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Cell Junctions: Lessons from a Broken Heart

In a case of the familiar being strange, new work shows that the integrity of the *Drosophila* cardiac system depends on septate-junction proteins even though the heart lacks discernable septate junctions.

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In cell signaling and cell adhesion, it is common to find groups of genes functioning as regulatory or structural 'cassettes' in different tissues. For example, the claudin-containing septate junctions that prevent paracellular diffusion across invertebrate epithelia are also used by glia cells to create the blood–brain barrier [1]. But cells are also very creative about adapting material at hand to new purposes. In a recent paper in *Developmental Cell*, Yi *et al.* [2] show that *Drosophila* cardiac integrity requires septate-junction proteins that are central components of epithelial and glial septate junctions, and that the subcellular localization of these proteins depends on G-protein signaling. At first glance, these observations are not remarkable as septate junctions are fairly well-characterized and have previously been shown to be regulated by G-protein signaling [1] (Figure 1). But the results of Yi *et al.* [2] are striking in light of the fact that *Drosophila* cardiac tissue lacks ultrastructurally discernable septate junctions and that cardiac septate-junction proteins are regulated through only Gα signaling, rather than the G$\beta\gamma$ and Gα signaling previously seen in glial tissues (Figure 1). These findings offer insight into heart development and may provide a greater understanding of the tissue-specific functions of junctional complexes.

Heart development in *Drosophila* and vertebrates is remarkably conserved at both the morphological and molecular level [3]. To better define the mechanisms of cardiac tube formation, Yi *et al.* [2] utilized a forward genetic screen to identify genes required for *Drosophila* heart morphogenesis [4]. The *Drosophila* heart is a simple contractile tube composed of two parallel rows of myoendothelial cardioblasts flanked on each side by a row of pericardial cells, which serve structural and excretory functions for the cardioblasts. In previously published work, Yi and colleagues [4] found multiple mutations that cause loss of cardioblast-pericardial cell adhesion, a phenotype which they termed *broken hearted* (Figure 1B). Characterization of these mutations showed that genes in the mevalonate pathway were required for geranylgeranylation of the G-protein subunit, Gγ1 [4]. In the present work, Yi *et al.* [2] define the nature and targets of the Gγ1-coupled signaling and find several unexpected results.

The first surprise was that the *broken hearted 6* mutation affected the gene *neurexin-IV* (*nrx*). Nrx is a central component of septate junctions, junctional complexes found in invertebrate epithelia and glial cells that are characterized by ladder-like 'septa' that span adjacent plasma membranes (reviewed in [5]). Septate junctions show similarity in terms of composition and function to vertebrate tight junctions in that both contain claudin-family proteins and provide paracellular diffusion barriers (reviewed in [6]). As detailed electron-microscopic characterization has shown that *Drosophila* heart tissue does not have septate junctions [7,8], finding Nrx in a screen for heart defects was disconcerting. But Nrx was not a singular anomaly, as Yi *et el.* [2] further showed that at least eight septate-junction proteins are required for cardiac integrity. These results suggest that the septate-junction proteins form a similar complex in the heart and in epithelial or glial cells. However, there must be significant differences between these complexes in different tissues as septa and paracellular barriers are formed in epithelia and glia, but not the heart (Figure 1C–E). Also, while septate-junction proteins localize to the specific junctional regions in the lateral surfaces of epithelial and glial cells [1,5], Yi *et al.* [2] find that septate-junction proteins are localized throughout the entire cell membrane in cardiac cells. Furthermore, although some septate-junction proteins have been demonstrated to be capable of mediating cell–cell adhesion *in vitro* [9,10], loss of septate junctions causes only minor cell–cell adhesion defects in a subset of epithelial tissues and glia [10,11]. By contrast, loss of the septate-junction proteins Nrx, Nrv2, Coracle and Sinuous dramatically compromises cardiac integrity in all mutant embryos (Figure 1B).

