Mini-Review

An End in Sight: Tropomodulin

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Actin filaments are required for cell locomotion and numerous intracellular activities in nonmuscle cells, and are a major component of the contractile apparatus in muscle cells. An understanding of the dynamics involved in the assembly and disassembly of actin filaments is fundamental to understanding many cellular functions. In this issue of *The Journal of Cell Biology*, Weber et al. (1994*b*) provide important new insights as to how actin assembly and disassembly is regulated in the cell with their demonstration of tropomodulin as a capping protein specific for the kinetically less active end of an actin filament.

Filament formation is a dynamic and polarized process (for review see Pollard and Cooper, 1986). In the absence of other proteins, actin monomers self-assemble in vitro into filaments reaching many microns in length. Assembly begins with the formation of nuclei containing three or four actin monomers. Elongation of the nuclei proceeds with the addition of monomers, but net growth at each end is not to the same extent. Monomers add to the kinetically more active ("barbed" or "preferred") end 10 times as quickly as to the less active ("pointed" or "nonpreferred") end. At equilibrium, the filaments are not static and monomers are both adding and dissociating from both ends of the filaments, albeit at different rates.

In vivo a number of actin accessory proteins exists which regulate filament assembly, for example, by associating with monomers thereby sequestering them from participating in assembly, or by capping the ends of actin filaments thereby preventing monomer exchange at that end (for a review on actin-binding proteins, see Hartwig and Kwiatkowski, 1991). Capping results in regulation of filament length and stabilization against disassembly. Proteins such as gelsolin (Yin and Stossel, 1979; Wang and Bryan, 1981) and villin (Bretscher and Weber, 1980; Glenney et al., 1981) are calcium-sensitive actin-binding proteins which both sever actin filaments and cap the barbed ends of actin filaments thereby preventing monomer exchange. Other capping proteins such as macrophage-capping protein (Southwick and DiNubile, 1986) or gcap39 (Yu et al., 1990) also cap the barbed ends of actin filaments in a calcium-sensitive manner, but do not sever actin filaments. Capping protein from *Acanthamoeba* (Isenberg et al., 1980) and capZ from muscle (Caldwell et al., 1989) bind to the barbed ends of actin filaments, but do not sever actin filaments and do not require Ca^{2+} for activity. Although over the years candidates for pointed end-capping proteins have been proposed, until now, none has been confirmed with the exception of DNaseI (Podolski and Steck, 1988; Weber et al., 1994*a*), whose relevance to actin assembly is unclear since it is not known to be cytoplasmic.

Several pieces of information indicate that the pointed end of actin filaments is capped in cells. For example, experiments with cytochalasin E indicate that actin monomers do not assemble onto the pointed ends of protofilaments in the erythrocyte (Pinder et al., 1986). Similarly, actin monomers will not add to the pointed ends of thin filaments in isolated myofibrils (Sanger et al., 1984; Ishiwata and Funatsu, 1985). Moreover, since the lengths of actin filaments in biological systems including muscle (Huxley, 1960), microvilli (Mooseker and Tilney, 1975), red blood cells (Byers and Branton, 1985), and stereocilia (Tilney et al., 1992) are uniform, one possibility is that length is partially determined by the existence of pointed end-capping proteins.

Identification of pointed end-capping proteins has been elusive not only because these ends of actin filaments are kinetically less active, but because of the limitations of the methods available to assay assembly (for a review of methods used to analyze actin assembly, see Gaertner et al., 1989). Most techniques including viscometry, light scattering, and flow birefringence measure bulk assembly which is dominated by growth at the preferred end because of the inherent polarity of the actin filament. Development of electron microscopy methods, in which actin assembly onto preformed nuclei such as microvillar core bundles or acrosomal bundles is monitored, has allowed for direct examination of activity at each end of the actin filament (Woodrum et al., 1975; Pollard and Mooseker, 1981; Coluccio and Tilney, 1983, 1984; Bonder et al., 1983), but these assays are not trivial to perform. The use of fluorescence probes such as pyrene iodoacetamide to label actin (Kouyama and Mihashi, 1981) has allowed for simple, quantitative analysis of actin polymerization and the interaction of actin accessory proteins with actin filaments; fluorescence intensity increases substantially when actin polymerizes. To distinguish events at the pointed ends of the actin filaments with this method, activity at the barbed ends must be squelched. Previously, filaments nucleated and capped at the barbed ends by villin (Northrop et al., 1986) have been used in pyrenyl-actin fluorescence assays designed to examine the effects of tropomyosin on actin assembly (Broschat et al., 1989; Broschat, 1990). Using a similar strategy, Weber et al. (1994*b*, this

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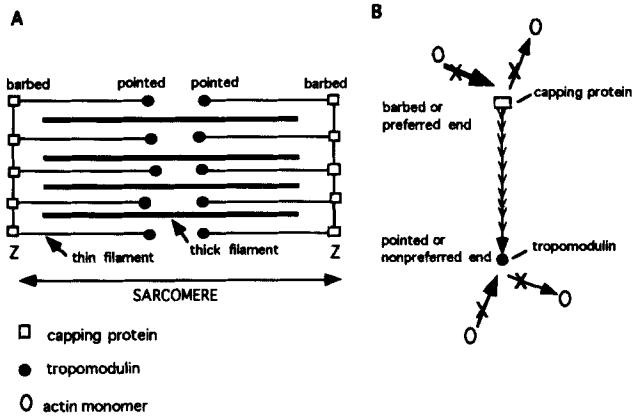

Figure 1. In both skeletal muscle (*A*) and nonmuscle cells (*B*), actin-containing filaments can be capped at the preferred end for assembly by capping protein (□) and at the nonpreferred end by tropomodulin (●).

issue) assay the effects of tropomodulin on the addition of pyrene-labeled actin monomers to filaments nucleated, and thereby capped at the preferred ends, by gelsolin (Bryan and Coluccio, 1985). The changes in fluorescence intensity therefore reflect activity at the nonpreferred ends of the actin filaments.

