

Balancing cell behavior at boundaries

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The restriction of cell intermingling across boundaries is essential for the establishment of discrete tissues. Eph receptor signaling prevents intermingling at many boundaries. In this issue, Luu et al. (2015. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201409026>) report a parallel pathway, mediated by Wnt signaling, Snail1, and paraxial protocadherin (PAPC). This pathway establishes a distinctive organization of cell adhesion and intercellular gaps at the interface between tissues.

The formation and maintenance of organized tissues is challenged by the intermingling of cells, which occurs as a consequence of cell proliferation and morphogenetic cell movements. Mechanisms that prevent intermingling across the boundaries of adjacent tissues thus have essential roles during development. Until recently, the principal mechanism was thought to be differential adhesion, created by tissue-specific expression of distinct cadherins, which mediate stronger homophilic compared with heterophilic cell adhesion (Steinberg, 1970). However, it is now known that at many boundaries, intermingling is prevented by other mechanisms, which are regulated by signaling between the adjacent cell populations (Dahmann et al., 2011; Battle and Wilkinson, 2012; Fagotto, 2014). Eph receptors and their ephrin ligands have emerged as major mediators of such signaling and may act in boundary formation by modulating cadherin-mediated adhesion, cell repulsion, and cortical tension (Cayuso et al., 2014; Fagotto et al., 2014). Important insights have come from studies of Brachet's cleft, which forms at the interface of migrating mesoderm cells and ectoderm during early stages of embryogenesis in *Xenopus laevis*. Brachet's cleft is an example of a boundary at which cell separation creates a gap between the tissues, within which extracellular matrix becomes deposited. The complementary expression of Eph/ephrin binding partners creates bidirectional Eph receptor activation at the mesoderm–ectoderm interface (Rohani et al., 2011, 2014). Eph activation underlies cycles of adhesion and repulsion, which enables mesoderm cells to migrate on ectoderm while also preventing them from invading (Rohani et al., 2011). The results of altering cadherin levels reveal that a balance is required: with too little adhesion, low level Eph receptor activation within mesoderm causes homotypic repulsion; with too much adhesion, mesoderm cells will invade ectoderm (Rohani et al., 2014).

Previous studies suggested that PAPC and noncanonical Wnt signaling in mesoderm cells act synergistically to promote boundary formation at Brachet's cleft (Kim et al., 1998; Winklbauer et al., 2001). In this issue, Luu et al. find that cell separation also requires Snail1, a transcription factor that regulates the motility of mesoderm cells (Blanco et al., 2007). The relationships between these components were investigated in an incisive series of gain- and loss-of-function experiments in *X. laevis* and zebrafish embryos, as well as tissue explant assays of cell separation (Luu et al., 2015). These experiments reveal that a Wnt receptor, XFz7, acts through a noncanonical pathway (Dvl2–RhoA–JNK–c-Jun) to up-regulate Snail1, which in turn enables PAPC to promote cell separation.

An essential feature of signaling in boundary formation is that it leads to distinct cell responses at heterotypic compared with homotypic contacts of cells. For Eph/ephrin signaling, this is achieved by complementary expression of high affinity binding partners such that strongest Eph receptor activation occurs at the heterotypic interface (Cayuso et al., 2014; Rohani et al., 2014). How then is this achieved for PAPC? Important evidence comes from the finding that overexpression of PAPC in the adjacent tissue prevents Snail1+PAPC from inducing cell separation (Luu et al., 2015). Cell separation thus requires interaction between PAPC-expressing and -nonexpressing cells. Because PAPC can bind to PAPC on adjacent cells, a simple model is that PAPC–PAPC trans-complexes are inactive, whereas free PAPC is present at the heterotypic interface and promotes cell separation. A clue for how PAPC activity regulates cell behavior came from apparently contradictory findings that although Dvl2 is required for Snail1 expression, cell separation still occurs after Dvl2 knockdown (Luu et al., 2015). This paradox was resolved by experiments using Dvl2 puncta as an indicator of Wnt planar cell polarity (PCP) pathway activity: Snail1+PAPC acts to decrease PCP activity at heterotypic but not at homotypic contacts of mesoderm cells. Because the PCP pathway can promote cell adhesion, this local inhibition alters cell behavior selectively at the mesoderm–ectoderm interface (Fig. 1).

