Actin-binding proteins

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Actin is an essential component of the cytoskeleton and plays a crucial role in eukaryotic cells. The actin cytoskeleton functions in the generation and maintenance of cell morphology and polarity, in endocytosis and intracellular trafficking, in contractility, motility and cell division. In cells, the assembly and disassembly of actin filaments, and also their organisation into functional higher-order networks, is regulated by a plethora of actin-binding proteins (ABPs) (dos Remedios et al., 2003; Maciver, 2004). The activities of these proteins are in turn under the control of specific signalling pathways.

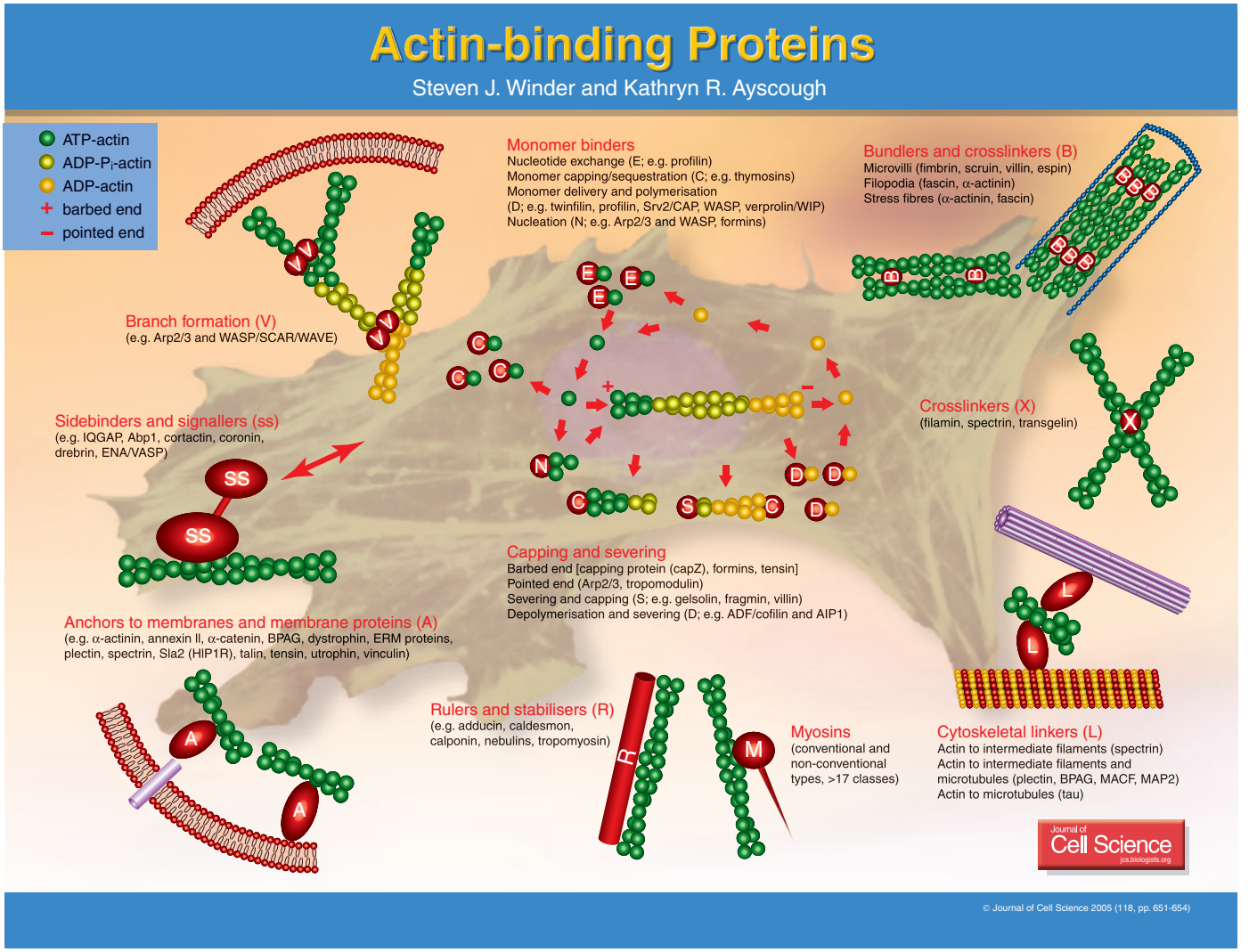
Here, we aim to highlight the main classes of ABP found in eukaryotic cells and indicate their likely mechanism of action as far as possible on the basis of both in vitro and in vivo studies.

Proteins regulating F-actin assembly and disassembly

Actin exists in two principal forms, globular, monomeric (G) actin, and filamentous polymeric (F) actin. Because of the arrowhead pattern observed when myosin decorates actin filaments, the fast-growing end of the polarised polymer is denoted the barbed end and the slow-growing end is donoted pointed end. Actin filaments extend when ATP-actin monomers are preferentially incorporated at the barbed end. As the filament matures, ATP bound in the central cleft of actin is hydrolysed, phosphate is released and the resulting ADP-actin filament is disassembled by loss of monomers from the pointed end. The released ADP-actin monomers then undergo nucleotide exchange to generate ATP-actin monomers that can be used for new rounds of polymerisation. This ATP-hydrolysis-driven, directional filament-growth is called actin treadmilling.

