Setting the pace of cell movement

Eukaryotic cells have many proteins that cap the barbed ends of actin filaments. Manipulation of their cellular concentration leads to changes in cell motility rates, actin dynamics and signal transduction reactions.

Although Oosawa and his associates extensively analyzed the kinetics of actin-filament polymerization from actin monomers in the 1960s, it was only with Tilney and Lindberg's experiments a decade later that attention focused on actin assembly and disassembly as an important aspect of non-muscle cell motility. Tilney and Lindberg correlated movements of marine sperm and human blood platelets, respectively, with large shifts in actin from an unpolymerized to a polymerized state. Cells undergo locomotion without necessarily changing their relative amounts of monomeric and polymeric actin, but actin assembly and disassembly rates are accelerated in moving cells, and the actin turnover rate correlates with the speed at which cells crawl [1].

Polarized actin assembly and disassembly

According to the convention originated by H.E. Huxley — based on his observations that myosin heads lean to confer an arrowhead structure on actin filaments that is visible in the electron microscope — actin filaments have 'barbed' and 'pointed' ends. Pollard and Ishiwata independently noted that actin monomers add more rapidly to the barbed than to the pointed ends of actin filaments, and exhaustive kinetic analyses of actin monomer exchange with actin filaments *in vitro* have established that the barbed end can elongate an order of magnitude faster than the pointed end, and that exchange of monomers with the pointed end is slow. ATP hydrolysis by actin subunits within a filament is responsible for maintaining a bias towards net assembly at the barbed end, and disassembly at the pointed end, of an actin filament [2].

One early clue integrating the complexity of actin dynamics with the regulation of actin assembly *in vivo* was the recognition by several groups of investigators that the predominant effect of a class of fungal metabolites, the cytochalasins, which inhibit cell movements and intracellular actin-filament assembly, is to inhibit exchange of actin monomers with actin-filament barbed ends. A consequence of barbed-end binding by cytochalasins is a substoichiometric reduction in actin-filament length, so that cytochalasin-treated filaments are more fluid (less gelled) than untreated filaments [3]. This insight facilitated the discovery of a large number of proteins that shorten actin filaments by binding to actin-filament barbed ends.

Binding of such agents to actin-filament barbed ends — 'capping' — shortens filaments, because it prevents filament growth but allows disassembly from the pointed end to proceed unchecked. As monomers dissociate from the pointed ends of the filaments, they can be withdrawn from the polymerization-competent pool by monomer-sequestration proteins, such as thymosin-β and profilin, that have higher affinities for actin monomers than does the pointed end. In contrast, the barbed end has a higher affinity for actin monomers than do the sequestering proteins, so that exposure of barbed ends is expected to cause the rapid assembly and elongation of filaments without other steps being required.

Barbed-end capping proteins

Two classes of proteins bind actin-filament barbed ends. One type, capping protein (CP; also known as capZ, Cap 32/34 or aginactin), is a highly conserved and ubiquitous heterodimer. CP constitutively binds actin-filament barbed ends with close to nanomolar affinity, and weakly nucleates actin monomers for elongation from the pointed end. The second, more diverse group, is the gelsolin family of severing and capping proteins, which includes fragmin, gelsolin, villin, capG, adseverin (also called scinderin) and severin. Multiple members of the gelsolin family may coexist in a single cell type. All proteins of this group require micromolar calcium to bind actin (protons also activate gelsolin). Once bound to actin, fragmin, gelsolin and severin become calcium-insensitive; the others, however, dissociate from filament ends when calcium falls to nanomolar concentrations. All nucleate actin assembly from the pointed end and, with the exception of capG, sever as well as cap the barbed ends of actin filaments. All barbed-end capping proteins are inhibited from capping *in vitro* by polyphosphoinositides, which under some circumstances remove capping proteins from actin-filament barbed ends *in vitro* and in permeabilized cells [4].

