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Orienting the Direction of EGFR Activation

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Morphogens are typically distributed symmetrically from their source of production. In this issue of *Developmental Cell*, Peng et al. (2012) demonstrate that a bias in the directionality of protrusions emanating from cells secreting the EGFR ligand Spitz leads to asymmetric activation of the pathway.

Morphogens play a key role in patterning tissues during development. They are commonly produced and secreted from a restricted source and spread to neighboring cells in order to induce cell fates in a concentration-dependent manner. The spatial organization of the source cells will dictate the resulting pattern of morphogen distribution within the tissue, while the biochemical properties of the morphogen itself will define the range. As a rule, a symmetric spreading of morphogen away from its source is envisaged. Thus, a linear source, such as the rows of cells secreting Dpp in the *Drosophila* wing disc, equally patterns both sides of the disc epithelium (Wartlick et al., 2011), while a point source, such as cell clusters secreting the EGF receptor (EGFR) ligand Spitz (Spi) in the *Drosophila* embryonic peripheral nervous system, induces a radial pattern of surrounding oenocyte cells (Brodu et al., 2004).

Can the symmetric propagation of a morphogen be biased? A report from the Vincent laboratory showed a different distribution of Wingless (Wg) on the anterior and posterior sides of the row of morphogen-secreting cells within each segment of the *Drosophila* embryo. This bias is induced by different properties of the cells receiving the signal, which impinge on the stability or transport of Wg (Sanson et al., 1999). The paper by Peng et al. (2012) in this issue of *Developmental Cell* presents and dissects an intriguing alternative paradigm for asymmetric propagation of a morphogen: polarized morphology of the source cells that generate and secrete the signal.

Bracts are specialized, thick, and pigmented hairs that emanate from distinct regions of the legs and wings of adult flies. They are always found in close association with mechanosensory bristles, the external manifestations of small (four-cell) sensory organs that cover the fly cuticle. An outstanding feature of bracts of the distal leg segments is that they are always positioned on the proximal side of the associated bristle (Hannah-Alava, 1958). While previous work has established that leg sensory organs induce the bract cell fate in neighboring epidermal cells via Spi-EGFR signaling (del Alamo et al., 2002; Held, 2002), the basis for the directional bias of this induction has remained a mystery that has now been solved.

Peng et al. first established that all epidermal cells are competent to adopt a bract cell fate, suggesting that the origin of the directional bias lies in the sending rather than the receiving cells. They then identified the socket cell, one of the four sensory organ cell types, as the specific source cell from which the Spi ligand is secreted, following processing by the Rhomboid protease. Using various markers to follow sensory organ and epidermal cell differentiation in developing pupae, the authors observed prominent cellular protrusions emanating specifically from the socket cell. While these lamellipodia-like extensions are highly dynamic, they maintain a persistent proximal orientation, in the direction where the bract cell fate is induced, and typically reach one or two cells away (Figure 1). Importantly, appearance of the projections precedes a gradual, proximally biased asymmetry of EGFR signaling, which culminates in a single proximal cell displaying exceptionally high signaling activity and adopting the bract cell fate. Spatially biased protrusions from the signaling cell therefore appear to provide the mechanism that induces bract cells in the proximal direction, a conclusion that was corroborated and extended by the following experiments.

Because the global polarity in developing epidermal tissues is controlled by the planar cell polarity (PCP) pathway, it is plausible that the bias in socket-cell extensions is also influenced by this pathway. Indeed, in mutants for the PCP pathway, the proximal bias in both directionality of the socket-cell protrusions and bract-cell positioning are partially compromised. A particularly striking observation made in this context is that the strong correlation between protrusion orientation and the site of an elevated EGFR signaling response is strictly maintained, regardless of the direction (proximal or otherwise) toward which the protrusions extend.