Nucleation

The first stage in de novo filament formation is nucleation. Polymerisation



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is energetically unfavourable until there is a nucleus of three associating monomers and in vitro this stage of filament formation is denoted the lag phase. In vivo, ABPs are crucial to ensure the rapid nucleation of filaments and these essentially function to remove the lag period. New filaments can form de novo, from the side of existing filaments, or by severing an existing filament. Different proteins function to promote each of these modes of nucleation.

The Arp2/3 complex can nucleate filaments from the side of existing filaments. This is important to allow the dendritic branching that is found at the leading edge of motile cells (Pollard and Borisy, 2003). Arp2 and Arp3 molecules themselves have a similar tertiary structure to actin itself, and when the Arp2/3 complex binds G-actin the effect is to generate a stable trimer and nucleus for the growth of a filament. Arp2/3 then acts as a pointed-end-capping protein that encourages rapid growth of the filament from its barbed end. Whereas the Arp2/3 complex can nucleate new filaments in vitro, it is likely that its activity in vivo is enhanced by functional interactions with other proteins, most importantly the WASP and SCAR/WAVE proteins. The G-actin-binding WASP homology2 (WH2) domain of WASP is important for the actin-nucleating activity of Arp2/3 and its role is probably to feed actin monomers to the filament barbed end. Verprolin/WIP binds both monomers and F-actin and has been shown to enhance Arp2/3-mediated actin polymerisation when bound to the Arp2/3 activator cortactin (reviewed in Paavilainen et al., 2004).

Actin polymerisation is also nucleated by the formin proteins. These proteins have only recently come under close scrutiny and there is still much to be understood about their mechanism of action in cells. However, studies indicate that the formin homology2 (FH2) domain dimer flexes to accommodate the processive addition of actin monomers to the barbed end of a filament (Xu et al., 2004). In yeast it is thought that the long actin cables that are required for cell polarity are generated by the nucleating action of the formins whereas the short branched networks in actin patches are generated by Arp2/3 nucleation.

Regulation of actin filament growth, stability and disassembly

Once nucleated, actin filaments are able to grow rapidly by addition of monomers at their barbed ends. Filaments are controlled by several mechanisms. Filament length is controlled by capping proteins. Barbed end cappers such as capping protein, gelsolin and tensin block addition of new monomers, so acting to decrease the overall length of the filament. In addition, gelsolin can sever actin filaments, thereby rapidly increasing actin dynamics. A mechanism for gelsolin severing has recently been proposed at the structural level (Burtnick et al., 2004). Depending on the environment in the cell this can have various outcomes, but it is usually a mechanism to disassemble F-actin-containing structures. Pointed end cappers, by contrast, reduce loss of monomer from the pointed end and thereby can lead to rapid filament extension.

The best characterised proteins that drive depolymerisation are the actin-depolymerizing factor (ADF) and the cofilin family members. This ubiquitous protein is highly conserved and plays a central role in actin turnover. It binds to ADP-F-actin and promotes dissociation of ADP-actin from the pointed end of the filament. ADF/cofilin also associates with AIP-1 (actin-interacting protein1). This interaction appears to increase the depolymerising activity of cofilin. The complex also promotes barbed end capping, although the details of this mechanism are not yet well understood (Paavilainen et al., 2004).

Another important and highly conserved family of ABPs are tropomyosins. These bind along the filament length and, like other proteins that bind along the side of actin filaments, stabilise the filament against spontaneous depolymerisation. Furthermore, tropomyosin has a protective effect against gelsolin severing and ADF/cofilin-mediated depolymerisation. In concert with troponins, tropomyosin also plays an important role in regulating the interaction of myosin with the actin filament in striated muscle. Other proteins that associate along the length of filaments act as rulers. Nebulin, for example, is a large elongated protein with numerous low-affinity actin-binding sites which act together to promote, stabilise and determine the length of actin-containing thin filaments in striated muscle. The length of the nebulin protein in different tissues and species corresponds with the length of the thin filaments in each half sarcomere; so nebulin is thought to have a molecular ruler function in determining filament length.

A numerous and diverse range of ABPs that we have denoted sidebinders and signallers are proteins that have been shown to bind specifically to F-actin. In addition, these proteins have domains that allow them to interact with other proteins and more specifically to function within signalling networks in cells to allow remodelling of the actin cytoskeleton at appropriate times and places within the cell. For example, several proteins that contain poly-proline motifs (e.g. VASP and vinculin) recruit components of the actin polymerisation machinery, such as profilin, whereas several SH3-domain-containing proteins are involved in membrane trafficking (e.g. Abp1) and association with adhesion complexes (e.g. cortactin).

Monomer binding proteins

The rapid growth of actin filaments that can be observed in motile cells and the reorganisation of actin that occurs in response to both intracellular and extracellular cues in other cell types requires the availability of actin monomers to be tightly regulated. This is achieved through a number of mechanisms by a group of highly conserved actin-monomer-binding proteins. Although there are a large number of monomer-binding proteins (>25 in mammalian cells alone), there are six major classes that are found in organisms from yeast to human and four of these classes are reported in plants.

