Cell surface actin remodeling

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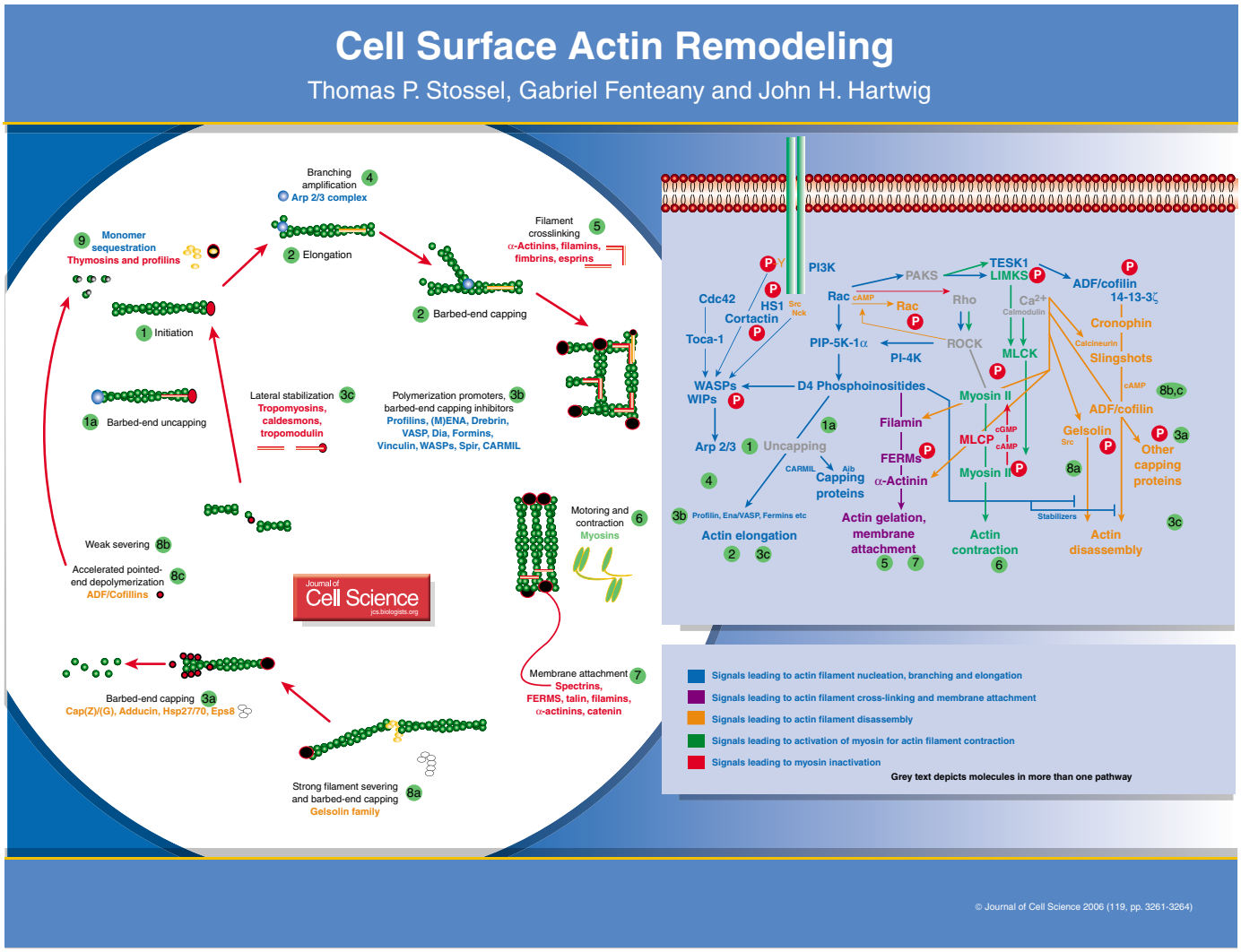
Actin filament remodeling at cell surfaces is a fundamental aspect of cellular life. Except for minor sequence variations, actin proteins are structurally identical. They have similar self-association properties and ATPase activities that accommodate their assembly into polarized semi-flexible filaments. The filament polarity defines the direction myosin motors move on them (from the pointed end to the barbed end). The barbed end is both more thermodynamically favored for new addition of actin monomer and more kinetically dynamic (fast exchanging) in the presence of ATP hydrolysis, which drives conformational changes in the exchanging subunits (Kuhn and Pollard, 2005). What makes actin truly interesting, however, is the variety of lengths and spatial conformations conferred upon it by hundreds of actin-binding proteins (ABPs). These control linear elongation, shortening and architectural organization of actin filaments in response to signaling cascades set in motion by environmental cues. The resulting exquisite variety of actin filament lengths and spatial configurations accounts for the diverse morphologies of eukaryotic cells and their highly specific changes in shape.

