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SPOTLIGHT

"Neur"al brain wave: Coordinating epithelial-to-neural stem cell transition in the fly optic lobe

Arnaud Ambrosini and Katja Röper

In the *Drosophila* larval optic lobe, the generation of neural stem cells involves an epithelial-to-mesenchymal–like transition of a continuous stripe of cells that sweeps across the neuroepithelium, but the dynamics at cell and tissue level were unknown until now. In this issue, Shard et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.202005035) identify that Neuralized controls a partial epithelial-to-mesenchymal transition through regulation of the apical Crumbs complex and through the coordination of cell behaviors such as apical constriction and cell alignment.

Neural stem cell (NSC) generation is a key aspect of neurogenesis in invertebrates and vertebrates. A well-defined balance between symmetric cell divisions generating a pool of NSCs and asymmetric cell divisions creating the required neuronal cell type diversity must be maintained (1). After larval hatching, *Drosophila* optic lobe cells proliferate and separate into inner and outer optic anlagen neuroepithelia (2), the latter investigated by Shard et al. in this issue (3). After initial proliferation of neuroepithelial (NE) cells at the end of larval development, a synchronous wave of differentiation sweeps across the NE sheet, leading to the progressive epithelial-to-mesenchymal transition (EMT) of all NE cells into NSCs (Fig. 1 A; 2). Newly generated NSCs remain in the same plane as the NE cells until they divide asymmetrically, generating a basal ganglion mother cell that in turn gives birth to several neural cell types (Fig. 1 A; 2).

Most studies have focused on molecular mechanisms underlying transition zone (TZ) generation and progression and the cell fate switch. However, how cell shape and polarity markers are modified to generate nonepithelial NSCs remain unknown. Shard et al. developed and applied endogenously tagged Neuralized (Neur) in both live imaging and fixed tissue studies, building on the superb ability to generate clonally mutant or transgenic cell patches in *Drosophila*. This revealed that the ubiquitin-ligase Neur is a marker of a single row of medial cells in the TZ, termed epi-NSCs, as they are the first cells to also express early markers of NSC fate, such as Worniu. Curiously, these cells retain certain epithelial features, such as E-cadherin expression, but lose apical Crumbs (Fig. 1 A).

The authors observed that epi-NSCs are the only cells in the TZ to constrict their apical surfaces. Using live imaging, they revealed that all NE cells undergo apical area fluctuations driven by apical–medial actomyosin, but that only epi-NSCs turn the myosin pulses into productive area reduction. Medial actomyosin pulsation requires coupling to adherens junctions for productive area shrinkage, as well as a type of ratcheting mechanism (4). What differentiates NE cells from epi-NSCs in this behavior remains unclear. Similar to mesoderm invagination in *Drosophila* embryos, where mesoderm-specific transcription factor Twist stabilizes the actomyosin network between pulses (4), changes to the expression profile of epi-NSCs may account for this difference.

The epi-NSCs's apical constriction is reminiscent of what occurs during other EMT processes, particularly embryonic neuroblast (NB) generation in the fly. NBs still residing in the embryonic epidermis reduce their apices through apical–medial actomyosin pulsation and sequential loss of neighbor contacts until they delaminate basally (Fig. 1 B) and then divide asymmetrically (5). Shard et al. discovered that, in contrast to NBs, epi-NSCs undergo their first asymmetric division while still being semi-epithelial with strong E-cadherin expression, and only then transition to the full NSC state. Both epi-NSCs and embryonic NBs lose apical Crumbs expression, and NBs, despite undergoing EMT, show no sign of transcriptional E-cadherin down-regulation before delamination (5). Thus, in contrast to the old "dogma" of E-cadherin down-regulation being a key aspect of EMT, its persistence in both epi-NSCs and embryonic NBs appears to be a feature among several EMT processes (6). This is supported by findings from oncology that a range of EMT states exist, with cells presenting hybrid characteristics of epithelial and mesenchymal cells (7).

