

SPOTLIGHT

Actomyosin fibers DAPLE epithelial apical junctions

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Epithelial cell morphology is essential for cellular homeostasis, but the mechanisms by which cell shape is established remain unclear. In this study, Marivin et al. (2022. *J. Cell Biol.* <https://doi.org/10.1083/jcb.202111002>) identify DAPLE as a linker between polarity complexes and the actomyosin network at apical junctions. By recruiting CD2P and activating Gαβγ-mediated RhoA signaling, DAPLE ensures proper cell shape and function.

Epithelial cells, the most common cell type in multicellular organisms, have a polarized organization with apical, lateral, and basal membrane domains. These domains compartmentalize the cellular functions and specify the interactions with other tissue cells and are thus crucial for normal cell physiology. Therefore, changes in cell shape can alter organ-specific function and homeostasis and are associated to aberrant processes, such as cancer (1). The apical and lateral borders of epithelial cells are defined by cellular junctions, including tight junctions and adherens junctions, which interact with the actin cytoskeleton. Cellular junctions are critical in defining the apicobasal epithelial polarity. In addition, there are different protein complexes involved in cell polarization, such as the partitioning defective protein (PAR) polarity complex, which comprises PAR3, aPKC, and PAR6 proteins and is located in apical junctions (2). One of the most characterized mechanisms by which cells acquire their shape is through actomyosin contraction. The actomyosin cytoskeleton arises from the assembly of actin fibers (F-actin) and non-muscular myosin II (NMIIB). Actomyosin contractility is regulated by the activity of GTPases such as RhoA, which drives contraction pulses that modulate junctional length thereby shaping the final cell pattern (3). However, how apical polarity complexes control the actomyosin cytoskeleton to

determine cell shape is not fully understood. In this study, Marivin et al. identify the protein DAPLE as the link between the actomyosin cytoskeleton and the apical-lateral cell junctions and thoroughly dissect the function of DAPLE in the acquisition of cell shape (4).

The authors first asked whether DAPLE is required to maintain the form and function of intercellular junctions. To answer this, they used DAPLE-depleted Madin-Darby Canine Kidney (MDCK) cells and measured their capacity to create an adequate epithelial barrier. They evaluated the transepithelial electrical resistance, which measures the ohmic resistance across the epithelial monolayer, in basal conditions and in cells treated with calyculin A, a protein phosphatase that increases tension at cell-cell junctions by blocking myosin phosphatases thereby heightening the forces generated by myosin II. They found that, without DAPLE, MDCKs lose not only their shape but also their barrier function.

Then, they went on to unravel the mechanism by which DAPLE safeguards epithelial cell shape and function. To identify which domain of DAPLE allows the association with apical junctions, they created C-terminal mutants of DAPLE and assessed cell junction morphology. Their results revealed that the C-terminal PDZ motif (PBM) allows DAPLE to establish the cell morphology on apical junctions (Fig. 1). Given

that the PAR complex is essential for epithelial cell polarization (2), the authors assessed the association of DAPLE with this polarity complex using co-immunoprecipitation (IP) experiments. While PAR3 was necessary for DAPLE recruitment to apical junctions, the other components of this complex (PAR6 and aPKC) were dispensable.

Once they had established the relationship of DAPLE with apical junctions, Marivin et al. moved to elucidate how DAPLE controls the apical actomyosin network. Previous work in the field has shown that RhoA regulates the formation of actin fibers and actomyosin contractility at the cellular junctions. Using a GTP-bound reporter to detect active RhoA in the presence or absence of DAPLE, the authors showed that DAPLE regulates the actomyosin contractility by increasing RhoA activity. Indeed, DAPLE-depleted MDCK cells showed reduced assembly of apical F-actin fibers and impaired recruitment and activation of myosin II. Next, they asked how junctional DAPLE drives actomyosin activation. The authors noticed that the loss of F-actin cables in cells lacking DAPLE resembled that of cells without CD2AP. CD2AP is a protein localized at the apical junctions that stabilizes F-actin fibers. CD2AP loss compromises actin stability and decreases actin accumulation at adherens junctions (5). They found that DAPLE also mediates the recruitment of CD2AP to apical junctions,