The survival and motility, albeit sometimes abnormal, of cells lacking ABPs that have powerful effects on actin in vitro imply that no single mechanism can explain surface actin remodeling for all cell types and occasions. This complexity is not surprising because the same or similar ABPs in highly motile amoeboid cells lacking permanent surface features can hardly be doing the same work that they do in a brush-border microvillus (which has a stable architecture) or within oocytes or yeast (which undergo relatively slow morphological changes). Indeed it is the details of actin remodeling that confer so much variety on cell behaviors.

The problem of cell surface (cortical) actin remodeling, however, is not hopelessly impenetrable. Four decades of research have revealed findings consistent with a nine-step cycle of



(See poster insert)

functions manifested by ABPs that can adequately explain many aspects of cortical actin remodeling, each step being responsive to signaling cascades. Here, we briefly summarize the key features of this cycle and their regulation. Notice that the outline of actin remodeling provided is based on information obtained from studies with different cell types but predominantly mammalian platelets, leukocytes, fibroblasts, epithelial cells, neuronal cells and tumor cells.

Initiation

Initiation (see poster, step 1) defines where and when actin filament elongation occurs at the cell surface. Cells have several strategies for initiating new actin polymerization, including de novo nucleation by the Arp2/3 complex, formins and Spir (Nicholson-Dykstra et al., 2005; Rafelski and Theriot, 2004). Polymerization of actin from newly exposed actin filament barbed ends is also a compelling mechanism for initiating new filament assembly. Free barbed ends elicit diffusion-limited polymerization of actin subunits bound to actin-monomer-sequestering proteins, the thymosins and profilins (step 9) (Yarmola and Bubb, 2004). Barbed-end exposure can result from uncapping – the removal of numerous barbed-end-capping proteins (gelsolin family, CapZ, Hsp70, ankyrins, Eps8) (step 1a) (Allen, 2003; Barkalow et al., 2003; Disanza et al., 2004) – or from the action of ABPs that sever actin filaments without capping them (step 7b) (DesMarais et al., 2005). One thing that makes these mechanisms attractive is the fact that half of the nearly millimolar actin in most eukaryotic non-muscle cells exists as short filaments, which provide ample barbed ends for rapid elongatation when uncapped and exposed to the large reservoir of sequestered actin monomers that cannot spontaneously nucleate. This mechanism is linked to signaling cascades that regulate ABPs to promote new polymerization from pre-existing barbed ends. Key participants in these are polyphosphoinositides, which remove all known capping proteins from barbed ends. Agonists that promote cortical actin assembly generally do so through activation of the small GTPases Rac, Rho and Cdc42 (Jaffe and Hall, 2005), that in turn stimulate enzymes leading to focal polyphosphoinositide accumulation and actin filament assembly and rearrangement (Niggli, 2005; Yin and Janmey, 2003).

Elongation

Actin filament barbed-end capping by factors that promote or inhibit capping (see below) regulates the extent of actin filament elongation (see poster, step 2).

Termination

Degradation of polyphosphoinositides, activation of the barbed-end-capping activity of CapG by Ca^{2+} and activation of Hsp70 or CapZ-interacting protein by phosphorylation (During et al., 2005; Eyers et al., 2005) terminate actin filament elongation (see poster, step 3a). By contrast, the capping protein inhibitors ENA, VASP, profilin, formins and CARMIL promote elongation even in the presence of active capping proteins (step 3b) (Barzik et al., 2005; Bubb et al., 2003; Higgs, 2005; Kovar, 2006; Yang et al., 2005). Actin-filament-stabilizing proteins such as tropomyosins, caldesmons, calponins, and tropomodulin (which also caps pointed ends in the presence of tropomyosin) (Fischer and Fowler, 2003) also promote elongation by retarding subunit depolymerization and inhibiting actin-depolymerizing ABPs, as described below (step 3c) (Bakin et al., 2004; Eyers et al., 2005; Mirzapoiazova et al., 2005).

Branching

Nucleation and transient 70° branching (see poster, step 4) of actin filaments mediated by the Arp2/3 complex (Pollard and Borisy, 2003; Stradal and Scita, 2006; Vicente-Manzanares et al., 2005) is essential for the intracellular movements of certain pathogens (*Listeria monocytogenes*, *Shigella*, *Salmonella* and *Rickettsia* species and poxviruses) (Gouin et al., 2005), some vesicles, and for the normal dynamics of adhesive podosomes and related structures (Linder and Kopp, 2005). A role for Arp2/3-mediated actin filament branching in leading-edge actin elongation is less certain, given high-resolution images preserving three-dimensionality (Medalia et al., 2002; Small et al., 2002), the absence of Arp2/3 from the leading edges of certain cells (Gupton et al., 2005; Strasser et al., 2004), the lack of an effect of Arp2/3 on actin filament network rigidity required for lamellar extension (Nakamura et al., 2002) and the results of experiments employing RNAi in fibroblasts (Di Nardo et al., 2005).