The authors then focused on the mechanism upstream of epi-NSCs's shape changes.

Medical Research Council Laboratory of Molecular Biology, Cambridge Biomedical Campus, Cambridge, UK.

Correspondence to Katja Röper: kroeper@mrc-lmb.cam.ac.uk.

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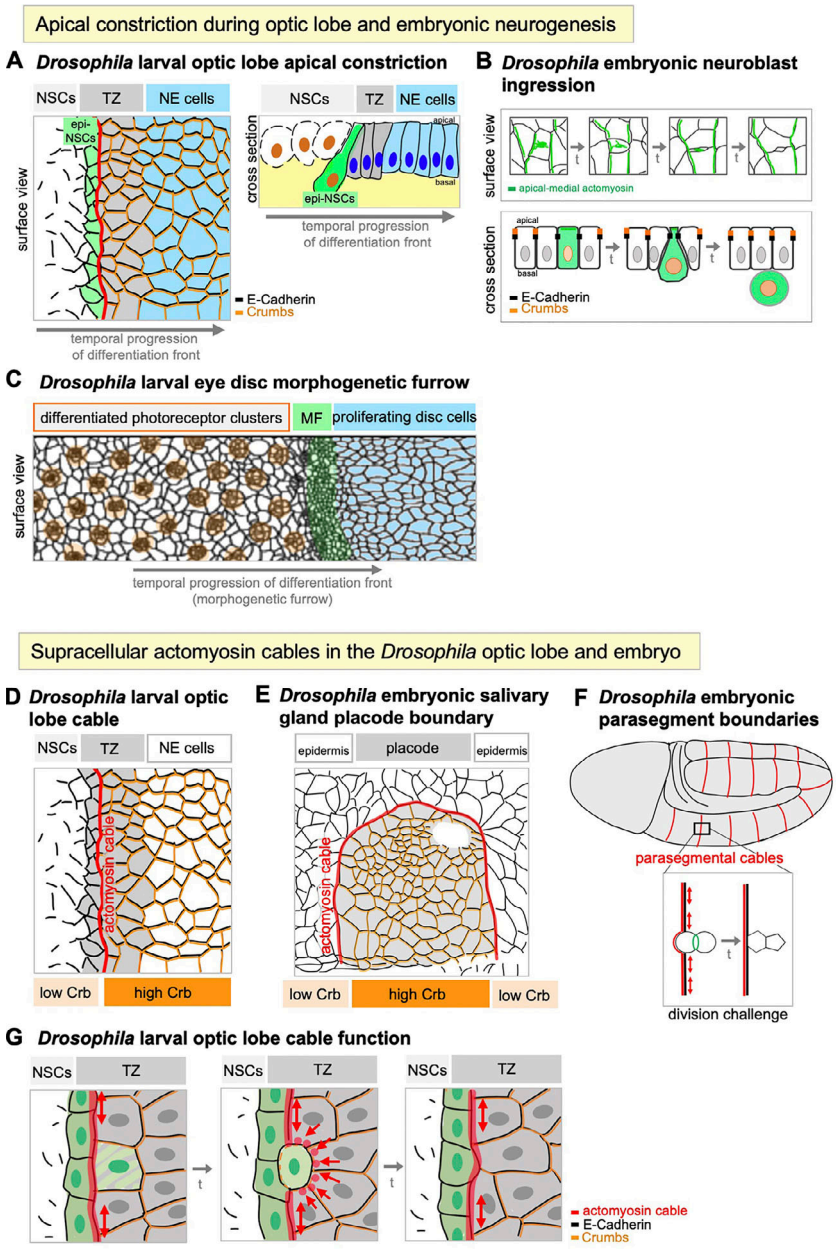