In principle, PAPC and Eph/ephrin activation could underlie boundary formation simply by establishing differential adhesion. However, there is strong evidence that this is not the case, for example, because PAPC overexpression does not alter

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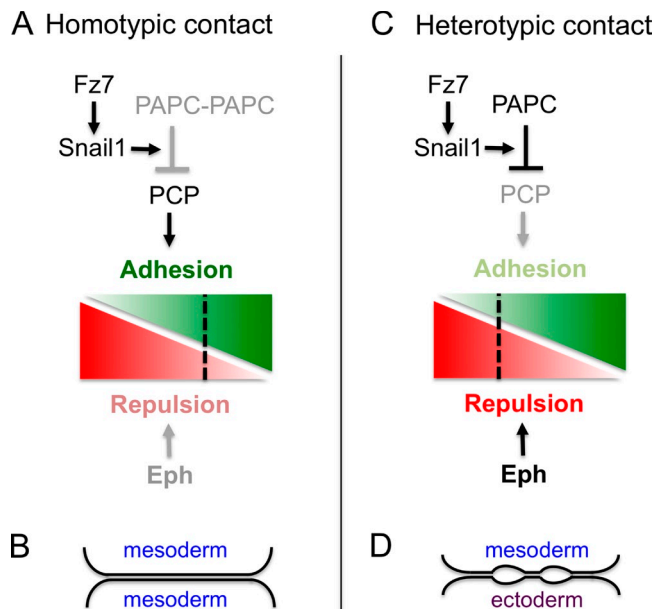


Figure 1. Signaling and responses to cell interactions. (A) At homotypic contacts of mesoderm cells, the PCP pathway can promote adhesion (green) because PAPC complexes form that have low PCP inhibitory activity. Eph receptor activation that promotes repulsion (red) is weak because coexpressed ephrins have low affinity. Consequently, the balance of cell responses favors adhesion (B). (C) At heterotypic contacts, free PAPC inhibits the PCP pathway. This PAPC activity requires Fz7-induced expression of Snail1. Eph receptors are strongly activated by high affinity ephrins expressed in ectoderm. Consequently, there is a balance of repulsion and adhesion that leads to formation of cleft contacts, characterized by interspersed stretches of adhesion and intercellular gaps (D).

cell adhesion as measured by tissue surface tension. In studies of the properties of the P^{APC+}-P^{APC-} interface, Luu et al. (2015) find that the cells form adhesive contacts yet can slide past each other easily. Electron microscopy reveals that the heterotypic interface is comprised of large intercellular gaps interspersed with stretches of membrane apposition consistent with adhesive interactions. This morphological organization—termed cleft contact—may enable adhesive interactions required for cell migration while preventing intermingling between the tissues. One way to think about cleft contacts is that they are a hybrid of cell adhesion and repulsion that requires input from P^{APC} and Eph receptor signaling to achieve the appropriate balance (Fig. 1).

These exciting findings set the stage for a number of further questions. One important issue is to understand at the biochemical level the activity of P^{APC}-P^{APC} complexes versus free P^{APC}, as well as the relationship between Snail1 and P^{APC}. Whereas Snail1 is required for full-length P^{APC} to promote cell separation, truncated P^{APC} lacking the cytoplasmic domain is sufficient in the absence of Snail1 (Luu et al., 2015). These observations suggest that a regulatory function of the cytoplasmic domain of P^{APC} is modulated by binding of a transcriptional target of Snail1. Another question is how Eph/ephrin signaling and P^{APC} synergize. This may involve convergence on Dvl activity because segregation of Eph receptor and ephrin-expressing cells requires interactions with Dvl (Tanaka et al., 2003). Finally, how does extracellular matrix accumulate in the intercellular gaps at cleft-like boundaries? During somitogenesis, Eph/ephrin signaling initiates boundary formation,

and then by inside-out integrin activation promotes accumulation of extracellular matrix at the border (Jülich et al., 2009). Extracellular matrix in turn promotes integrin signaling required for boundary maintenance (Jülich et al., 2005; Koshida et al., 2005). It will be interesting to uncover whether there is a similar role of Eph/ephrin signaling in extracellular matrix deposition at Brachet's cleft and other cleft-like boundaries.

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