Actin filament crosslinking

Bivalent actin-filament-crosslinking proteins either abet or repel the inherent parallel alignment of actin filaments promoted by thermodynamic and ionic factors (see poster, step 5). Relatively small globular or rod-like ABPs, such as fimbrin, scruin, α-actinins and espins, stabilize actin bundles, whereas larger ABPs that have inherent spring-like properties, such as the filamins, instead promote high-angle (orthogonal) filament organization (Gardel et al., 2006; Gardel et al., 2004). α-Actinins, filamins and spectrins, a family of membrane-associated crosslinking proteins, also function as scaffolds for signaling intermediates that stimulate actin elongation; so they are well positioned to direct the orientation of elongating actin filaments (Broderick and Winder, 2005; Feng and Walsh, 2004).

Actin filament contraction, cargo motoring, and membrane binding

Parallel bundles and orthogonal networks represent extremes of the highly complex actin filament arrangements observable at the cell surface by electron microscopy and other high-resolution techniques. Actin filament configurations are susceptible to deformation by contractile forces generated by bipolar myosin filaments (predominantly myosin II) (Landsverk and Epstein, 2005), which act especially on actin networks attached to membranes. Unconventional myosins primarily move vesicles and other cargoes along actin filaments (‘motoring’) (see poster, step 6). Signals contributing to actin elongation, such as polyphosphoinositides, also increase binding of actin filaments and intermediary ABPs, including talin, vinculin, filamins, catenins, α-actinins, and zyxin to certain receptors, including integrins (Ginsberg et al., 2005), cadherins (Drees et al., 2005), and proteins of the FERM family (Cho and Stahelin, 2005) (step 7). This brings actin filament barbed ends close to the same signals that promote their elongation,

potentially amplifying the mass of elongating actin at the surface.

The linkage between actin filaments and membranes is important for mechanical traction against substrates and retraction of membranes for shape changes and locomotion in response to contractile forces. This linkage is also essential for localizing signaling factors to initiate the formation of cell-substratum and cell-cell adhesions, as well as other cellular processes.

Actin filament disassembly

The most efficient way to break down a network dominated by thread-like elements is to cut the threads. This approach disperses lattices of long actin filaments immobilized by interpenetration of filaments and shorter filaments cross-linked by ABPs. Two ABP families accomplish this task. The most efficient are proteins of the gelsolin family, which disrupt the interactions between actin subunits in filaments in response to Ca^{2+} or phosphorylation by Src kinase and then tightly cap the barbed ends of the severed filaments (Kumar et al., 2004) (see poster, step 8a). Ca^{2+} can also interfere with binding of crosslinking ABPs and thus destabilize actin networks. Ca^{2+} works with calmodulin to inhibit binding of filamin to actin (Nakamura et al., 2005) and directly inhibits the binding of some α-actinins (Broderick and Winder, 2005).

The second major actin-filament-severing ABPs are proteins of the actin-depolymerizing factor (ADF)/cofilin family, which weakly sever but do not cap the barbed ends of actin filaments (step 8b) (Fass et al., 2004). Barbed ends generated by cofilin either serve as initiation sites for new elongation or become capped (step 3a), depending upon the signals present. A cofilin-binding protein, Aip1, enhances cofilin activity (Okada et al., 2002), as do two families of phosphatases, the slingshots and cronophin. The adaptor protein 14-3-3ζ antagonizes this effect. Phosphorylation of cofilin by LIM kinase, downstream of Rac activation, inactivates cofilin (Huang et al., 2005; Nishita et al., 2005). Actin-filament-stabilizing proteins, particularly tropomyosins, also inhibit severing of actin filaments by cofilin but are less effective against gelsolin family members, and different tropomyosin isoforms generated by alternative mRNA splicing confer subtlety on this inhibition (Gunning et al., 2005). Polyphosphoinositides strongly inhibit actin filament severing by both protein families, which is consistent with their general propensity to promote actin filament assembly.

Although efficient at breaking down actin networks and providing more filament ends, actin filament severing does not directly contribute to maintenance of an actin monomer pool required for new filament assembly. However, the cofilin proteins, by accelerating subunit dissociation from pointed ends (‘nibbling’) (step 8c) are the major drivers for this (Carlier et al., 1999).

Monomer sequestration

Profilins and, in mammalian cells, thymosins bind to actin monomers (see poster, step 9), preventing them from spontaneous nucleation.

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