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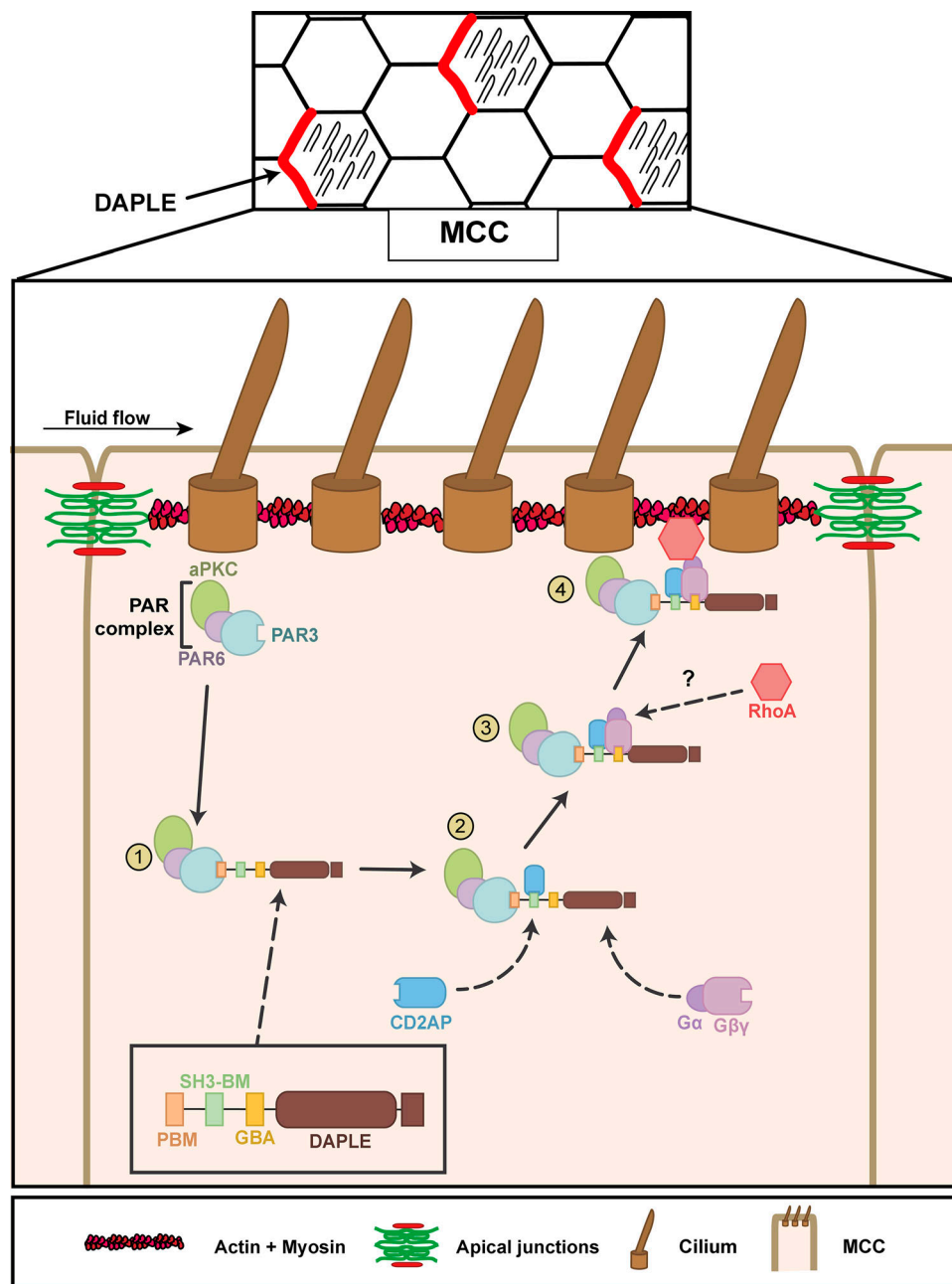


Figure 1. **Schematic model representing DAPLE interactions with actomyosin network in apical junctions at MCC cells.** DAPLE is asymmetrically located across the horizontal plane in the MCCs. At the bottom, DAPLE is depicted with its three domains: GBA, SH3-BM, and PBM highlighted. Discontinuous arrows represent the translocation of specific proteins towards DAPLE, and continuous arrows and numbers represent subsequent interactions. (1) The PAR polarity complex in the apical membrane interacts with DAPLE through its SH3-BM domain; (2) CD2AP and the heterotrimeric protein Gαβγ binds to the SH3-BM and GBA motif of DAPLE, respectively; (3) RhoA is recruited, but it remains unclear whether RhoA interacts directly with Gαβγ or with p114RhoGEF, as previously proposed; and (4) RhoA activation drives apical actomyosin assembly to allow cilia positioning and function.

where both proteins, CD2AP and DAPLE, reside at sites of actin nucleation. Finally, by the means of protein-domain co-IP experiments, the authors reported that the interaction of DAPLE with CD2AP is mediated by the SH3 binding motif (SH3-BM) of DAPLE and the SH3-2 domain of CD2AP.

