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. (1994b) 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 DiNobile, 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 Ca²⁺ 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., 1994a), 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 (Moosiker 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; Bender 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. (1994b, this
binding molecule associated with the red blood cell mem-
pyrene-labeled actin monomers to filaments nucleated, and
ments and is directly responsible for altering the rate of
provide compelling biochemical evidence to demonstrate
thin filaments (Fowler et al., 1993). In this new report,
capZ (CaseUa et al., 1987). Separation of this doublet upon
ments are located at the Z-discs, the main component being
tai proteins including actin, myosin II, tropomyosin (Mat-
pies. In addition, tropomodulin joins a long list of cytoskele-
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 rela-
tive 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., 1994b, this issue) demon-
strates 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 exam-
pies. In addition, tropomodulin joins a long list of cytoskele-
tal proteins including actin, myosin II, tropomyosin (Mat-
sunura et al., 1983; Fowler, 1987), α-actinin (Maruyama
and Ebashi, 1965; Burridge and Feramisco, 1981; Duhai-
man 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 fila-
ments resembling those present in erythrocytes exist in other
nonmuscle cells. One example might be provided by the dy-
nactin complex whose major component is an actin-related
protein that forms a short filament in association with cap-
ping 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 for-
motion, whereas drug studies have indicated that the fila-
ments involved in the contractile ring are dynamic. The com-
pletion 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 (Bretsch
er and Weber, 1980), and macrophage-capping protein (South-
wick and DiNuble, 1986) exhibit calcium-sensitive proper-
ties; moreover, the interaction of actin with some accessory
proteins including gelsolin (Janmey and Stossel, 1987), vil-
lin (Janmey and Matsudaira, 1988), gcanp (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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