COMMENTARY

Actions by actin: reciprocal regulation of cortactin activity by tyrosine kinases and F-actin

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The polymerization of actin is catalysed by the Arp (actin-related protein) 2/3 complex, which acts downstream of a variety of receptors and signalling cascades. Intermediary molecules such as cortactin bind to the Arp2/3 complex and stimulate its activity, thus promoting actin polymerization and actin filament stabilization. New data in this issue of the *Biochemical Journal* by the Kapus group suggest that cortactin is reciprocally regulated by filament-

ous (F) actin and tyrosine kinases. This suggests a new paradigm for considering the cellular processes that regulate the dynamic organization of the actin cytoskeleton.

Key words: actin cytoskeleton, actin polymerization, Arp2/3 complex, Fyn/Fer kinases, tyrosine phosphorylation.

For many years, the view of the actin cytoskeleton was that its role was primarily structural – that it provided the support needed to maintain the shape and organization of the cell, and the scaffolding for the actions of catalytic molecules such as motor proteins. The actin cytoskeleton is now widely recognized as an enormously dynamic structure that undergoes constant remodelling and reorganization in response to external stimuli. It is believed to play a critical role in virtually every cellular process and function. The central role of the actin cytoskeleton in cellular functions such as motility, contraction, changes in cell shape and rigidity, signal transduction, membrane trafficking, and cell division is well established. Central to the dynamic nature of actin is its ability to switch rapidly between a filamentous polymeric form (F-actin) and a monomeric globular (G-actin) form. This dynamism enables it to rapidly remodel its structure to respond to cellular stimuli, and to participate in localized responses to external stimuli or regional events within a cell. In recent years, there has been tremendous progress in identifying the signalling pathways and effector molecules by which actin cytoskeletal structures are regulated. In this issue of the *Biochemical Journal*, Fan et al. [1] provide important evidence for a new notion in cytoskeletal regulation: that the cytoskeleton modulates and fine-tunes its own organization by feeding back on the molecular processes and signalling pathways that regulate it.

Actin filaments exhibit a polarity in their structure: G-actin monomers bind to ATP and add to the ‘barbed’ end of an existing actin filament; the ATP is then hydrolysed rapidly after polymerization. This creates a polarity to the actin filament in which one end, the barbed end, contains ATP-bound monomers, and the other, the ‘pointed end’, is composed of ADP-bound monomers from which the phosphate has been released. New ATP-bound monomers preferentially add to the barbed end and ‘old’ monomers are released from the pointed end. This results in growth of the filament in the direction of the barbed end. Although this process can proceed spontaneously at a slow rate, actin filament assembly is markedly enhanced by the presence of a 7-protein complex known as the Arp (actin-related protein) 2/3 complex, which is ubiquitous in all cell types from yeast to humans [2]. Since its discovery in 1994, the Arp2/3 complex has been established as a central regulator of actin polymerization, and it is

now known to be activated downstream of various receptors for extracellular stimuli. The Arp2/3 complex stimulates actin polymerization in the presence of ATP–G-actin by mimicking the barbed end of the actin filament; it can provide a nucleus for initiation of new actin filaments, or it can bind to F-actin and catalyse the assembly of actin monomers on to existing actin filaments to form branches.

The actin-polymerizing activity of the Arp2/3 complex alone is weak; however, it is increased dramatically by the presence of one of a number of endogenous activators. The most well-characterized are the WASP (Wiskott–Aldrich syndrome protein) and Scar family proteins [3]. The type I myosin Myo1p, and most recently cortactin and Abp1p, have also been demonstrated to activate the Arp2/3 complex [4–6]. All of these activators interact with the Arp2/3 complex through a conserved sequence, an acidic ‘A’ domain at the N- or C-terminus of the molecule. All WASP/Scar family members require a monomeric G-actin-binding region to activate the Arp2/3 complex. In contrast, Abp1p and cortactin require an F-actin-binding region, rather than a G-actin-binding region, demonstrating that the binding of G-actin monomers is not a requirement for Arp2/3 complex activation [2].

