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SPOTLIGHT

Strength in numbers: Proteins travel as condensates to reinforce cell–cell junctions under stress

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Cell–cell junctions under stress recruit proteins to reinforce adhesion, but how these proteins are rapidly delivered is unclear. In this issue, Zheng et al. (https://doi.org/10.1083/jcb.202601182) identify a mechanism whereby the adhesion protein Canoe/Afadin travels along cell boundaries to vertices under tension in optimal-sized condensates.

In multicellular organisms, epithelial tissues create barriers to line organs and separate the inside of the organism from the outside world. Epithelial tissues are composed of many cells tightly connected to each other through specialized cell–cell attachments called junctions or adhesions. These cell–cell junctions are critical to the ability of epithelia to maintain impermeability and tissue integrity. Epithelial cells experience many conditions in which their ability to stay connected is challenged, including extrusion of dying cells, cell division, and morphogenetic cell movements. In response to mechanical stress, cell–cell junctions undergo a phenomenon called adhesion strengthening. Adhesion strengthening involves rapid recruitment of additional junctional adhesion protein components to the site under stress, which is thought to mitigate the amount of force experienced by any one protein. This process is not unique to epithelial cell–cell junctions but occurs broadly across cell adhesion structures and cell types (1, 2, 3). Many key structural proteins at adhesions undergo force-dependent conformational changes that expose additional protein-binding sites to accommodate adhesion growth (4, 5). However, it remains unclear how material is rapidly delivered to sites of tension.

Tricellular junctions (TCJs) are locations where three cells meet and are sites of high tension, especially during tissue remodeling (Fig. 1 A). Recent studies, using the *Drosophila* embryonic epidermis as a model epithelium, showed that junctional adhesion proteins are recruited to TCJs in a tension-dependent manner to reinforce cell–cell adhesion and regulate transmission of mechanical signals (Fig. 1 B) (6, 7). However, it is not clear how these proteins arrive at the right place at the right time, given that these changes occur on a timescale of seconds to minutes. In a new study (8), Zheng and colleagues uncover a novel mechanism called edge-vertex flow by which the adhesion adaptor protein Canoe (known as Afadin in vertebrates) moves along cell edges toward TCJs under tension. Transport of junctional components is conventionally thought to occur through cytoplasmic diffusion or vesicular trafficking. Instead, the new study shows Canoe moves along bicellular junctions (BCJs) in liquid-like puncta and is packaged into optimal-sized condensates. These condensates are delivered to TCJs through a directed, actomyosin-coupled transport mechanism to facilitate rapid and precise adhesion reinforcement under tension (Fig. 1 C, center). These findings introduce biomolecular condensates as functional transport units for adhesion components and expand the conceptual framework of intracellular transport beyond classical vesicular pathways.

TCJs contain unique adhesion proteins whose functions are not fully understood and may contribute to the specific mechanical and signaling needs of the vertex (9). Zheng et al. performed a screen for TCJ-enriched proteins and identified mushroom body tiny (Mbt/p21-activated kinase [PAK]), a conserved serine/threonine kinase and the only group II PAK in *Drosophila*. Loss of Mbt/PAK disrupts Canoe enrichment at TCJs and leads to accumulation of enlarged condensates at BCJs (Fig. 1 C, right). This hyper-condensation stalls edge-vertex flow and impairs adhesion reinforcement. Mechanistically, the authors found that Mbt/PAK localization is regulated through relief of autoinhibition and exposure of newly identified adherens junction–localizing motifs. Surprisingly, its kinase activity is dispensable for this function. Instead, Mbt/PAK acts primarily as a scaffold that promotes the formation and proper spatial organization of Canoe condensates. This expands the capabilities of PAK proteins from signaling kinases to structural regulators of condensates at cell junctions.

Molecular condensates are found throughout cells and have diverse roles, including concentrating molecules to enhance biochemical reactions, establishing mesoscale architecture, and regulating macromolecule folding. However, condensates occur in a wide variety of sizes, and it has been unclear how condensate size and function are related (10). Zheng et al. further show that condensate size determines Canoe function and transport efficiency. Both excessive and insufficient condensation impair adhesion strengthening by disrupting edge-vertex flow (Fig. 1 C). Hyper-condensation via forced oligomerization produces large, immobile condensates that