Strategic barbed-end exposure and cellular actin assembly

The extensive evidence relating signal transduction intermediates to capping protein activity *in vitro* led to the proposal that diverse messengers allow the same set of capping proteins to control different actin dynamics in space and time. Diffusible messengers such as ions and (possibly) protein kinases were suggested to promote actin disassembly, whereas membrane phosphoinositides were suggested to promote actin assembly (Fig. 1) [5].

Resting cells contain large amounts of polymerized actin, and evidence has now accumulated showing a strong correlation between stimulus-induced cell motility and actin assembly on exposed barbed ends (reviewed in [6]).

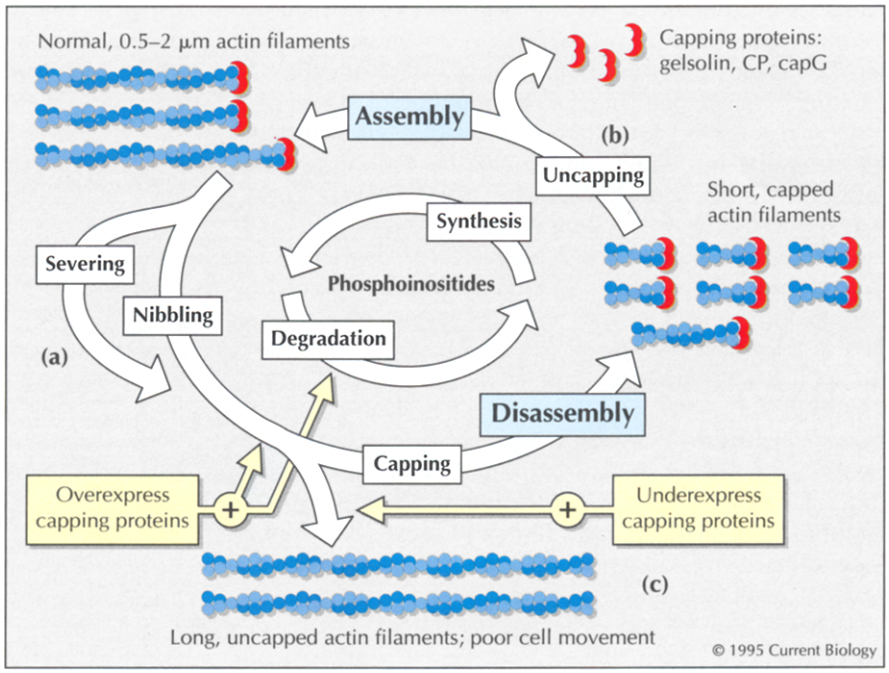

Fig. 1. Interrelated actin assembly reactions and metabolic cycles affected by the expression of capping and severing proteins. Cytoplasmic actin-filament assembly and disassembly is controlled by filament capping and severing, and the accessibility of filament barbed ends. These proteins are regulated by ions, such as Ca^{2+} and H^+, and phosphoinositides. **(a)** Severing promotes and accelerates filament disassembly above the background provided by nibbling proteins. **(b)** Uncapping of filament barbed ends leads to net filament assembly. **(c)** Diminished capping activity increases the length and content of cytoplasmic actin filaments. Overexpression of capping proteins increases the rate of phosphoinositide turnover.

In resting cells, most filament barbed ends are capped by barbed-end binding proteins, preventing elongation. Activation results in the dissociation of these proteins from the barbed end ('uncapping'), a process that occurs just below the plasma membrane at the leading edge of moving cells, providing spatial control of actin assembly for cell motility. According to this model, the 'nucleation' activity of barbed-end capping proteins is not aimed directly at the creation of elongated filaments, but rather provides a reservoir of cryptic barbed ends that are available for uncapping.

