So far, yet so close: α-Catenin dimers help migrating cells get together

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Epithelial cells in tissues use their actin cytoskeletons to stick together, whereas unattached cells make active plasma membrane protrusions to migrate. In this issue, Wood et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201612006) show that the junction component α-catenin is critical in freely moving cells to promote adhesion and migration.

Actin-binding proteins regulate the assembly, organization, and contractility of actin filaments. Distinct sets of actin-binding proteins are found at cell–cell adhesions or cell–extracellular matrix attachments, with unique functions in organizing actin to scaffold, generate force, or signal. However, when cells change between free moving and tethered in a polarized epithelium, they need to repurpose their actin cytoskeletons. Thus, many actin-binding proteins change location and function. In truth, most actin-binding proteins have multiple functions, which may be in the insoluble cytoskeleton, cytoplasm, or nucleus. This helps the cell to coordinate environmental cues with the activities or functions of each protein. It also raises interesting questions about the mechanisms by which cells regulate the same proteins in different locations to perform specialized activities, depending on the context.

The F-actin binding and bundling protein α-catenin is a prime example of a multifunctional protein with varied intracellular localization. It was originally identified as the indirect link between the E-cadherin receptor cytoplasmic tail and the actin cytoskeleton. The α-catenin pool associated with E-cadherin complexes interacts with the cytoskeleton under force (Benjamin et al., 2010), which facilitates α-catenin binding to other F-actin binding proteins to support cell–cell adhesion. However, α-catenin also localizes in the cytoplasm and in the nucleus. In the nucleus, α-catenin modulates transcriptional activity (Klezovitch and Vasioukhin, 2015), thereby controlling cell proliferation. In the cytoplasm, α-catenin may regulate vesicular trafficking via its interaction with dynamitin, a microtubule-binding protein (Lien et al., 2008). These functions of α-catenin in the cytosol are thought to be adhesion independent: the cell maintains different pools of α-catenin at distinct intracellular sites to be used in various processes. In this issue, Wood et al. shed light on a hidden facet of α-catenin: its localization at membrane protrusions and local modification of actin structures underlying migration.

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By forcibly inducing the dimerization of α-catenin, the researchers found that it preferentially localizes at the plasma membrane, away from cadherin adhesive complexes at junctions. That α-catenin can dimerize is already known (Drees et al., 2005). However, how α-catenin dimers are formed, their precise function, or localization is uncharted territory. Using surface plasmon resonance, Wood et al. (2017) demonstrate a selective affinity of α-catenin homodimers for vesicles loaded with phospholipids, in particular phosphatidylinositol-3,4,5-trisphosphate (PIP_3), whereas α-catenin monomers had no detectable binding (Fig. 1).

α-Catenin mutants unable to interact with PIP_3 maintained their ability to form homodimers, suggesting that phosphoinositide binding is not essential for interaction with membranes. However, PIP_3 binding may also facilitate α-catenin homodimer formation via an increase in the local effective concentration of α-catenin at PIP_3-rich sites on the plasma membrane. The authors speculate that, alternatively, phosphoinositide binding may induce a conformational change that favors dimerization and restricts free diffusion (Wood et al., 2017). This interpretation is consistent with the reduced ability of monomers to bind PIP_3 and the fact that the dissociation constant of α-catenin dimers is higher than the concentration of cytosolic α-catenin (Drees et al., 2005). Alternatively, it is feasible that a lipid-binding pocket in the dimeric structure forms through contributions of residues from the interface between the two α-catenin monomers. Although the authors could not confirm the latter because of the resolution of the α-catenin dimer crystal structures, a shared pocket between monomers has been previously shown to mediate phosphatidylinositol-4,5-bisphosphate (PIP_2) binding in the close homologue vinculin (Chinthalapudi et al., 2014).

Indeed, phospholipid association with cytoskeletal proteins is a known mechanism to modulate their function. For example, capping protein is thought to competitively bind to either the fast-growing end of actin filaments or PIP_2 in the plasma membrane (Edwards et al., 2014). This regulation allows cells to rapidly trigger a burst of polymerization upon phosphoinositide production, such as during platelet activation. Conversely, it also allows cells to rapidly clear actin off the plasma membrane after events such as phagocytic engulfment, when the actin coat is no longer needed and might interfere with trafficking.

The findings by Wood et al. (2017) support the proposed contribution of phospholipids to organize and regulate

