Actin Dynamics: Tropomyosin Provides Stability

Dispatch

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Tropomyosin stabilizes actin filaments, while ADF/cofilin promotes their disassembly. These two proteins compete with each other for binding to the actin filament, providing a critical balance for actin assembly in vivo.

The field of actin assembly and cell motility has made great strides in recent years, on several fronts. There have been advances in understanding the mechanisms of actin filament assembly and disassembly, as well as the regulatory processes by which extracellular signals are transduced to the actin cytoskeleton. The discovery of the Arp2/3 complex and its stimulation by WASp and cortactin relatives has influenced the research focus of many in the field and promoted connections with a large body of signaling research. With this information on Arp2/3 complex and actin dynamics in hand, many groups are taking new looks at their favorite proteins. These new experiments are particular exciting because they involve a higher level of complexity than we have seen in the past. Researchers are now able to investigate the combined action of multiple purified proteins *in vitro* and multiple mutations *in vivo*.

Tropomyosin is one such protein where new information is available. In striated muscle, tropomyosin helps mediate the signal for contraction, controlling access of myosin heads to the actin filament. Non-muscle cells and lower organisms, including yeasts, also contain tropomyosin. In those cells, tropomyosin's primary role appears to be stabilizing actin filaments and regulating access of actin-binding proteins, including 'unconventional' myosin [1], to the actin filament. Recent studies have shed new light on these functions of tropomyosin [2–4].

Tropomyosin is a long, thin protein with two α-helical polypeptides which form a coiled coil in a parallel orientation. Vertebrates have 17 tropomyosin isoforms, produced by alternative splicing from four genes. Tropomyosin can exist as a homodimer, and some pairs of isoforms can form heterodimers, providing even more potential complexity [5]. Tropomyosin binds along the side of the actin filament, spanning six or seven actin subunits. The actin filament is polar, so the tropomyosin molecules are all oriented in the same direction. They interact with each other at their ends — head-to-tail — which makes their binding highly cooperative.

As one might expect from the nature of the binding, tropomyosin inhibits the dissociation of actin subunits from the end of the actin filament [6,7]. Also, the presence of tropomyosin makes the actin filament stronger and less likely to bend or break [8,9]. These properties may be important in non-muscle cells where actin filaments rapidly assemble, move and disassemble at different times and places. Tropomyosin also stabilizes actin filaments by interacting with tropomodulin at the pointed end of the actin thin filament in muscle. The combination of tropomodulin and tropomyosin caps the pointed end of the actin filament *in vitro* and *in vivo* [10], helping to control thin filament length and turnover in the sarcomere [11]. Non-muscle cells also have tropomodulins, where their role is just beginning to emerge.

Tropomyosin has long been known to protect actin filaments from the depolymerizing action of DNase I [12] and ADF/cofilin [13] and the severing action of gelsolin [14]. In a recent study, Ono and Ono [2] explored these interactions in *Caenorhabditis elegans*. They found that tropomyosin stabilizes actin filaments and antagonizes the action of ADF/cofilin, which promotes filament disassembly. In biochemical experiments with purified proteins, the binding of tropomyosin and ADF to the actin filament were mutually exclusive. Tropomyosin enhanced actin filament assembly and inhibited the ability of ADF/cofilin to disassemble actin filaments. Native actin (thin) filaments isolated from muscle were found to contain tropomyosin but not ADF/cofilin, and purified ADF/cofilin did not interact with the native filaments unless tropomyosin was removed. Tropomyosin and ADF/cofilin were localized by fluorescence staining to different places in muscle, indicating that their mutual exclusivity is also exhibited *in vivo*.

Taking a molecular genetic approach, Ono and Ono [2] obtained evidence that these same functional interactions exist in the whole organism. They found that suppression of tropomyosin by RNA interference (RNAi) led to a decrease in muscle-based motility and to disorganization of the muscle actin filaments. ADF/cofilin loss-of-function mutants also showed decreased motility, with some (but less) disorganization of the actin filaments. In these ADF/cofilin mutants, suppression of tropomyosin had no additional effects on the actin, suggesting that the presence of tropomyosin protects the actin from the effects of ADF/cofilin, as seen *in vitro*.

Tropomyosin was also recently found to inhibit the ability of Arp2/3 complex to nucleate actin polymerization [3]. The Arp2/3 complex binds to the side of an existing (mother) actin filament, nucleating a new (daughter) filament to form a branch. The presence of tropomyosin on an existing filament inhibits the action of Arp2/3 complex. In this context, tropomyosin might inhibit new actin filament assembly *in vivo*. Tropomyosin would decorate older actin filaments, restricting the nucleating action of Arp2/3 complex to newer actin filaments. These interactions would help to maintain new actin filament assembly near the leading edge of the cell, which supports cell protrusion.
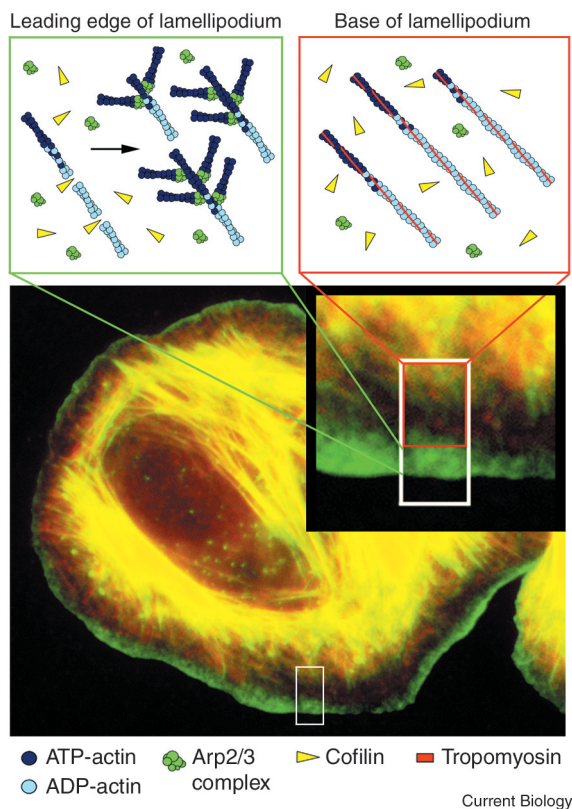
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Figure 1. Models for the action of tropomyosin and other actin-binding proteins on actin assembly near the edge of a migrating cell.

