Double JMY: making actin fast

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The assembly of actin networks is dependent on nucleation-promoting factors. A new study identifies JMY as a protein containing two separate nucleation-promoting activities that shuttles between the nucleus and the cytoplasm and promotes cell migration. These observations indicate that JMY is an important factor controlling actin dynamics in motile cells.

Actin filaments provide the structural basis for much of cell motility and are therefore critical to numerous physiological processes such as morphogenesis, wound healing and immune response. Abnormal cell migration also has a role in disease states such as autoimmune disorders and metastatic cancer. To understand these processes better, a comprehensive knowledge of the mechanisms for promoting, inhibiting and regulating actin dynamics is required. The first, rate-limiting, step in forming actin filaments is the *de novo* nucleation of actin filaments from actin monomers. This reaction is strongly kinetically disfavoured by the presence of proteins that sequester actin monomers within cells. Thus, protein cofactors that promote actin filament nucleation are required for the generation of actin networks at specific locations within the cell, such as at the distal lamellipodium.

One mechanism by which new filaments are nucleated is by creating branches off the sides of existing filaments by means of the Arp2/3 complex. This highly conserved seven-protein complex has intrinsically low activity and requires activating protein cofactors. The best-studied of these cofactors is the WASP family of nucleation-promoting factors (NPFs), which is regulated by Rho-family GTPases[1]. Originally there were only two known activators of the Arp2/3 complex (WASP and SCAR), but that number has grown recently to include several other proteins[2,3]. This increasing complexity of Arp2/3-complex activators suggests that cells use subcellular and context-specific activation of Arp2/3 for a more robust and precise regulation of branched actin networks.

A more recently discovered mechanism for generating new actin filaments is through a protein called Spire[4], which promotes filament nucleation by bringing monomers together with four tandem actin-monomer-binding WH2 domains. The four bound actin monomers are lined up end-to-end and mimic a short single strand of a nascent filament. Together, these monomers form the pointed end of a new filament to which free monomers then bind to grow the nascent filament. Spire-mediated nucleation does not result in branched actin filaments, and the mechanism may thus be used either to jump-start network formation or in circumstances in which a stiff branched network is not necessary.

Using protein homology searching, Zuchero *et al.*[5] have identified the p53 cofactor JMY as possessing a potential Arp2/3 regulatory sequence. JMY is known to bind to p300/CBP and cooperates with it to activate p53-dependent transcription[6], but no connection with the actin cytoskeleton had previously been suspected. Zuchero and colleagues purified JMY and demonstrated biochemically that it activates Arp2/3-induced actin polymerization in a dose-dependent fashion. Somewhat surprisingly, they also found that JMY was able to catalyse actin polymerization in the absence of Arp2/3. Further examination revealed that JMY, like Spire, was able to nucleate new filament formation through tandem WH2 domains. This is the first instance of these two biochemical activities being united in one protein. By both increasing the speed at which new filaments are formed and harnessing the amplification of polymerization that occurs after activation of Arp2/3, JMY seems to be capable of inducing very rapid assembly of new actin networks.

Zuchero and colleagues' examination of JMY in a cellular context reveals a primarily nuclear localization for JMY in most cell types, as would be expected for a p53 regulator. In primary human neutrophils, however, JMY co-localizes with actin filaments at the leading edge and is excluded from the nucleus. This localization pattern correlates with motility, because JMY moves from the nucleus to the cytoplasmic compartment when HL60 cells are differentiated from non-motile cells into highly motile neutrophil-like cells. Furthermore, overexpression and knockdown studies demonstrated that JMY expression promotes the rate of cell migration in wound-healing assays. These data are consistent with a role for JMY in controlling actin dynamics in highly motile cells (Fig. 1).

As with any newly discovered protein activity, questions quickly outstrip available answers. One interesting question is whether JMY has a role in regulating nuclear actin in addition to its known function as a transcriptional regulator. Although not well understood, nuclear actin has been linked to transcription, chromatin structure and nuclear transport[7]. The characteristics of nuclear actin networks, however, are fundamentally different from those used during cell motility, so it is unclear how JMY may function in this context, or what consequences any activity would have.