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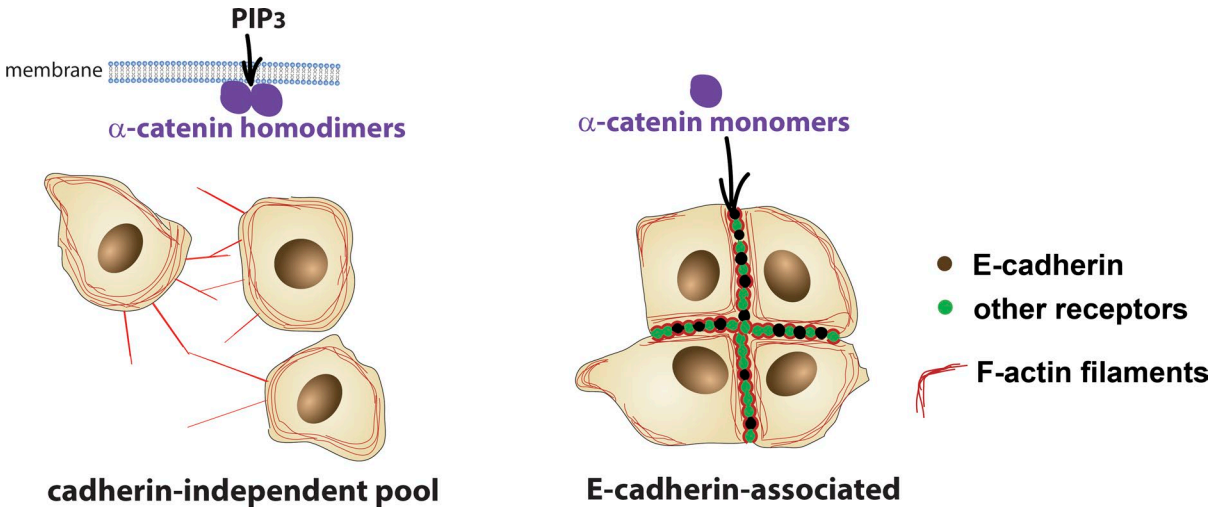

Figure 1. **The dimeric form of α-catenin interacts preferentially with PIP_3 at the membrane of single cells and promotes the formation of long, extended filopodia that may establish attachment with neighboring cells.** Monomeric α-catenin is the form that interacts with β-catenin in cadherin complexes at adhesion sites. Forcible dimerization of α-catenin mutants that do not bind PIP_3 prevents filopodia formation and promotes faster wound closure of epithelial sheets. Strikingly, expression of these PIP_3-binding defective mutants also interferes with the stability of cadherin-based adhesions.

cell architecture (Shewan et al., 2011). The results also add a new twist, by demonstrating the profound influence of PIP_3-dependent localization of the α-catenin dimer on its cytoskeletal functions. Previous work has shown that α-catenin dimers can attenuate the barbed-end polymerization and actin-branching activity of Arp2/3 complexes in vitro (Drees et al., 2005) and protect filaments from severing (Hansen et al., 2013), favoring unbranched actin bundles. By forcibly inducing α-catenin dimerization, Wood et al. (2017) observed that dimers localize at nonjunctional membranes and strongly induce filopodia that are both longer and more abundant than in controls. These filopodia are thought to be key structures that initiate cell–cell contacts when epithelial cells approach each other. Thus, the plasma membrane recruitment via PIP_3 and dimer formation of α-catenin may occur when cells are free of cell–cell contacts as a way of promoting cell–cell attachment and catalyzing epithelial tissue formation. This dual function for α-catenin thus nicely fits with a role in both promoting and stabilizing cell–cell adhesion.

α-Catenin mutated to disable its interaction with PIP_3 incorporated into E-cadherin complexes and cosedimented with F-actin in vitro similar to wild type. Intriguingly, forcibly dimerizing this mutant weakened the mechanical strength of cell–cell junctions. Perhaps the filopodia that precede cell–cell junction formation, previously described as a zippering mechanism (Vasioukhin et al., 2000), are important for correct assembly of junctions and stability. In polarized epithelial cells, it is possible that PIP_3 localization at the basolateral domain (Shewan et al., 2011) potentially provides docking sites for α-catenin dimers at the cell–cell interface, independently of cadherin complexes. One can speculate that such events could modulate the properties of the actin cytoskeleton at junctions in a way to facilitate more stable adhesions (i.e., promoting linear bundles of F-actin).

In addition to the formation of extended filopodia displayed by α-catenin dimers, it is possible that α-catenin dimerization may impact the recruitment of signaling regulators to cell–cell contacts. Small GTPase regulators such as the exchange factor ECT2 or the GTPase-activating proteins (GAPs) MgcRacGAP and DLC1 require α-catenin for their localization at junctions (Braga, 2017). However, whether their recruitment or retention could be modulated by α-catenin homodimers has not yet been established. Alternatively, as most GTPase regulators contain a pleckstrin homology domain, it is also conceivable that an interaction with lipids at the plasma membrane plays a role in their localization, and α-catenin interaction could modulate their activity locally. Such regulation has been shown for the junction-resident proteins cingulin and Ajuba to inactivate the exchange factor GEF-H1 and the GAP CdGAP, respectively (Braga, 2017).

Overall, the newly described dimerization and switch in function of α-catenin between the plasma membrane and cell–cell contacts suggest an important role in the establishment of new cell–cell contacts as well as maintenance of existing ones, which could contribute to tissue development and remodeling. It makes sense that actin-binding proteins would have different localizations and functions in cells when they are either freely migrating before tissue establishment or coming together to form multicellular tissues. It will be interesting to test whether α-catenin dimers are also formed when tissues are injured and participate in filopodia formation in the context of wound healing and repair.

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