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SPOTLIGHT

PAK4: A vanguard of epithelial vertex remodeling

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How epithelial tissues remodel while preserving their barrier remains a fundamental question in epithelial biology. In this issue, Adhikary et al. (https://doi.org/10.1083/jcb.202510024) identify PAK4 as a dynamic regulator of vertex remodeling that fine-tunes actomyosin contractility to maintain tissue integrity.

Epithelial tissues form the protective lining of our organs. Although they appear stable, they are remarkably dynamic. Throughout life, epithelial cells divide, exchange neighbors, integrate newly born cells, and eliminate damaged or unfit cells. Despite this continuous remodeling, the tissue must remain mechanically intact and function as a selective barrier that allows passage of water, ions, and solutes while blocking harmful substances. How epithelia achieve this balance between dynamic remodeling and uninterrupted barrier function is a central question in epithelial biology.

This remarkable adaptability of epithelial tissues relies on an intricate network of cell–cell junctions that physically link neighboring cells into a continuous sheet (1) (Fig. 1). These junctions are organized along the apicobasal axis, with specialized roles (2) (Fig. 1). Basally, hemidesmosomes anchor cells to the basement membrane. Moving toward the apical surface, desmosomes provide mechanical strength, gap junctions enable communication between neighboring cells, adherens junctions connect the actomyosin cytoskeleton across cells, and finally, tight junctions seal the space between adjacent cells to establish the epithelial barrier (Fig. 1). While this organization is repeated at every cell–cell interface, the geometry of an epithelial sheet inevitably creates points where three or more cells meet (Fig. 1). These multicellular junctions on the apical side called the vertices are more than simple meeting points (3). They are topological hubs where multiple junctional interfaces converge, making them critical for both tissue organization and barrier integrity (4).

Unlike the relatively stable junctions between two neighboring cells, vertices are among the most dynamic regions of an epithelial tissue (1, 5). Every time a cell divides, intercalates, or extrudes, the architecture of these multicellular junctions must be reorganized (1, 6): a stable tricellular vertex can transiently transform into a four-way vertex or higher order rosette before resolving back into a stable configuration (7) (Fig. 1). These rearrangements underlie processes such as T1 transitions, where neighboring cells exchange contacts, and T2 transitions, driven by cell extrusion (8). Such transitions rely on actomyosin-generated contractile forces, while junctional proteins enriched at vertices like Angulin and MARVEL-family proteins preserve tight junction continuity as vertices are dismantled and rebuilt (3, 9). Yet, an important question remains: how is this contractile machinery precisely controlled so that vertices stay flexible enough to remodel, yet robust enough to preserve epithelial integrity?

One protein that has attracted attention in this context is PAK4 (p21-activated kinase 4), a serine/threonine kinase downstream of the small GTPase Cdc42 (10). PAK4 was known to localize to cell–cell junctions, with particularly strong enrichment at multicellular vertices, and to interact with the actin-associated scaffolding protein Afadin at adherens junctions (11). These observations place PAK4 at the crossroads of junctional organization and cytoskeletal regulation. Adhikary et al. (12) set out to test this idea using complementary approaches in cultured MDCK cells, *Xenopus* embryonic epithelia, and mouse intestinal tissue. Their work reveals that PAK4 fine-tunes actomyosin contractility during vertex remodeling, allowing epithelial tissues to remain both dynamic and mechanically robust while preserving barrier function.

The study begins by asking a simple question: is PAK4 truly a vertex-associated protein? Rather than being uniformly distributed along cell–cell junctions, PAK4 is strongly enriched at tricellular and higher order vertices. Live imaging reveals that PAK4 is recruited only while a vertex is actively remodeling and disappears once remodeling is complete, suggesting that it actively participates in this process rather than serving as a static structural component. Consistent with this idea, loss of PAK4 disrupts epithelial organization, leading to persistent higher order vertices, unresolved rosettes, and defective postcytokinetic junction remodeling. Restoring wild-type PAK4 rescues these defects, establishing PAK4 as a dynamic regulator that drives remodeling vertices back to stable tricellular junctions.

