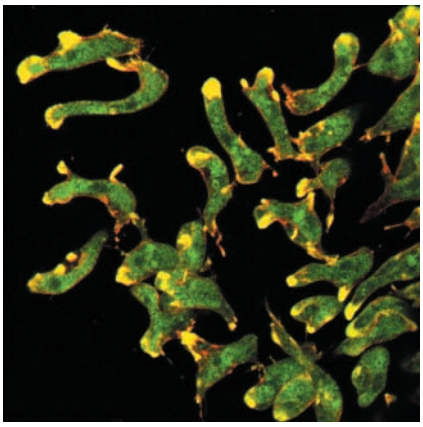
Moving the Nucleators

A moving cell needs to make new actin filaments at its ever-advancing leading edge. How does the cell's actin-polymerizing machinery keep up with this shifting demand? Jung et al. come up with a tantalizing clue in this issue (page 1479). They report that the *Dictyostelium* protein CARMIL (Capping protein, Arp2/3, Myosin I Linker) links certain type I myosins to the actin-nucleating Arp2/3 complex. Type I myosins are nonprocessive motors, but the high concentration of actin at the leading edge, or a higher-order complex containing multiple myosins, may allow these motors to transport Arp2/3 forwards. Additionally, CARMIL appears to be a novel activator of Arp2/3's actin-nucleating activity.

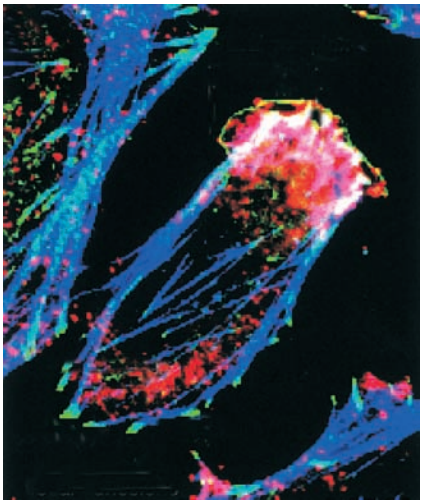


Jung et al. believe that CARMIL is the first cytoskeletal protein besides actin known to interact with capping protein. Given its high concentration in the cell, CARMIL could be important in sequestering capping protein from the growing ends of actin filaments. Alternatively, CARMIL may bind to capping protein on the end of actin filaments and use Arp2/3 to nucleate new actin filaments off those ends.

Cells lacking CARMIL show a reduction in endocytosis and motility. Similar genes exist in flies, worms, mice, and humans, suggesting that CARMIL may be important in a wide variety of actin-based events.

On the Move with Amyloid Precursor Protein

A series of papers has proposed varying, and sometimes conflicting, functions for Alzheimer amyloid precursor protein (APP). On page 1403, Sabo et al. show that APP and the associated protein FE65 cooperate to increase cell movement, as measured in a wound-healing assay.

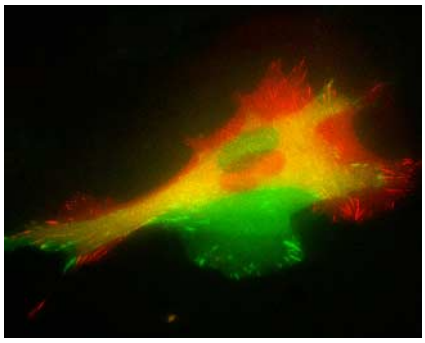


During the wounding assay, and in other motile cells, APP, FE65, and the adhesion-associated protein Mena all colocalize in lamellipodia, and the tripartite complex can be precipitated from cells. If cells are ripped up from a substrate, integrins and the tripartite complex are left behind on the surface, suggesting that these components are members of adhesion complexes.

The mechanism by which APP and FE65 increase movement is yet to be explained. Overproduction of FE65 has been shown to increase proteolysis of APP, and Sabo et al. suggest that the liberated fragments of APP could compete with integrin binding sites, thus freeing the cell for locomotion. FE65 binding to Mena, meanwhile, may release profilin, given that Mena's profilin- and FE65-binding sites overlap. This displacement of profilin should also favor movement. Sabo et al. are now testing neuronal precursor cells and nerve growth cones to see whether these are the physiologically relevant sites for APP and FE65 function.