At the bottom is a micrograph of a carcinoma cell stimulated with EGF, labeled for F-actin (green) and tropomyosin (red). The inset shows how the leading edge of the lamellipodium lacks tropomyosin, relative to the base of the lamellipodium. The diagrams at the top illustrate models for these two regions. The leading edge (top left) contains a branching network of short actin filaments, with nucleation by Arp2/3 complex and severing by cofilin. The base of the lamellipodium (top right) contains longer, unbranched actin filaments decorated with tropomyosin, which prevents binding of cofilin and Arp2/3 complex. (Figure kindly provided by Vera DesMarais [4].)

Recent subcellular localization studies lend further support to this notion. DesMarais and co-workers [4] found that tropomyosin does not occur in association with the newly polymerized actin filaments near the leading edge of cells. Dawe and Cramer found that tropomyosin is enriched in the rear portion of lamellipodia in migrating cells (L. Cramer, personal communication). Tropomyosin also prevents the binding and action of an unconventional myosin, myo 1b (myr-1), on actin filaments *in vitro* [1]. *In vivo*, myo 1b localizes to a migrating cell's leading edge, which lacks tropo-myosin. On the other hand, tropomyosin had no effect on the ability of capping protein to cap the barbed ends of actin filaments *in vitro* (M. Wear, personal communication).

One might speculate on how to integrate these observations into a model for actin assembly (Figure 1). Actin filaments nucleated by Arp2/3 complex at the leading edge probably lose their attachment to

Arp2/3 over time, as the filaments hydrolyze ATP to ADP and move away from the membrane-based signals that activate Arp2/3. When the Arp2/3 complex is lost, actin filament branchpoints must break and the filaments are inclined to disassemble. The hydrolysis of ATP to ADP on the aging filament promotes the cooperative binding of ADF/cofilin, which in turn promotes filament disassembly by severing the filament and by increasing the subunit dissociation rate from the pointed end. On the other hand, as the filaments age, tropomyosin may bind to them. Tropomyosin binding is also cooperative along a filament, but the presence of tropomyosin and ADF/cofilin should be mutually exclusive along a given filament. Tropomyosin makes filaments more stable — the opposite effect from ADF/cofilin. In this model, competition between tropo-myosin and ADF/cofilin becomes a critical variable that pitches the balance between assembly and disassembly for each actin filament.

An alternative view is that ADF/cofilin functions at the leading edge to promote assembly, not disassembly, of actin filaments (Figure 1). In this model, ADF/cofilin severs filaments to create new ends, which support polymerization [15]. *In vitro*, growing barbed ends, created by ADF/cofilin, provide a fertile substrate for the nucleation of branched filaments by Arp2/3 complex [16]. These two models may both be correct. ADF/cofilin may have opposing effects at different places and times.

As noted above, even yeasts have tropomyosin. Studies in yeast have underpinned the notion that tropomyosin stabilizes actin filaments. At first glance, the actin cytoskeleton of a yeast cell looks rather different from that of a mammalian cell migrating in culture; there are, however, important functional parallels between the two types of cell. In budding yeast, tropomyosin stabilizes the filaments that compose the actin 'cables' that course through the cytoplasm [17]. These cables are the tracks on which secretory vesicles move to sites of polarized exocytosis and cell growth, powered by an unconventional myosin [18]. When tropomyosin function is rapidly lost and gained in a exquisitely temperature-sensitive mutant, the cables disappear and reappear, respectively, in a matter of minutes. In yeast, cofilin is found in cortical actin patches [19] where it promotes disassembly of actin filaments [20]. Thus, yeast cells represent another important case where tropomyosin and ADF/cofilin have opposing functional effects and decorate different subsets of actin filaments *in vivo*.

We are in a time rich with discoveries of how actin filaments assemble and disassemble in cells, and how these processes are controlled in response to cell signals. Future experiments with biochemical reconstitutions of increasing complexity, ultrastructural studies of filament architecture, and finer temporal and spatial resolution in control of the activity of proteins in cells will produce more new and exciting insights. Lastly, I would like to thank Louise Cramer and Sarah Hitchcock-DeGregori for communicating unpublished results and reading the manuscript, and to acknowledge support by NIH grant GM38542.

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