The authors next asked what recruits PAK4 to these dynamic junctions? They identified Afadin as the candidate. Like PAK4, Afadin is enriched at active vertices, and removing Afadin produces remarkably similar defects while simultaneously abolishing junctional PAK4 localization. Yet, Afadin itself remained junctional, suggesting that its primary role is to recruit PAK4

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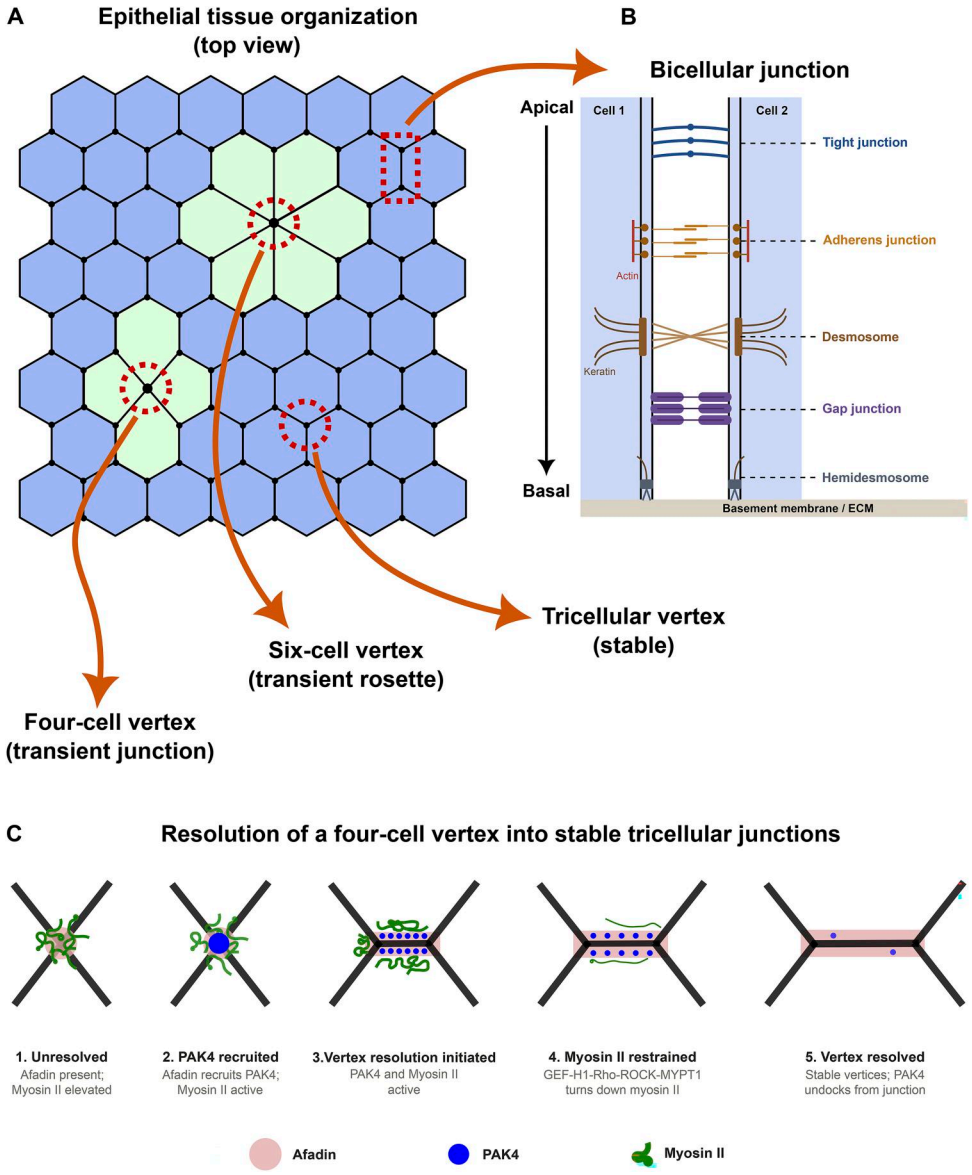