Multicolored Movement

With so much going on during cell movement, Laukaitis et al. have resorted to tracking multiple fluorescent fusion proteins at once. On page 1427, they describe the results of their analysis. Paxillin, α-actinin, and α5-integrin are recruited to cell adhesions at the leading edge sequentially, thus building a complex that may first become competent to signal, then to act as structural scaffold, and finally to transduce force.



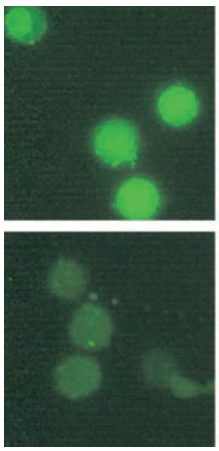
Small clusters of paxillin appear early during assembly of the complex. Formation of these clusters is dependent on integrin function, although integrins are present at levels that cannot be detected in the clusters. The paxillin clusters are subject to rapid turnover, with old clusters perhaps recycling their components to newer clusters at the leading edge. The arrival of the structural protein α-actinin stabilizes the paxillin clusters and initiates their centripetal movement. Later still, the majority of the α5-integrin joins the complexes, which then cease moving centripetally and become fixed relative to the substrate. Excess, uncomplexed integrin is probably incorporated into the integrin-containing vesicles that Laukaitis et al. detect moving from the leading edge to the perinuclear area.

At the back of the cell, integrins remain attached to the substrate and are therefore left behind the advancing cell. In contrast, paxillin and α-actinin clusters stay inside the cell

and are transported towards the cell body before finally dispersing.

Keeping Crm1 from the Pore

Lindsay et al. report (page 1391) that Ran-binding protein 3 (RanBP3) is an accessory factor that increases the efficiency of Crm1-mediated nuclear export. They suggest that RanBP3 works by preventing the binding of Crm1 to the nuclear pore complex (NPC) until Crm1 has been loaded with both substrate and Ran:GTP.



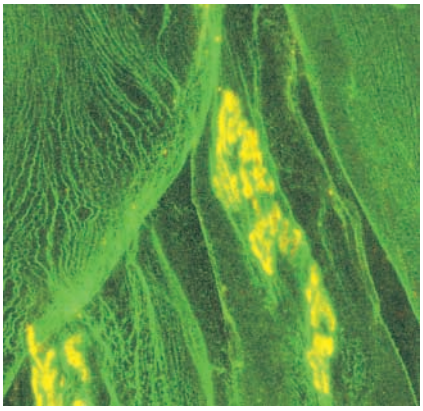
RanBP3 stabilizes the association of Crm1 and Ran:GTP, an association that is necessary for subsequent loading of substrates containing a nuclear export sequence. If this loading event is prevented with the drug leptomycin, binding to the NPC is prevented in the presence of RanBP3 but still occurs if RanBP3 is not present.

Lindsay et al. analyze the RanBP3 domains required for binding to Crm1 both in the absence and presence of Ran:GTP and export substrate. They conclude that the binding mode switches during export-complex formation. Initially, the nucleoporin-like F domain of RanBP3 is important. But after Ran:GTP and export substrate are added, binding to Crm1 is primarily indirect, via RanBP3's association with Ran:GTP. This may expose Crm1's nucleoporin-binding site (formerly associated with RanBP3's F domain) and initiate nuclear export.

Plastic Muscles

Bezakova and Lømo report (page 1453) that either muscle activity or addition of agrin protein (especially the muscle isoform) can reorient a large proportion of the muscle cytoskeleton. The reorientation may be part of the process by which active muscles sense that they have connected to their surrounding environment.

The cytoskeletal elements under study are costameres, which contain proteins such as dystrophin and the dystroglycans. Costameres link the actin cytoskeleton to the surrounding basal lamina, thus protecting muscles against mechanical stresses. In innervated muscles, costameres form as transverse stripes relative to the muscle fibers. But Bezakova and Lømo find



that the orientation of costameric proteins in denervated muscles is longitudinal, although the transverse orientation can be induced by electrical stimulation or application of agrin protein.

As Hagiwara and Fallon explain in a Comment article (page F39), the neural isoform of agrin induces the formation of neuromuscular junctions. Neural and muscle isoforms of agrin bind both α-dystroglycan and laminin, suggesting that agrin could be a bridge that strengthens the interaction between these two molecules. This interaction should be sufficient to generate the transverse orientation, as laminin remains transverse under all conditions. The longitudinal orientation of costameres in denervated muscle may be a result of some other underlying muscle structure.

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