for determining network density and size (Burke et al., 2014; Suarez and Kovar, 2016). To understand the landscape of the monomer pool in a complex actively polymerizing network, as found in keratocyte fragments (Raz-Ben Aroush et al., 2017), it will be necessary to use methods such as fluorescence recovery after photobleaching (FRAP) or photoactivation and/or photoconversion experiments coupled with mathematical modeling to assess actin monomer dynamics in different *in vivo* contexts (Skruber et al., 2018) and/or to use cell-size confinement to generate reconstituted systems with well-defined but limited sources of actin monomers. Given the importance of the local polymerizable actin concentration for determining actin dynamics, evaluating the pool of polymerizable actin – and potentially its gradients – in different cellular contexts is one of the key challenges of the coming years.

Nucleation

Profilin prevents the spontaneous association of actin monomers into actin dimers and trimers, which are necessary intermediates prior to the formation of actin filaments (Dominguez, 2009; Sept and McCammon, 2001). These nucleation steps, which are thermodynamically unfavorable, are accelerated by actin nucleators (Fig. 1). Three main classes of actin nucleators have been characterized: the Arp2/3 complex, the formin family and the tandem monomer-binding protein family, including the proteins

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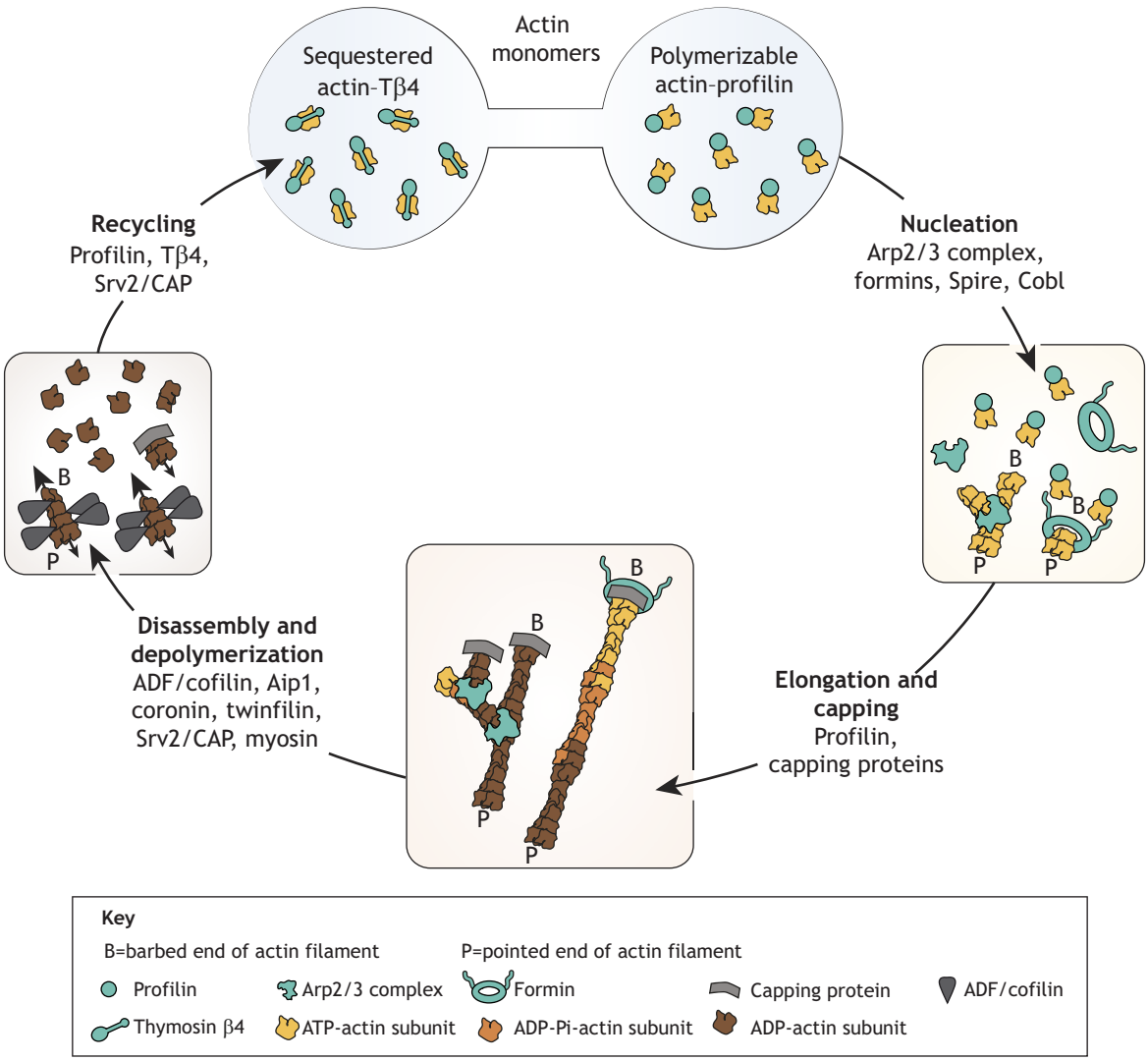