The strong circumstantial case for involvement of proximally biased socket-cell protrusions in bract-cell induction now led the authors to ask whether the protrusions per se are necessary to induce EGFR signaling. The extensions are rich in actin, and their elongated morphology suggests that they are based on actin cables, rather than branched actin structures. Indeed, partially compromising the activity of the formin-family actin nucleator Diaphanous (Dia) resulted in significant reduction in the size of the protrusions. Induction of bract cells was dramatically reduced under these circumstances. While EGFR signaling was still active in the epidermal cells, it remained at low levels and failed to elevate within proximal neighbors of the sensory organ. Overexpression of active Spi in sensory organs, in the *dia* mutant background, was able to restore bract-cell induction, but these were no longer confined to the proximal side. It thus appears that the protrusions allow the socket cell to present the active ligand more effectively to the receiving cells. Bias in the directionality of these extensions, dependent in part on the PCP pathway, leads to selective activation of EGFR and induction of bract cell fate preferentially proximal to the

mechanosensory bristles. It will be interesting to further explore the detailed cytoskeletal basis underlying formation of the socket-cell protrusions and the global mechanisms that, together with the PCP pathway, influence their directionality.

The position of the socket-cell extensions is very dynamic, such that they typically spend an average time of minutes (or less) over a given cell. It follows that the level and diffusibility of Spi must be restricted so that it leads to EGFR activation only in close proximity to the position where it was presented, similar to other Spi-based induction events (Yogev et al., 2011). The polarity bias is evident when the directionality of the protrusions is examined and summed over time. By an unknown mechanism, the cells that receive the signal must be doing just that. This filtering mechanism would give rise to persistent EGFR activation and response only in cells that have contacted the protrusions for an extended period of time. The nature of such a mechanism capable of creating a sharp threshold and bistable switch, so that only the cells that experienced extensive contact with the ligand-producing cell will exhibit a transcriptional response to EGFR activation, poses an intriguing challenge for future research.

This work adds another twist to previous studies that examined the role of cell protrusions in developmental signaling. The initial activation of Notch signaling in the *Drosophila* pupal epidermis that determined the spacing of the sensory organ precursors (SOPs) was shown to rely on dynamic protrusions that are sent by the Delta ligand-producing cells (Cohen et al., 2010). The ligand is membrane anchored in this case, and the final arrangement and spacing of the SOPs depend on the dynamics and length of the extensions that present Delta. Specialized and elongated extensions, termed cytonemes, were implicated in signaling by a variety of developmental pathways in *Drosophila* (Roy et al., 2011). They were originally identified as extensions that are sent by receiving cells toward the morphogen source, but they can also be generated by the ligand-producing cells toward the target tissue. In conclusion, the employment of dynamic cell protrusions by sending or receiving cells during development could not only affect the patterns generated in space but could also add the element of summation of biased signaling over time.

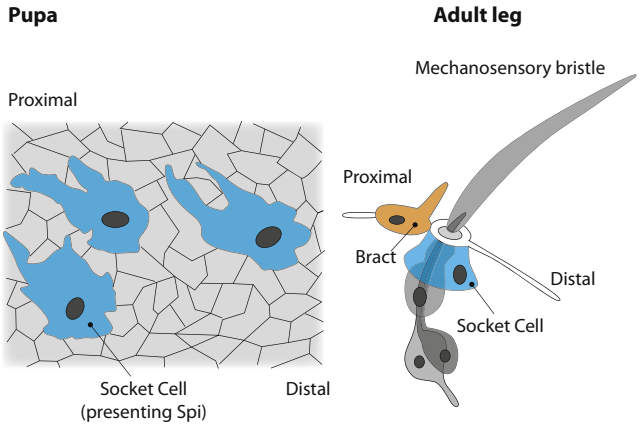

Figure 1. Proximally Oriented Protrusions of Socket Cells Present Spitz and Break the Symmetry of EGFR Activation

During the pupal stages, socket cells of mechanosensory organs on the leg process the EGF receptor (EGFR) ligand Spitz (Spi) and present it to neighboring cells. The socket cells generate dynamic proximally biased protrusions. The longer time periods that these extensions project in the proximal orientation lead to preferential activation of EGFR in the proximal cells and the induction of bract cells at that position.

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