JMY's relationship with other nucleators also remains unclear. There may be cytoplasmic competition for actin monomers, and

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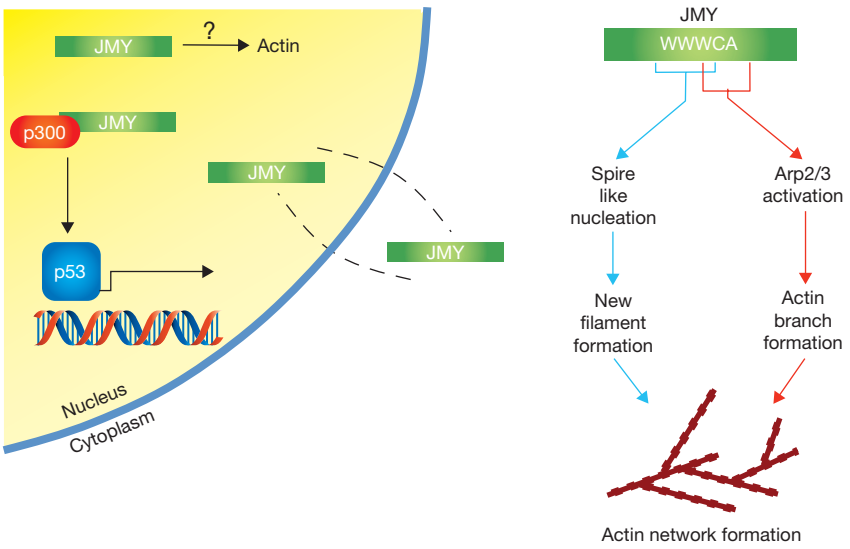

Figure 1 JMY functions both in and out of the nucleus. JMY functions in concert with p300 to activate p53-dependent transcription. In highly motile cells JMY is transported to the cytoplasm, where it promotes the formation of actin filament networks by means of two separate biochemical activities. It is able to nucleate new filament formation through a Spire-like mechanism that is dependent on its tandem actin-monomer-binding WH2 domains. In addition, JMY promotes actin branch formation by activating the Arp2/3 complex that is dependent on its three tandem actin-monomer-binding WH2 domains (WWW). In addition, JMY promotes actin branch formation by activating the Arp2/3 complex with at least one WH2 domain, a central domain (C) that binds actin and Arp2/3, and an Arp2/3-binding acidic domain (A). It remains undetermined whether JMY regulates nuclear actin dynamics.

migration of JMY into that compartment could diminish the supply of actin available for other nucleators such as WASP–Arp2/3 or the formins (another class of actin-nucleating factors). Such competition might complicate the analysis of JMY depletion phenotypes as a result of potential over-activation of other nucleators in the absence of JMY. An alternate possibility is that the nucleators act independently, and introduction of JMY can be used simply as a context-dependent catalyst. In this fashion, JMY may be part of a network of nucleators and NPFs that act in concert to fine-tune cytoskeletal dynamics.

Another unanswered question concerns the mechanism by which JMY activity is regulated. Given its potent biochemical activity, perhaps it is not surprising that the primary localization of JMY is in the nucleus. Rather than regulating JMY by phosphorylation or other post-translational modification, sequestration of the protein away from most actin could be an ideal way to keep its activity moderated until required. Both the method of transport from the nucleus and its potential triggers remain to be discovered. Similarly, the supply of JMY protein could be regulated by proteasome-mediated degradation. DNA damage causes an accumulation of JMY protein, whereas Mdm2-catalysed ubiquitylation targets JMY for proteasome-dependent degradation[8]. Cell motility-related cues could also tap into this mechanism and contribute to its availability to alter actin dynamics. It will be interesting to determine whether localization and protein degradation are indeed used to control JMY activity, and to see whether other mechanisms also contribute.

Another complex issue is reconciling the involvement of JMY in two very different cellular processes: transcription and the regulation of actin dynamics. It will be important to examine both pathways in the future when examining the function of JMY. Specifically, it will be necessary to test whether any given phenotype is attributable to one activity or a combination of both. Such dichotomy is not unprecedented, because the β-catenin protein is known to have important roles as a cytoskeletal linker mediating cell adhesion, as well as acting as a component of the Wnt signalling pathway that translocates to the nucleus after pathway activation[9]. Perhaps this system could offer insights into the best path to follow in understanding how JMY balances such discrete functions.

Although there are many unanswered questions about how JMY functions and is regulated, it is clear that Zuchero *et al.* have added another entry to the list of important actin regulatory proteins. Their work has therefore helped refine our understanding of actin dynamics and cell motility.

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