Fig. 1. The dynamic steady state of actin. The mininal steps needed to reach a dynamic steady state of different actin architectures are illustrated. Sequestered and polymerizable actin represent the pool of actin monomers. Nucleation is the formation of actin dimers or trimers. Elongation and capping modulate controlled growth of the different forms of actin organization (branched networks that are generated by the Arp2/3 complex or bundles generated by formins). Disassembly and depolymerization results in the breakdown of actin structures to monomer subunits. Recycling renews the pool of actin monomers that are charged with ATP. The different nucleotide states of actin, barbed and pointed ends and different proteins or complexes are represented by the indicated symbols. The arrows at barbed (B) and pointed (P) ends indicate depolymerization, with larger arrows representing faster dissociation.

Spire and Cobl, which are proposed to cause nucleation by tethering three or more actin monomers together (Campellone and Welch, 2010). The different mechanisms of action and the cellular localization of these nucleators influence their physiological properties and abilities to build specific actin structures. The Arp2/3 complex and formins are also influenced by external signals as their activity is controlled either directly or indirectly by membrane-bound Rho GTPases (Lawson and Ridley, 2018; Ridley, 2015). In addition, profilin has been shown to favor formin-mediated assembly over Arp2/3 complex-based nucleation, thus modulating homeostasis between different networks (Suarez et al., 2015; Rotty et al., 2015).

Elongation and capping

Pointed ends do not grow in profilin-actin since profilin masks the barbed face of the actin monomer, preventing addition to pointed ends (Pollard et al., 2000). Therefore, in cellular conditions of profilin-actin, networks that are generated by the Arp2/3 complex grow with a rate that depends on the association rate constant for monomer addition at the barbed ends (which is typically 10 per μM per second, Pollard, 1986) and the concentration of polymerizable actin monomers. Growth is terminated by a lack of monomers or by capping proteins (Fig. 1). Therefore, the balance between rates of growth and capping needs to be well-adjusted for the formation of defined actin networks (Akin and Mullins, 2008; Blanchoin et al., 2000a; Kawska et al., 2012). This balance is illustrated by a comparison of the dynamics of two similar structures found in different cells, where the actin monomer concentrations are very different. For example, actin patches in yeast and cell lamellipodia are both generated by the Arp2/3 complex and consist of highly branched and intertangled actin networks (Young et al., 2004; Svitkina and Borisy, 1999). Based on the concentration of available monomers, the growth of a lamellipodium should be orders of magnitude faster than a patch, but this is not the case because capping proteins regulate growth in lamellipodia (Moseley and Goode, 2006). Indeed, capping proteins are in fact necessary

for correct lamellipodia formation in motile cells with a high concentration of monomers (Iwasa and Mullins, 2007). In contrast, in *S. cerevisiae*, where monomer concentration is very low, capping protein can be removed and actin patches still show a qualitatively normal organization (Young et al., 2004). Growth of actin filaments that is mediated by formin is even more complex because different formins produce different association rate constants at filament barbed ends (Chesarone and Goode, 2009). In addition, formin and capping proteins function antagonistically at the barbed ends of actin filaments to control their length through the formation of a 'decision complex', where capping protein and formin are simultaneously bound to a paused barbed end. Depending on how the complex decomposes, growth will resume or the filament will be permanently capped, lending an extra layer of control to the dynamics of the barbed end (Fig. 1, Bombardier et al., 2015; Shekhar et al., 2015). Finally, formins are known to cooperate with the Arp2/3 complex for the elongation of protrusive networks (Block et al., 2012; Kage et al., 2017). Taken together, elongation and capping, in the context of the amount of polymerizable monomeric actin and the nature of the nucleating agent, are what defines the dynamics of actin network growth.