Figure 1. **Mechanisms of NSC differentiation in the *Drosophila* larval optic lobe in comparison with other developmental processes. (A)** Schematic of the differentiation wave in the optic lobe turning NE cells into NSCs via the intermediate state of epi-NSCs. Epi-NSCs have lost Crumbs and apically constrict. **(B)** Embryonic NSCs/neuroblasts delamination also involves apical constriction. **(C)** A differentiation wave termed the morphogenetic furrow (MF) sweeps across the larval eye disc. **(D)** Epi-NSCs align due to a high/low Crumbs boundary and hence anisotropy, leading to a complementary actomyosin accumulation. **(E)** Similar Crumbs anisotropy drives actomyosin cable assembly at the boundary of the salivary gland placode. **(F)** Actomyosin cables at parasegmental boundaries in the embryo prevent cell mixing across compartment boundaries during cell division challenges. **(G)** NE cells acquiring NSC fate down-regulate Crumbs, thereby triggering Crumbs anisotropy and actomyosin accumulation driving continuous alignment of epi-NSCs.

They uncovered that Neur controls apical constriction of epi-NSCs due to its capacity to target specific isoforms of Stardust (Sdt; and thus, the whole Crumbs complex) for degradation. Using an elegant genetic system based on expression of Neur-resistant Sdt isoforms, Shard et al. showed that this impairs apical constriction. Furthermore, inhibition of Neur activity through BrdR overexpression leads to a stronger reduction in apical constriction, suggesting Neur controls apical constriction through several additive mechanisms. Apical constriction is also affected by loss of Rho-GEF3, another NCS and epi-NSC–specific factor.

Apical constriction in the context of EMT is a conserved feature of delaminating cells, as well as during extrusion of apoptotic cells from an epithelium (8). As epi-NSCs do not, in fact, delaminate but remain situated level with NE cells, the function of the apical constriction of epi-NSCs is unclear, be it an evolutionary remainder or fly specific. It is also noteworthy that during another morphogenetic process in the fly that involves a wave of differentiation, the generation of photoreceptor clusters in the larval eye disc, apical constriction of cells is the physical manifestation of the differentiation wave passing (Fig. 1 C; 9). Future research will reveal whether apical constriction conveys further important features to differentiating cells in both processes.

Going from cells to tissues, Shard et al. additionally discovered that Neur also controls the formation of a supracellular actomyosin cable at the interface between epi-NSCs and NE cells. This is downstream of the generation of a high/low Crumbs boundary (Fig. 1 D), highly reminiscent of what was observed at the boundary of the salivary gland placode in the fly embryo (Fig. 1 E; 10, 11). The presence of this cable correlates with an alignment of cell junctions along the boundary, suggesting it is under tension. Supracellular actomyosin cables have been described in many contexts, notably during *Drosophila* development but also in vertebrate development (12, 13). Such cables can fulfil functions from static boundaries that prevent unwanted cell mixing (Fig. 1 F; 14) to dynamic assemblies involved in larger-scale morphogenesis (10, 12, 13). While the mechanism defining cable position via a high/low Crumbs boundary is similar to the salivary gland cable, the role of the epi-NSC/NE cable seems different: it is continuously regenerated as the TZ progresses, and a new high/low Crumbs boundary is generated. Shard et al. observed in live imaging that cells switch to epi-NSC fate on an individual basis and proposed that the cable ensures the continuous alignment of newly generated epi-NSCs by "pushing" them into the correct line of cells (Fig. 1 G). Such behavior is reminiscent of parasegmental cables in the embryo that prevent cell mixing across compartment boundaries (Fig. 1 F; 14).

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The study by Shard et al. beautifully analyzes and dissects the cell biology of epithelial-to-NSC transition in the *Drosophila* optic lobe. The elegant use of transgenic tools and genetic perturbation allowed the authors to reveal a multitude of new aspects of this process, while simultaneously demonstrating that EMT processes can combine many different features. Some of these features appear conserved between related processes (e.g., epi-NSCs and NBs), while others are specific to a certain process (the here-observed actomyosin cable). It remains to be resolved which function the apical constriction plays in this and other differentiation processes, and why a continuous wave of differentiation is advantageous to a tissue.

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