Considering prior studies of the Garcia-Marcos lab and their current work, DAPLE

appears to function as a scaffold that requires its PBM domain to bind apical Par3 and to favor the localization of different actors at the apical junctions to stabilize the actomyosin network. On one hand, DAPLE, through its SH3-BM, recruits CD2AP, thereby stabilizing actin. On the other hand, DAPLE GBA (Gα binding and activating) motif binds activated heterotrimeric G

proteins, inducing myosin-dependent apical constriction in epithelial cells. Thus, they evaluated how the different DAPLE domains contribute to the acquisition of proper epithelial cell shape. They observed that DAPLE requires both its SH3-BM and GBA interaction domains to generate normal junction linearity and apical cell area. However, while the distribution of junctional F-actin

requires all the DAPLE interaction domains, RhoA location does not depend on the interaction of DAPLE via its SH3BM domain with CD2AP. Indeed, whereas SH3-BM and GBA motif are both required for F-actin assembly, only the GBA motif is critical for RhoA and myosin II regulation.

Finally, to assess the role of DAPLE in a more physiological model, Marivin et al. had a look at epidermal multiciliated cells (MCCs) in *Xenopus* embryos. MCCs present a robust F-actin network to ensure proper cilia orientation in order to drive fluid flow across the epithelium. MCCs are essential for toxin clearance and mucociliary action, among other essential functions, in *Xenopus* epidermis (6). The authors observed that, in DAPLE morphants, the number, length, and organization of cilia were significantly affected, which could compromise epithelial function.

Although this work establishes that DAPLE links the apical PAR polarity complex with the actomyosin networks, the molecular mechanisms by which DAPLE activates RhoA at cellular junctions remains unresolved here. Previous work from the authors established a link between the p114RhoGEF protein and DAPLE. DAPLE, an atypical, non-GPCR activator of heterotrimeric G proteins that localizes at apical cell junctions, triggers G $\beta\gamma$ -dependent activation of p114RhoGEF to promote apical cell constriction in the neural tube of *Xenopus* (7). This was further corroborated in *Drosophila* but with a classical GPCR activation (8). However, the activation of p114RhoGEF

turns out to be far more complex. p114RhoGEF associates with junctions through the adaptor cingulin, which drives RhoA signaling at the junctional complex and regulates tight junction assembly and epithelial morphogenesis (9). Furthermore, Myosin VI promotes recruitment of the heterotrimeric G α 12 protein to E-cadherin, where it signals p114RhoGEF to activate RhoA (10). Thus, additional experiments are needed to understand how p114GEF is activated at cellular junctions and under what conditions which of these mechanisms is required for its activation.

Lastly, the current work confirms previous observations reporting DAPLE colocalization with Disheveled, a protein involved in the planar cell polarity (PCP) machinery in MCCs. PCP, a polarity pathway in which cells are organized across the plane of a tissue perpendicular to the apicobasal axis, is essential for the function of specific structures such as cilia (11). As shown by Marivin et al., DAPLE localizes with planar polarization in MCCs; thus, DAPLE could control not only cilia formation and organization but, as it would be expected, the contractility of the cellular junctions in an asymmetric way. If this is confirmed, the link between PAR3-driven apicobasal polarity and PCP, which has been suggested in the literature, would be firmly established.

Overall, the work by Marivin et al. substantially contributes to understanding the mechanisms that determine epithelial cell shape and reveals one of the

missing links between the polarity protein complexes and the actomyosin network, establishing DAPLE as an essential connector of apical polarity and junction contractility.

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References

- Jung, H.Y., et al. 2019. *Nat. Cell Biol.* <https://doi.org/10.1038/s41556-019-0291-8>
- Chen, J., and M. Zhang. 2013. *Exp. Cell Res.* <https://doi.org/10.1016/j.yexcr.2013.03.021>
- Coravos, J.S., et al. 2017. *Trends Cell Biol.* <https://doi.org/10.1016/j.tcb.2016.11.008>
- Marivin, A., et al. 2022. *J. Cell Biol.* <https://doi.org/10.1083/jcb.202111002>
- Tang, V.W., and W.M. Brieher. 2013. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201304143>
- Brooks, E.R., and J.B. Wallingford. 2014. *Curr. Biol.* <https://doi.org/10.1016/j.cub.2014.08.047>
- Marivin, A., et al. 2019. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201811174>
- Garcia De Las Bayonas, A., et al. 2019. *Curr. Biol.* <https://doi.org/10.1016/j.cub.2019.08.017>
- Terry, S.J., et al. 2011. *Nat. Cell Biol.* <https://doi.org/10.1038/ncb2156>
- Acharya, B.R., et al. 2017. *Cell Rep.* <https://doi.org/10.1016/j.celrep.2017.02.078>
- Bernascone, I., et al. 2017. *Cold Spring Harb. Perspect. Biol.* <https://doi.org/10.1101/cshperspect.a027946>