The second unexpected finding arises from the nature of septate junction regulation. While G-protein

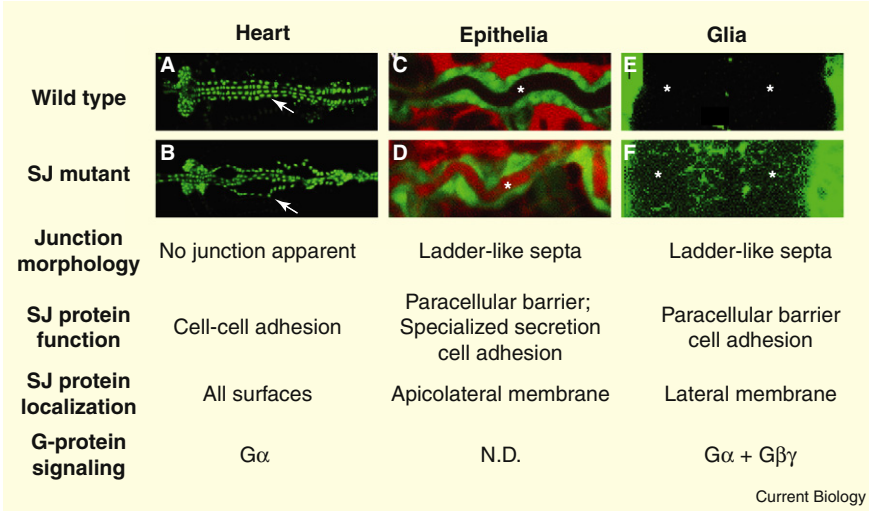

Figure 1. *Drosophila* heart morphogenesis utilizes non-canonical functions of septate junctions regulated by different G-protein signaling compared to glial cells.

(A) In wild-type *Drosophila* hearts, lateral pericardial cells adhere tightly to the central cardioblasts. (B) In septate junction (SJ) mutants, pericardial cells detach from the cardioblasts (arrow). (C) Wild-type tracheal epithelia (green) are impermeable to a fluorescent dye (red) injected into the body cavity of embryos. Note the lack of dye in the tracheal lumen (asterisk). (D) Loss of septate-junction proteins causes dye leakage into the lumen (asterisk). (E) In glial cells, septate junctions also provide a paracellular barrier for the enclosed nervous system. A fluoresent dye injected into late stage wild-type embryos is excluded from the central nerve cord (asterisks), but leaks into the nervous system in septate junction mutants (F). (C,D) with permission from [17]. (E,F) with permission from [1].

signaling has previously been demonstrated to regulate septate junction organization and function in glial cells, important details of this regulation are different in the heart. G-proteins act as effectors of G-protein coupled receptors (GPCR) and are composed of three subunits, Gα, β, and γ. When activated by the receptor, the α subunit releases the βγ dimer, allowing both α and βγ to initiate separate signaling cascades (reviewed in [12]). Through genetic manipulation, Yi *et al.* [2] demonstrated that Gα signaling alone is required for correct localization of septate-junction proteins in heart cells. This differs from the situation in glial cells, where both Gα and Gβγ signaling are required for correct septate junction assembly (Figure 1). Furthermore, while the GPCR Moody is necessary to initiate G-protein signaling in glial cells [1], no obvious GPCR was found to be required for cardiac integrity. Thus, although G-protein coupled signaling regulates septate junction components in both heart and glia, the mechanisms are significantly different.

One of the most interesting aspects of the results of Yi *et al.* [2] are the possible insights they might provide into heart development in other systems. The conservation already observed in heart development suggests that G-protein signaling pathways could be conserved [13], and the new results suggest a possible common target of claudin-based junctions — septate-junction complexes in the case of *Drosophila* and tight-junction complexes in the case of vertebrates [14,15]. Interestingly, human claudin-5 localizes to the lateral membranes of cardiomyocytes, not just the apical tight-junction region, and is implicated in human cardiomypathy [16]. Alternatively, as septate junctions also have both structural and molecular similarity to vertebrate paranodal junctions (reviewed in [5]), it is possible that the paranodal-junction proteins such as NCP-1 and Contactin will also have non-canonical roles in vertebrate heart development. While the details of septate-junction protein function and G-protein signaling in the *Drosophila* heart remain to be determined, the work of Yi *et al.* [2] defines critical pathways controlling heart morphogenesis and provides a starting point for understanding non-canonical roles of conserved junctional proteins.

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