Disassembly

Actin disassembly takes place in two steps: fragmentation of actin networks into small filaments, and depolymerization into monomers of the small actin fragments that were generated in this process (Fig. 1, Blanchoin et al., 2014). Like nucleation or elongation, disassembly depends on the nature of the network (Gressin et al., 2015). A branched network disassembles mainly through debranching that is mediated by the actin depolymerizing factor (ADF)/cofilin family proteins or glia maturation factor-like protein (GMF) (Blanchoin et al., 2000b; Chan et al., 2009; Gandhi et al., 2010; Gressin et al., 2015). For parallel or antiparallel networks of bundled filaments, the severing of filaments by ADF/cofilin on its own is not sufficient to dismantle the structure entirely (Gressin et al., 2015), but enough to maintain a steady state length, at least for parallel bundles that are initiated by formin (Michelot et al., 2007). Actin-interacting protein 1 (Aip1) is a necessary cofactor that synergizes with ADF/cofilin and coronin proteins to disassemble actin bundles (Nadkarni and Brieher, 2014; Gressin et al., 2015; Jansen et al., 2015). For antiparallel contractile actin networks, the contribution of the myosin motor protein during disassembly is unclear, but actin filament buckling that is produced by myosin contraction has been shown to lead to filament breakage (Murrell and Gardel, 2012), and buckling might also favor severing by ADF/cofilin. To be fully efficient, ADF/cofilin must work in concert with capping proteins (Suarez et al., 2011). Indeed, the presence of ATP or ADP-Pi-loaded subunits near a growing barbed end prevents this region of the filament from being decorated by ADF/cofilin (Suarez et al., 2011; Wioland et al., 2017). Barbed-end capping terminates the growth of filaments within a structure, and thus favors decoration of filaments by ADF/cofilin (Suarez et al., 2011). Therefore, the disassembly of actin networks is intricately linked to the growth and capping balance of the actin steady state, as discussed above.

Until recently, a few puzzling questions remained concerning the disassembly step. First, why do fragments that are generated by ADF/cofilin not elongate rapidly until capped, thus reversing the disassembly effect of ADF/cofilin? Second, how does rapid disassembly from capped fragments occur? The rate constant of depolymerization at pointed ends is only 0.27 per second, and depolymerization would be much more efficient if it occurred at the barbed end with a rate constant of 7.2 per second (Pollard, 1986). Recently, these two questions have been elegantly addressed by Wioland and co-workers, who showed that decoration with ADF/cofilin – upon nearing the barbed end – dissociates capping protein from that end (Wioland et al., 2017). Even more striking, barbed ends of filaments that are saturated with ADF/cofilin do not grow because ADF/cofilin prevents monomer addition (Wioland et al., 2017). In other words, ADF/cofilin alters barbed end dynamics by binding to the sides of the filament and changing its structure (Tanaka et al., 2018), thus preventing capping protein binding and monomer addition, while still allowing subunit dissociation (Fig. 1). Other proteins, such as twinfilin and Srv2/cyclase-associated protein (CAP), which accelerate depolymerization at the barbed and pointed ends, can also participate in actin disassembly (Johnston et al., 2015). Their collaborative effort depolymerizes a filament of one micrometer length in less than a minute (Johnston et al., 2015). How such depolymerization occurs on ADF/cofilin-decorated filaments, or on small fragments that are generated by both ADF/cofilin and Aip1, needs to be investigated. Srv2/CAP has also been shown to enhance severing by ADF/cofilin (Chaudhry et al., 2013). It is still unclear exactly how small fragments depolymerize into single subunits. Recent advances in time-resolved electron microscopy, combined with static and dynamic light scattering, might help in the observation of these entities, which fall below the diffraction limit (Frank, 2017; Lopez et al., 2016).

