Neurulation: coordinating cell polarisation and lumen formation

Julien Compagnon and Carl-Philipp Heisenberg*

IST Austria, Am Campus 1, Klosterneuburg, Austria

*Correspondence to: heisenberg@ist.ac.at

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Cell polarisation in development is a common and fundamental process underlying embryo patterning and morphogenesis, and has been extensively studied over the past years. Our current knowledge of cell polarisation in development is predominantly based on studies that have analysed polarisation of single cells, such as eggs, or cellular aggregates with a stable polarising interface, such as cultured epithelial cells (St Johnston and Ahringer, 2010). However, in embryonic development, particularly of vertebrates, cell polarisation processes often encompass large numbers of cells that are placed within moving and proliferating tissues, and undergo mesenchymal-to-epithelial transitions with a highly complex spatiotemporal choreography. How such intricate cell polarisation processes in embryonic development are achieved has only started to be analysed. By using live imaging of neurulation in the transparent zebrafish embryo, Buckley *et al* (2013) now describe a novel polarisation strategy by which cells assemble an apical domain in the part of their cell body that intersects with the midline of the forming neural rod. This mechanism, along with the previously described mirror-symmetric divisions (Tawk *et al*, 2007), is thought to trigger formation of both neural rod midline and lumen.

Brain morphogenesis in zebrafish is initiated by the convergence movements of yet apicobasally unpolarised neural progenitors towards the dorsal side of the embryo, where they accumulate along the forming body axis giving rise to the neural rod. It is only after the formation of the neural rod, that the neurocoel is formed by cavitation (Papan and Campos-Ortega, 1994). A prerequisite for lumen opening is the formation of a continuous apical surface at the neural rod midline that is free of midline-crossing cell bodies and cellular protrusions (Figure 1). Previous work has suggested that apical midline formation and clearance is achieved by a mode of polarised cell division that gives rise to two mirror-symmetric daughter cells placed on both sides of the neural rod midline. Since these divisions allow cells to cross the midline, they have also been named crossing divisions (c-division). C-divisions not only distribute cells over the midline but also contribute to cell polarisation by accumulating the apical marker Pard3 at their abscission site (Geldmacher-Voss *et al*, 2003; Tawk *et al*, 2007). An instructive function of c-divisions in neural rod midline

formation is supported by experiments showing that ectopic c-divisions within the neural rod give rise to ectopic apical midline structures (Tawk *et al*, 2007; Quesada-Hernández *et al*, 2010; Zigman *et al*, 2011) (Figure 1). Yet, in embryos where the c-divisions are inhibited, neural rod midline formation and lumen opening still occur (Ciruna *et al*, 2006; Tawk *et al*, 2007; Quesada-Hernández *et al*, 2010; Zigman *et al*, 2011) (Figure 1).

To understand how the neural rod midline forms in the absence of c-divisions, Buckley *et al* (2013) have now used mosaic labelling of Pard3 to follow the apicobasal polarisation of neural rod cells in embryos where c-divisions are inhibited. They found that in progenitor cells that cross the neural rod midline region, Pard3 accumulates in the region of the cell that intersects with the position of the future midline. Similar accumulations were also observed in progenitor cells of normal embryos undergoing c-divisions before they enter into mitosis. With cell division progressing in those cells, the Pard3 accumulation domain then localises to the emerging cleavage plane. Together, these observations suggest that neural progenitors initiate apicobasal polarisation before and independently of division (Figure 1).

The authors also show that along with the accumulation of Pard3 in the midline-intersecting region of progenitor cells, two more apical structures—the centrosome and Rab11-positive intracellular compartments—also localise to this region. Accumulation of Pard3 and Rab11 at the midline is prevented by depolymerisation of microtubules. Moreover, tissue-specific expression of a dominant-negative Rab11 impaired lumen opening while leaving midline polarisation unaffected, suggesting a novel role for Rab11 in lumen opening independently of cell polarisation. In progenitors crossing the neural rod midline, the microtubule organising centre (MTOC) is initially located centrally at the midline-intersecting region of the cell and organises a mirror-symmetric microtubule network on both sides of the midline. In cases where midline-crossing cells are not undergoing c-division, one side of the microtubule network reorganises and the cell protrusion associated with this network shrinks progressively until the cell is restricted to one side of the forming midline only.

Taken together, these observations suggest that neural progenitors are able to sense the midline. This is the region of the neural rod where the converging progenitor cells from both sides of the neural plate meet and interdigitate. To

Figure 1 Formation of the neural rod midline during zebrafish neurulation. Interference with cell divisions and neural plate convergence reveal that neural progenitors use at least three distinct and partially redundant mechanisms to form the apical neural rod midline mirror-symmetric c-divisions; interdigitation at the neural rod midline; and positioning the apical domain away for the basement membrane.

determine if this interdigitation might play a role in apicobasal progenitor cell polarisation and midline formation, the authors separated both sides of the neural plate to prevent converging progenitor cells from both sides from meeting at the midline. In addition, they blocked cell divisions within the neural plate so that cells in the separated half of the neural plate cannot form ectopic midlines by undergoing c-divisions. Neural progenitors in embryos with separated neural plates and blocked cell divisions were still able to polarise; however, they did not form apical midline structures, but instead became apical on the edge of the neural tissue. Moreover, apical protein did not enrich in distinct domains along the length of the progenitor cell, but exclusively on the end of the cell that is furthest away from

the basement membrane. This suggests that in the absence of c-divisions and progenitor cell interdigitation at the midline, apicobasal cell polarisation is determined by the position of the ventral basement membrane of the neural plate/rod (Figure 1). Consistent with this suggestion, the authors further show that knockdown of laminin, a prominent component of the basement membrane, lead to aberrant neural rod/tube morphology.

In conclusion, this work, together with previous studies on the same topic (Ciruna *et al.*, 2006; Tawk *et al.*, 2007; Quesada-Hernández *et al.*, 2010; Zigman *et al.*, 2011), indicates that there are at least three distinct and partially redundant mechanisms by which neural progenitors form the neural rod midline: (1) by undergoing c-divisions independently

from the cell's position within the forming neural rod; (2) by undergoing interdigitation at the forming neural rod midline; and (3) by positioning the apical domain away from the laminin-containing basement membrane (Figure 1). How these different mechanisms function together in neural rod midline formation and lumen opening is still not entirely clear. For instance in embryos with reduced convergence, the midline is duplicated due to progenitor cells entering c-divisions before reaching the midline. However, later, when the converging progenitor cells are expected to meet and interdigitate at the middle of the neural rod, a third midline is not induced (Tawk *et al*, 2007). One possibility to explain this phenomenon is that ectopic midline formation by c-divisions is inhibiting cell interdigitation at the midline and/or

apicobasal polarisation through cell interdigitation. Further studies are required to understand the molecular and cellular basis for such interaction between the different midline-forming mechanisms.

Generally, the study by Buckley *et al* (2013) is a perfect example of how challenging but also rewarding it can be to study cell polarisation and epithelial morphogenesis within the developing zebrafish embryo rather than in the culture dish. While some of the observations are still subject to interpretation, it will not be long before further progress in the development of genetic, cell biological and imaging tools will allow to design and conduct even more sophisticated functional experiments narrowing down the interpretation range for those exciting observations.

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