Cortactin localizes primarily within peripheral cell structures, such as lamellipodia, pseudopodia and membrane ruffles, and is strongly associated with F-actin [7,8]. It has been implicated in cytoskeletal signalling mediated by multiple extracellular stimuli such as fibroblast growth factor, thrombin, integrins and mechanical strain. Cortactin is a prominent substrate for Src-related tyrosine kinases, including c-Src, the Fyn/Fer pathway, c-Met and Syk. By binding to actin filaments, cortactin may recruit the Arp2/3 complex and thereby promote the branching of F-actin, F-actin cross-linking and the formation of actin-filament networks. A study by Huang et al. [9] demonstrated that the *in vitro* tyrosine phosphorylation of cortactin results in a dramatic down-regulation of its F-actin cross-linking activity, suggesting that tyrosine phosphorylation may be a mechanism by which extracellular stimuli regulate the activity of cortactin towards the actin cytoskeleton. This observation is analogous to previous observations that the activity of other Arp2/3 complex activators, such as the WASP family proteins, can be regulated by tyrosine kinases [10].

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The report of Fan et al. [1] in this issue of the *Biochemical Journal* provides evidence for a novel mechanism by which the polymerization state of actin can modify the activity of molecules that regulate its own assembly. Fan et al. [1] present evidence that F-actin depolymerization induced by latrunculin B induces a marked tyrosine phosphorylation of the C-terminal residues of cortactin, and that this can be inhibited by jasplakinolide, a stabilizer of F-actin. The cortactin tyrosine phosphorylation induced by latrunculin B is mediated by the Fyn/Fer kinase pathway, and is independent of Src. Furthermore, by expressing truncated forms of cortactin, the authors demonstrate that the actin-binding repeat region of cortactin, but not its Arp2/3-binding region, is essential for the latrunculin-induced Fyn/Fer-mediated tyrosine phosphorylation of cortactin's C-terminal tyrosine residues. This suggests a mechanism by which the activity of cortactin can be reciprocally regulated by extracellular stimuli and the actin cytoskeleton.

The authors propose an intriguing hypothesis for the function of the reciprocal regulation of Fyn/Fer-mediated cortactin phosphorylation. They suggest that, under resting conditions, the N-terminus of cortactin may bind to an F-actin meshwork consisting of short actin filaments (not visible as stress fibres) and the Arp2/3 complex. This might be confined to localized regions within the cell where the activity of cortactin could serve to sustain the stability of the local actin architecture. During such processes as cell migration or growth, stimuli that induce the depolymerization of actin may liberate cortactin from the actin meshwork and stimulate its tyrosine phosphorylation, thereby inhibiting its ability to activate the Arp2/3 complex and promote actin bundling. During cell migration, the cortactin might subsequently be re-incorporated into the actin meshwork at a new location nearer the moving cell boundary, thus stabilizing the newly forming boundary of the cell. The reciprocal inactivation and activation of cortactin activity might therefore be essential for cell migration. Such a paradigm might also be extended to other physiological conditions in which cell shape or rigidity is modulated dynamically in response to external stimuli or events.

The reciprocal regulation of cortactin activity by F-actin may be part of a larger pattern of feedback by the cytoskeleton on cellular processes that regulate cytoskeletal organization. In one report, Woodring et al. [11] proposed a dynamic interplay between the tyrosine kinase c-Abl and F-actin. These investigators demonstrated that the catalytic activity of the c-Abl tyrosine kinase, which is regulated by cell adhesion, is reduced when F-actin is bound to its C-terminal actin-binding region, and that latrunculin A increases the activity of c-Abl *in vivo*. The cytoplasmic

c-Abl is involved in physiological processes that involve cytoskeletal reorganization, and there is evidence that it affects actin polymerization and F-actin bundling. Thus this suggests another instance in which the regulation of cytoskeletal organization may involve a dynamic exchange in which a signalling protein that regulates actin polymerization receives feedback from the actin cytoskeleton to modulate its activity.

Additional studies will be required to determine whether reciprocal signalling mechanisms such as those suggested by the observations of Fan et al. [1] and Woodring et al. [11] represent a blueprint for a more pervasive pattern for the regulation of cytoskeletal processes. However, a model of cytoskeletal regulation in which a stimulus initiates a cascade of signalling events culminating in a downstream cytoskeletal event such as actin polymerization is likely to represent an overly simplistic framework for understanding the cellular mechanisms that regulate the dynamic organization of the actin cytoskeleton.

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