Recycling

An assembly-competent actin oligomer pool has previously been proposed (Okreglak and Drubin, 2010; Smith et al., 2013); however, for most reassembly processes, actin filaments must be broken down into their individual monomers. Actin monomers are bound to ADP when they dissociate from a filament (Blanchoin and Pollard, 1999). These subunits therefore need to be reloaded with ATP to reintegrate into the pool of sequestered or polymerizable actin (Fig. 1). As ADF/cofilin bound to an actin subunit blocks nucleotide exchange (the rate of nucleotide dissociation of ADF/cofilin-bound ADP-actin is 0.006 per second; Blanchoin and Pollard, 1998), profilin or Srv2/CAP act as nucleotide exchanging factors: they dissociate ADF/cofilin from ADP-actin subunits and load subunits with ATP (Blanchoin and Pollard, 1998; Chaudhry et al., 2010; Gurel et al., 2015; Kotila et al., 2018). This replenishes the pool of polymerizable actin (Fig. 1). In the presence of high concentrations of Tβ4, as in platelets, the situation is more complex, because thymosins also block nucleotide exchange (Goldschmidt-Clermont et al., 1992; Xue et al., 2014). However. Tβ4 has a 100-fold higher affinity for ATP-actin compared with ADP-actin monomers (Jean et al., 1994), so nucleotide exchange probably occurs before thymosin binds monomers. This might occur through formation of a transient ternary complex between Tβ4, actin monomer and profilin, or other nucleotide-exchanging factors (Yarmola et al., 2001). Overall, a complex choreography of actin-binding proteins controls the recycling of ADP-actin monomers to the polymerizable or sequestered ATP form.

Feedback

The huge variability in the structure of different forms of actin organization, their growth rates and lifetimes beg the question as to how the perfect match between assembly and disassembly rates and maintenance of the pool of actin monomers is ensured in these different contexts. This must be controlled by as yet unidentified feedback mechanisms. Is it a structural feedback, where network size, structure and filament density affect actin dynamics, a mechanical feedback, where tension and pressure regulate dynamics, or even a

biochemical feedback, where polymerization depletes factors, thus limiting assembly or disassembly? It is likely that it is a combination of these different types of feedback. Structural feedback has been observed recently in a reconstituted lamellipodium, where network size and filament density have been shown to control network growth rate (Boujemaa-Paterski et al., 2017). Force-dependent feedback controls both the growth of branched networks generated by the Arp2/3 complex (Bieling et al., 2016; Mueller et al., 2017; Plastino and Blanchoin, 2017) and formin-mediated actin filament assembly (Courtemanche et al., 2013; Jégou et al., 2013; Zimmermann et al., 2017). Biochemical control is seen in the competition for actin monomers between formin-based and Arp2/3-based actin networks (Burke et al., 2014) or in local monomer depletion at sites of active assembly that negatively impacts growth rate (Boujemaa-Paterski et al., 2017). The identification and mechanistic understanding of the different feedback loops that control cellular actin dynamics will require a huge effort from both top-down and bottom-up approaches, bridging the gap between investigations at molecular, cellular and tissue levels.

Conclusions and perspectives

What are the limitations to achieving a complete understanding of the dynamic steady state of actin networks? *In vivo*, the biggest limitation is the observation of individual actin filaments whose average lengths are 10 to 100 nm, which is below the diffraction limit of light microscopy (Anderson et al., 2017). The development of new super-resolution approaches and new fluorescent markers, combined with electron microscopy, might help to fill this gap (Gao et al., 2018; Skruber et al., 2018). However, a true understanding will require visualization of the actin cytoskeleton in its native state, imaging the coordinated dynamics of different subcellular actin organizations. A step in this direction is the *in vitro* reconstitution of a complete dynamic system, where branched networks, parallel bundles and contractile antiparallel structures maintain a coordinated steady state regime in a cell-sized environment that mimics the limited supply of biochemical components in a real cell (Burke et al., 2014). Growing different actin organizations has been partially achieved using micropatterning approaches (Reymann et al., 2010), but never with a combination of different nucleation machineries. Growth must be initiated in the presence of both the disassembly machinery and the proteins necessary to recycle actin subunits back to the pool of polymerizable actin. Ideally, this reconstituted system would allow for the modulation in real time of the different actin organizations, such as changing the pattern of nucleation to evaluate how the system responds and adapts to this new configuration. One of the biggest challenges is determining the operating concentrations for the different components of a complex mixture incorporating nucleation, turnover and actin recycling machinery. Parallelizing the experiments by means of microfluidics will probably be necessary. The field is technically ready to tackle this challenge, both *in vitro* and *in vivo*, but it will be necessary to join forces, as the task is too complex for a single laboratory.

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Competing interests

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