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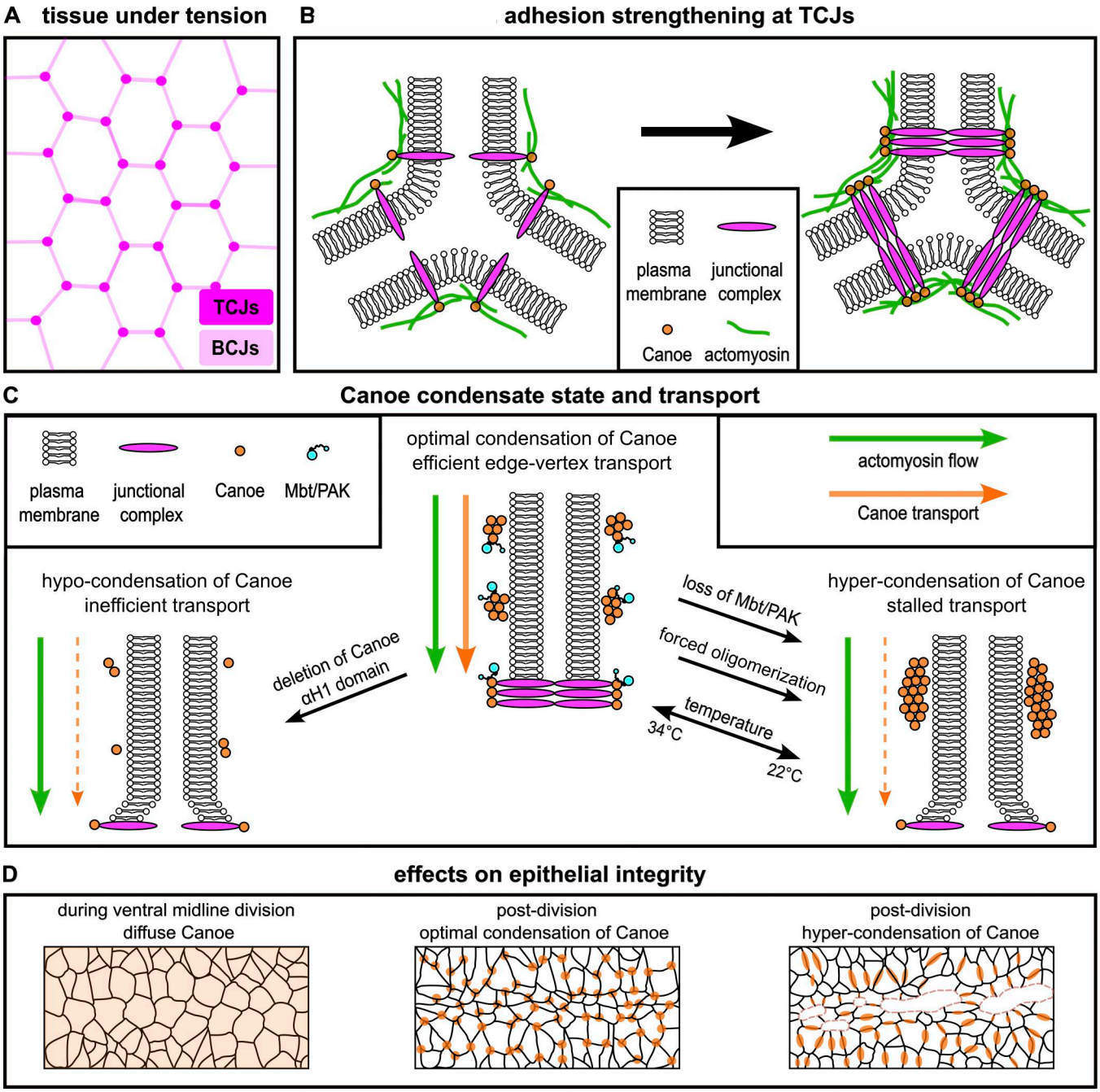

Figure 1. **Canoe/Afadin is delivered via edge-vertex flow to epithelial cell–cell junctions under stress. (A)** Epithelial cells under tension exhibit reinforced adhesion at TCJs, relative to BCJs. **(B)** The adhesion protein Canoe is recruited to TCJs in a force-dependent manner, contributing to adhesion strengthening. **(C)** Mbt/PAK helps organize Canoe into optimal-sized condensates that are transported to TCJs. Hypo-condensation or hyper-condensation of Canoe results in inefficient transport and lack of adhesion reinforcement. **(D)** Following cell division at the *Drosophila* ventral midline, Mbt/PAK and Canoe restore adhesion. When Canoe is hyper-condensed due to loss of Mbt/PAK, epithelial gaps emerge due to defective adhesion reinforcement.

fail to undergo directional transport, whereas hypo-condensation through the deletion of the Canoe α-helix (αH1) leads to inefficient transport and fails to rescue adhesion defects in Canoe null mutants (Fig. 1 C, left). Together, these results indicate that adhesion strengthening is regulated not by Canoe presence alone but by the physical state of its condensates. Consistent with this, condensate states are tunable. Increasing temperature to 34°C dissolves large Canoe condensates formed via loss of Mbt/PAK to smaller dynamic puncta and partially restores adhesion strengthening. This supports that phase behavior is a key regulator of adhesion reinforcement.

The consequences of defective adhesion reinforcement in this model are evident in the emergence of epithelial gaps at multicellular vertices during early gastrulation, a period of tissue-scale morphogenetic stress, as well as during post-ventral midline cell division (Fig. 1 D). The synchronous cell divisions that take

place along the ventral midline during stage 9 of *Drosophila* embryonic development require epithelial adhesion remodeling and re-establishment. Mechanisms of how epithelial cells maintain junctional integrity while undergoing division have been incompletely understood (11). Zheng et al. show that both Mbt/PAK and Canoe are actively regulated across the cell cycle. As cells progress from interphase to metaphase, Canoe's enrichment at TCJs decreases, and Mbt/PAK levels are reduced. After division, Canoe relocalizes to junctions in a Mbt/PAK-dependent manner and restores adhesion. When Canoe is in a hyper-condensed state, adhesion is not restored after division, and epithelial gaps emerge. This supports a model in which adhesion assembly is actively downregulated during cell division and then rapidly rebuilt through condensate-based transport.

Overall, this work by Zheng et al. advances understanding of mechanosensitive protein transport by revealing a nonvesicle–based mechanism for Canoe, where regulated phase transitions via biomolecular condensation enable efficient transport along cell cortices. More broadly, it reveals that biomolecular condensation can function not only in spatial organization but also as a physical transport strategy and identifies postmitotic adhesion reassembly as a key process governed by this mechanism. This research further implies that similar phase separation and regulated condensation processes may be employed by other force-sensitive proteins such as Ajuba (7), vinculin (5), and ZO-1 (3) to achieve swift, precise responses. Altogether, the work links physical phase transition principles to cellular mechanotransduction. It proposes a new paradigm in tissue morphogenesis, where the material states of proteins mediate precise responses to mechanical forces to maintain tissue architecture during dynamic cellular movements.

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