Text by Ben Short
bshort@rockefeller.edu

Building up actin at adherens junctions

A biochemical approach reveals that α-actinin-4 and Arp2/3 team up to assemble actin at intercellular adhesions.

Adherens junctions—the cadherin-based adhesions that connect neighboring cells—are intimately associated with actin filaments and work with the cytoskeleton to polarize and shape epithelial tissues. Though the junctions contain numerous actin-binding proteins, how they recruit and/or assemble actin at intercellular contacts remains unclear (1). Tang and Brieher reconstitute junctional actin assembly in vitro to identify roles for both α-actinin-4 and the Arp2/3 complex in the process (2).

For many years, the actin-binding and cadherin-associated protein α-catenin was thought to provide a direct link between adherens junctions and the actin cytoskeleton. But this dogma was strongly challenged when James Nelson and colleagues found that α-catenin couldn't bind actin at the same time as it bound to another junctional protein, β-catenin (3, 4).

Since then, researchers have proposed various other ways for adherens junctions to bind actin fibers. Vivian Tang and William Brieher, from the University of Illinois at Urbana-Champaign, approached this topic from a different angle by investigating whether junctional components promote the assembly of new actin filaments at intercellular adhesions. Tang and Brieher depolymerized actin in MDCK kidney epithelial cells using the drug latrunculin and, after washing out the drug, watched to see where actin filaments reassembled (2). "Cadherin junctions at the apical surface marked the major sites of actin assembly," Brieher recalls. Assembly was blocked by inhibitors of the Arp2/3 actin-nucleation complex, but it was unclear whether actin polymerized at the adhesions themselves or whether filaments were assembled nearby before being recruited to the junctions.

Tang and Brieher took an in vitro approach to answer this question, adding actin to purified cell membranes in the hope of reconstituting actin assembly. Sure enough, actin monomers assembled into filaments that accumulated in foci on cadherin-enriched membranes, and this process was blocked by Arp2/3 inhibitors. Pre-formed actin filaments, on the other hand, didn't aggregate on the purified membranes, indicating that adherens junctions nucleate, rather than capture, actin fibers. "These experiments were relatively easy to do in vitro but would be difficult to do inside the cell," Brieher explains. "We then wanted to push the system and use it to search for unknowns."

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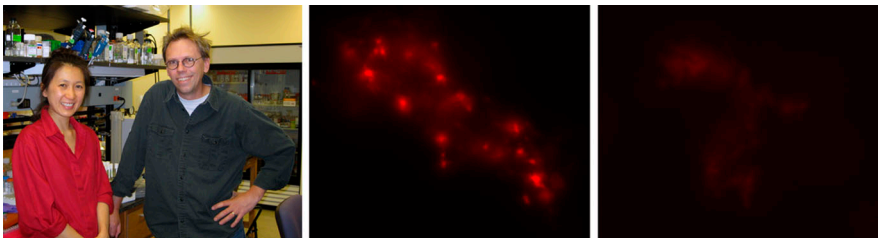
The researchers found that pretreating purified membranes with KCl stripped away a factor required for actin assembly. Tang and Brieher identified this factor as the actin-bundling protein α-actinin-4; adding back this protein—or any other member of the α-actinin family—restored the ability of stripped cell membranes to support actin accumulation. Knocking down α-actinin-4 reduced the amount of apical actin in MDCK cells and reduced the cells' ability to reassemble actin at adherens junctions after latrunculin washout.

Mutations in α-actinin-4 are associated with the human kidney disease focal segmental glomerulosclerosis (FSGS) (5). These mutations increase α-actinin-4's affinity for actin, but how this results in disease is unknown. Tang and Brieher investigated one such mutant—K255E—and found that, although it still bundled actin filaments and localized to cell adhesions, it couldn't support actin assembly on cadherin-enriched membranes in vitro. Indeed, the K255E mutant acted as a dominant negative, inhibiting wild-type α-actinin's ability to promote actin accumulation in vitro and at adherens junctions in MDCK cells. If α-actinin-4 K255E also blocks actin assembly at intercellular adhesions in patient kidneys, it may explain why this mutation is associated with an autosomal dominant form of FSGS.

How does α-actinin-4 K255E suppress actin accumulation when it still binds and bundles actin efficiently? And how does wild-type α-actinin work with Arp2/3 to promote junctional actin assembly? These are questions that Tang and Brieher now plan to investigate using their biochemical reconstitution approach. "The in vitro system got us this far," says Brieher. "We'll stay with it and see where it takes us next."

Focal Point



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Vivian Tang (on left) and William Brieher (on right) reconstitute actin filament assembly at adherens junctions in vitro and reveal that this process—on purified membranes and in cells—is dependent on the Arp2/3 actin-nucleation complex and the filament-bundling protein α-actinin-4. Isolated cadherin-enriched membranes assemble actin foci (red) in the presence of wild-type α-actinin-4 (center) but not in the presence of a mutant version of the protein associated with the kidney disease focal segmental glomerulosclerosis (far right).

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