As a group, the monomer-binding proteins are involved in binding ADP-actin as it is released from filament ends (e.g. twinfilin, ADF/cofilin), facilitating the nucleotide exchange of ADP for

ATP (e.g. profilin and CAP) and delivering the monomer to barbed ends (or to Arp2/3) to facilitate new rounds of polymerisation (e.g. twinfilin, Srv2/CAP, profilin, verprolin/WIP and WASP). As well as promoting polymerisation of existing filaments, some monomer-binding proteins are involved in nucleating the formation of filaments de novo and from the side of existing filaments (see nucleation above).

In motile cells it is imperative that there is a large pool of monomer that can be released to allow rapid filament extension. This can be achieved by monomer-sequestering proteins, the best studied of which is the thymosin family. These proteins act by clamping ATP-actin top to bottom, to effectively cap at both barbed and pointed ends and prevent incorporation into filaments (Hertzog et al., 2004; Irobi et al., 2004). Appropriate signals at the cell cortex can then trigger activation of profilin, leading to a rapid release of thymosin binding, and result in a massive increase in the amount of actin available for polymerisation.

Proteins regulating higher-order F-actin structures

The organisation of actin into networks and higher-order structures is crucial to both the form and function of cells. These structures can be responsible for the overall shape and order in a cell, for example, the terminal web and microvilli of brush border epithelial cells or the ordered architecture of striated muscle – as well as more transient or dynamic structures relating to a particular cellular function, such as the acrosomal processes of some invertebrate spermatozoa, and filopodia at the leading edge of locomoting cells. With the exception of dendritic branches formed by the action of the Arp2/3 complex mentioned above, all other higher-order F-actin structures are formed by two broad actin-binding activities; F-actin bundling and F-actin crosslinking.

Actin-bundling proteins

Actin bundling is the parallel or antiparallel alignment of F-actin into linear arrays and is generally achieved by proteins that either contain two discrete actin-binding domains within their sequence or by multimeric proteins that contain only a single binding domain per subunit. Actin bundles are also usually further subdivided into loose or tight bundles, the topography of which is more or less dependent on the architecture of the bundling protein. The presence of two actin-binding domains in close proximity, such as in the protein fimbrin, leads to the formation of tight actin bundles – as found in microvilli. The more loosely ordered structures of actin stress fibres, however, are organised by the dimeric and antiparallel protein α-actinin, which has a single actin-binding site per subunit. These are separated by a helical spacer region, placing the two actin-binding domains of the dimer some distance apart, which results in a looser association of actin filaments.

Actin crosslinking proteins

The arrangement of actin filaments into orthogonal arrays is also mediated by proteins or protein complexes containing multiple actin-binding domains, but in general the two domains are separated by longer, more flexible spacer regions, which allows a more perpendicular arrangement of actin filaments. Examples of this type of protein are the large flexible dimeric filamin or tetrameric spectrin complexes, but crosslinking is also achieved by small monomeric proteins, such as transgelin, which under certain conditions organises actin filaments into dense meshworks. Interestingly, the yeast homologue of transgelin, known as Scp1p, induces tight actin bundles rather than meshworks (Winder et al., 2003).

Actin as a mechanical framework

Rather than affecting actin dynamics or regulating actin structure per se, an enormous number of ABPs use actin as a scaffold, physical support or track. Whilst some of these proteins may indirectly alter actin dynamics and structure, this is not their primary role in the cell. We have classified these proteins into three main categories; myosins, anchors to membrane complexes, and linkers between actin and other cytoskeletal elements.

Myosins

Myosins are actin-dependent molecular motors that produce movement (and force) through the hydrolysis of ATP. As such, myosins simply use actin as a track along which to move. Most people are familiar with the two-headed myosin II involved in contractility and tension generation, but there are now over 17 different classes of myosins with various diverse functions (Hodge and Cope, 2000). Nonetheless, where tested, they all use actin as a track to move their specific cargo; whether it be membranes or vesicles, actin filaments or a host of other proteins – and mostly but not exclusively from the pointed to the barbed end of the filament.

Cytoskeletal linkers and membrane anchors

The utility of F-actin as a structural framework within cells necessitates its connection to other cellular elements. Most of these individual proteins have quite specific functions relating to their (sub)cellular and organismal context. But as alluded to above they can be subdivided into two broad groups: proteins that connect actin to membranes or membrane proteins and those that interconnect different cytoskeletal elements. In the former category are proteins such as dystrophin and utrophin or talin and vinculin, which connect the actin cytoskeleton to the cell adhesion receptors dystroglycan or integrin, respectively; and proteins that can bind directly to membranes and also interact with actin such as annexins. The latter category comprises a small but important group of proteins that can link actin to microtubules, actin to intermediate filaments or, in the case of plectin, actin to both microtubules and intermediate filaments. Clearly such proteins are of great importance to the cell in the integration of structure and signalling between the cytoskeletal elements and the maintenance of cell integrity.

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