Manipulating expression levels of capping proteins

To assess the position of these barbed-end capping proteins in cellular actin assembly, biologists have investigated the effects of experimentally manipulating their cellular concentrations (Fig. 1). Cunningham *et al.* [7] isolated clonal cell lines of fibroblasts stably transfected with the cDNA encoding human gelsolin, which express 25–125 % more gelsolin than control cells, and observed that the gelsolin-overexpressing cells crawled faster. In fact, the rate of chemotaxis was directly proportional to the increased gelsolin concentration, suggesting that the increased gelsolin content accelerated actin remodeling. This study proved that modifying the intracellular actin-related machinery can influence cell locomotion, and suggested that other reactions coupling gelsolin's effects on actin to signal transduction are not rate-limiting — that is, they can accommodate modest increases in gelsolin concentration by turning over more rapidly

Sun *et al.* [8] and Hug *et al.* [9] recently demonstrated that overexpression of capG or CP accelerates the motility of fibroblasts or *Dictyostelium* amoebae, respectively. Sun *et al.* [8], using the overexpression techniques employed for gelsolin, observed phenotypes remarkably similar to those caused by gelsolin overexpression. Stable transfection resulting in 30–80 % more capG than normal cells increased, in proportion to the capG expression level, the rates of both random migration and cell chemotaxis. CapG-overexpressing cells also gave exaggerated responses to stimulation by platelet-derived growth factor (PDGF) — the ruffling response of their dorsal surfaces, the elevated inositol phosphate production and intracellular calcium transients were all more marked than normal. As capG and CP do not sever filamentous actin, these studies focused attention on the quantitative importance of barbed-end capping for cell movement, and linked capping proteins to polyphosphoinositide turnover. The effects of capG overexpression on signal transduction reactions confirmed the earlier inference that these reactions are not inherently rate-limiting.

Lowering the level of CP in *Dictyostelium* amoebae, using antisense mRNA, caused cells to move more slowly. In cells expressing ~ 25 % of normal CP levels, the rate of actin-filament assembly was increased, and a greater percentage of actin was assembled into filaments. While the latter finding is expected from loss of capping activity, the former may link actin gene regulation to either actin monomer or CP concentration in cells. Cytoskeletal organization was also disturbed in cells with low CP levels: actin filaments were long and distorted in shape, characterized by the appearance of unusual surface spikes filled with actin-filament bundles. Similarly, deletion of the CP gene in yeast cells also results in highly abnormal cell morphology and disruption of their cytoplasmic actin cables [10]. *Dictyostelium* cells expressing low levels of CP, however, retained their ability to respond in normal fashion to the chemotactic challenge by cAMP and increased their actin-filament content 1.5-fold after cAMP-stimulation. CP-overexpressing cells still responded to cAMP, but with a diminished increase in actin-filament content.

Gene 'knockout' mice lacking gelsolin expression have recently been produced [11]. The absence of gelsolin had no deleterious affect on mouse development or survival, attesting to the redundancy of capping proteins. Gelsolin-null mouse embryos have no detectable actin-filament severing activity. This finding highlights the importance of CP and weak actin-filament severing ('nibbling') proteins, such as actin-depolymerizing factor (ADF) and cofilin, for the constitutive actin-filament depolymerization associated with the relatively slow cell movements required for morphogenesis.

Individual cells derived from adult gelsolin-null mice, however, show significant abnormalities. Isolated fibroblasts spread slowly and develop excessively thick actin stress fiber bundles, which are stable to treatments that normally cause their rapid disruption, such as cytochalasin B. Once spread, these cells fail to move. Neutrophil migration *in vivo* and *in vitro* is modestly impaired. Compared to normal mouse platelets, resting platelets from gelsolin-null mice have an increased content of filamentous actin, and they are slow to remodel their actin cytoskeleton when activated. These findings point to gelsolin as a key component of rapid actin-filament disassembly, required for fast cellular responses involved in host defense and hemostasis.

Molecular manipulations of actin-filament barbed-end capping proteins thus demonstrate pivotal roles for these molecules in cell motility, in accelerating the rate of actin flux through filaments, and in the modulation of phosphoinositide metabolism and signaling. The ability of all these proteins to interact with phosphoinositides, and of some to affect phosphoinositide metabolism directly, places these proteins in a position to regulate cell motility by responding to changes in the production or exposure of these important cellular signals.

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