Previously, Fowler (1987) identified a 40-kD tropomyosin-binding molecule associated with the red blood cell membrane; the molecule was subsequently named tropomodulin (Fowler, 1990). Immunofluorescence microscopy on skeletal muscle with anti-tropomodulin antibodies stained a doublet in the region of the A-bands, the portion of the sarcomere in which the pointed ends of the thin filaments reside (Fowler et al., 1993); the barbed ends of the muscle thin filaments are located at the Z-discs, the main component being capZ (Casella et al., 1987). Separation of this doublet upon stretching of the myofibrils suggested, but did not prove, that tropomodulin was associated with the pointed ends of the thin filaments (Fowler et al., 1993). In this new report, Fowler and colleagues (Weber et al., 1994*b*; this issue) now provide compelling biochemical evidence to demonstrate that tropomodulin binds to the pointed ends of actin filaments and is directly responsible for altering the rate of monomer assembly and disassembly at that end (Fig. 1).

The basis for this conclusion is founded in a number of experimental observations. In the presence of tropomodulin, assembly and disassembly of actin monomers at the pointed ends of actin filaments are only partially blocked; however, when tropomodulin is added to tropomyosin-coated actin filaments, both assembly and disassembly at the pointed ends are completely prevented. This would suggest that the affinity for tropomodulin to the end of a filament increases when tropomyosin is present. Tropomyosin, an elongated molecule that lies along the length of actin filaments, is found in the thin filaments of muscle where in association with troponin, it regulates muscle contraction. In nonmuscle cells, a variety of tropomyosin isoforms exists where they are proposed to stabilize actin filaments (for a review on the various isoforms of tropomyosin, see Pittenger et al., 1994). Previous studies have shown that although tropomyosin stabilizes actin filaments from disassembly, elongation is not blocked (Broschat et al., 1990). Thus, the cooperation of tropomyosin and tropomodulin completely blocks activity at the pointed end.

Tropomyosin molecules associate head to tail along the length of the thin filaments in muscle. In the erythrocyte, the limited length of the actin filaments can accommodate only two tropomyosin molecules, one on each side of a short actin filament (Fowler, 1987). The amounts of tropomodulin relative to actin in both muscle and red blood cells indicate that in both cases each actin filament would be associated with one or two tropomodulin molecules (Fowler et al., 1993).

The initial identification of tropomodulin in red blood cells (Fowler, 1987) and its recognition as the elusive pointed end–capping protein long expected to be present in skeletal muscle (Fowler, 1990; Weber et al., 1994*b*, this issue) demonstrates once again how the simple paradigm of the red blood cell can provide information about the organization of the cytoskeleton in more complex cells (see Bennett, 1985). Expression of the erythrocyte membrane skeletal proteins spectrin, ankyrin, protein 4.1, and the adducins (or closely related proteins) in other nonmuscle cells are earlier examples. In addition, tropomodulin joins a long list of cytoskeletal proteins including actin, myosin II, tropomyosin (Matsumura et al., 1983; Fowler, 1987), α-actinin (Maruyama and Ebashi, 1965; Burridge and Feramisco, 1981; Duhaiman and Bamburg, 1984), and capping proteins (Casella et al., 1987; Isenberg et al., 1980) common to both skeletal muscle and nonmuscle cells.

Future studies will determine whether short-capped filaments resembling those present in erythrocytes exist in other nonmuscle cells. One example might be provided by the dynactin complex whose major component is an actin-related protein that forms a short filament in association with capping protein at the barbed end of the filament and a potential tropomodulin homologue at the pointed end (Schafer et al., 1994). Equally exciting will be how to reconcile the prospect of cytoplasmic actin filaments stabilized at both ends with capping proteins with the dramatic and rapid reorganization of the actin cytoskeleton that accompanies cell locomotion or cytokinesis (for reviews see Wang, 1991; Satterwhite and Pollard, 1992). Microinjection experiments have indicated that preexisting actin filaments are recruited for cleavage formation, whereas drug studies have indicated that the filaments involved in the contractile ring are dynamic. The completion of cleavage is characterized by the quick disassembly of these filaments. One explanation relies on the reversibility of these capping proteins. Members of the gelsolin family including gelsolin (Yin and Stossel, 1979), villin (Bretscher and Weber, 1980), and macrophage-capping protein (Southwick and DiNubile, 1986) exhibit calcium-sensitive properties; moreover, the interaction of actin with some accessory proteins including gelsolin (Janmey and Stossel, 1987), villin (Janmey and Matsudaira, 1988), gcap39 (Yu et al., 1990), and capZ (Heiss and Cooper, 1991) is regulated in vitro by polyphosphoinositides. Determining how binding of tropomodulin to actin and tropomyosin is regulated will yield important clues as to the precise mechanism of actin assembly and disassembly in cells.

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