Figure 1. **PAK4 resolves transient vertices. (A)** Schematic of the top view of an epithelial tissue showing bicellular junctions and different vertex configurations. Bicellular junctions, stable tricellular vertices, and transient higher order vertices are indicated. Green cells participate in transient vertices, whereas blue cells form stable tricellular vertices. **(B)** Schematic of a vertebrate bicellular junction showing the organization of the tight junction and adherens junction in the apical region. **(C)** Sequential steps during the resolution of a transient four-way vertex into a stable tricellular vertex. The schematic illustrates the proposed roles of Afadin, PAK4, and Myosin II during vertex remodeling.

rather than maintain junctional architecture. Artificially restoring PAK4 to junctions partially rescues Afadin-deficient cells, but only when PAK4 retains its kinase activity, indicating that localization alone is not sufficient. Structural modeling further supports a direct interaction between the N-terminal region of PAK4 and the RA1 domain of Afadin, while overexpressing the isolated PAK4 N terminus competitively displaces endogenous PAK4 and phenocopies PAK4 loss. Together, these findings place Afadin upstream of PAK4 and suggest additional PAK4-independent functions.

If the Afadin–PAK4 pathway coordinates vertex remodeling, disrupting it should compromise the epithelial barrier. Using two complementary assays, TER and ZnUMBA, the authors confirmed that disrupting this pathway consistently compromises barrier function. But why does the loss of PAK4 cause such dramatic defects? The answer appears to lie in the actomyosin cytoskeleton: loss of PAK4 reorganizes Myosin II into striking sarcomere-like assemblies along cell–cell junctions, indicating excessive contractility. Inhibiting Myosin II largely restores barrier function, demonstrating that uncontrolled actomyosin activity underlies much of the phenotype. Interestingly, wild-type neighboring cells suppress these abnormalities in mosaic tissues, revealing that epithelial integrity emerges as a collective property of the tissue rather than the responsibility of any single cell.

The final piece of the puzzle was to explain how PAK4 controls actomyosin

contractility. Loss of either PAK4 or Afadin causes GEF-H1 accumulation, pointing to excessive activation of the Rho-ROCK pathway and inhibition of the myosin phosphatase MYPT1. Restoring constitutively active MYPT1 rescues the remodeling defects, placing MYPT1 downstream of PAK4. Together, these findings show that PAK4 promotes GEF-H1 turnover to restrain Myosin II activation. Rather than simply driving contraction, PAK4 fine-tunes when and where contractile forces are generated, allowing remodeling to proceed without compromising epithelial integrity.

Thus, Adhikary et al. identify PAK4 as a central regulator of epithelial vertex remodeling. Recruited by Afadin, PAK4 orchestrates the timely resolution of multicellular vertices by fine-tuning actomyosin contractility through the GEF-H1-Rho-ROCK-MYPT1 axis, enabling epithelial tissues to continuously remodel without compromising mechanical integrity or barrier function (Fig. 1). However, several open questions remain: what distinguishes a remodeling vertex from a stable one, and how is PAK4 recruited with such spatial and temporal precision? Does local geometry, mechanical forces, or vertex lifetime govern its recruitment, and what are its direct phosphorylation substrates? Answering these questions will further establish PAK4 as a key player in epithelial biology, with potential therapeutic prospects toward wound repair and disease.

More broadly, this study reframes how we think about epithelial remodeling. Rather than viewing actomyosin contractility solely as the engine driving junction rearrangements, it highlights an equally important need to precisely limit and redistribute these forces in space and time. Successful remodeling depends not only on generating force, but also on knowing when and where to stop. This work positions multicellular vertices as dynamic hubs, where biochemical signaling, mechanics, and tissue topology converge. Epithelial integrity thus emerges not from static cell–cell adhesion, but as a process continuously regulated as tissues remodel.

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