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Cell polarity pathways converge and extend to regulate neural tube closure

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Neural tube defects, such as spinabifida, craniorachischisis and anencephaly, are some of the most common birth defects in humans. Recent studies in mouse model systems suggest that craniorachischisis is associated with mutations in genes that regulate cell polarity. Using *Xenopus* as a model system, Wallingford and Harland have now shed light on the mechanism by which these pathways affect neural tube closure.

Planar cell polarity (PCP) pathways were originally identified in *Drosophila* as crucial regulators of epithelial cell polarity, controlling the orientation of ommatidia in the eye and bristle hairs in the wing [1,2]. Recent studies in fish and frogs demonstrate that similar PCP pathway components regulate the polarized cell movements that occur in convergent extension (CE) movements during gastrulation [3–5]. In zebrafish, gastrulation-defective strains were found to carry mutations in genes encoding PCP homologs. Concurrently, the similarities between the polarized cell movements that drive gastrulation in *Xenopus* and the polarization of an epithelium in flies inspired investigators to test the role of PCP homologs in CE movements in *Xenopus*. From these studies a vertebrate pathway regulating CE movements has emerged (Box 1). Significantly, disruptions of PCP genes in the dorsal mesoderm and ectoderm during *Xenopus* gastrulation often elicited defects in NT closure [6–8]. Furthermore, explant studies have demonstrated that CE movements in both neural and mesodermal tissues are affected by alterations in PCP genes [7,8]. This left open the question of whether CE movements are necessary for proper closure of the NT and, if so, in what tissues are they essential. Here, we explore the mechanism and role of PCP-like pathways in neural tube (NT) closure. We review the recent data in vertebrates implicating a PCP-like pathway that regulates CE movements in the NT. These cell movements are required for proper NT closure. Additionally, defects in PCP signaling result in neural tube defects (NTDs) during embryogenesis, possibly contributing to human birth defects.

NT closure in vertebrates

Formation of the NT in the brain and all but the most caudal aspects of the spinal cord in birds, mammals and amphibians occurs through primary neurulation [9,10] (Fig. 1). Rolling of the neural plate into a tube occurs as neural epithelial cells undergo dramatic cell shape

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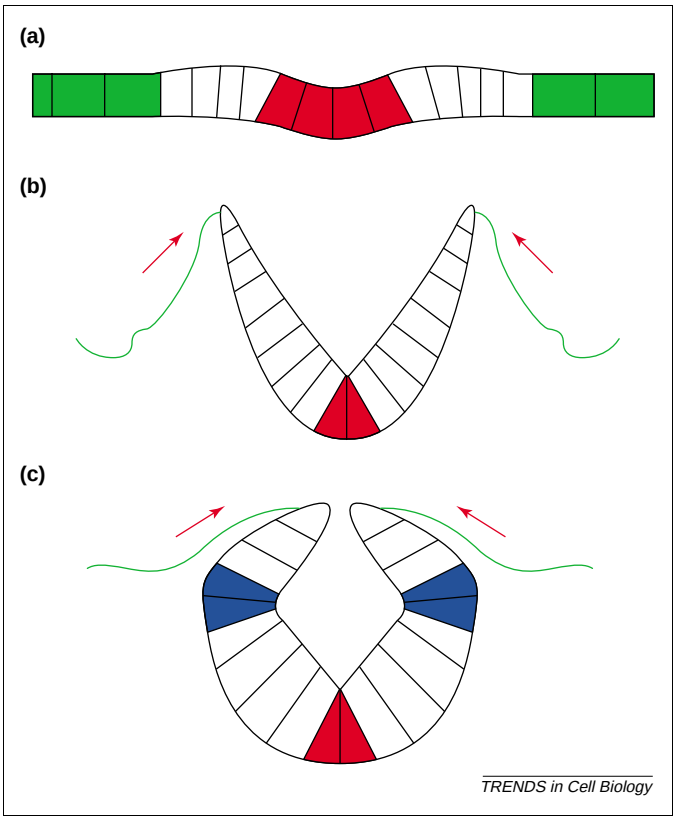

Fig. 1. Morphogenic events during neural tube (NT) closure. (a) Cells in the medial region of the neural plate undergo an apical constriction that results in formation of the medial hinge point (MHP, red). (b) Folding of the neural plate around the MHP occurs as the neural folds form at the edges of the neural plate (*) and elevate. Epidermal cells rearrange to force the neural folds to converge in the dorsal midline (red arrows). (c) Dorsal–lateral hinge points (DLHP, blue) form as neural folds come in contact with the epidermis, which continues to drive convergence of the neural folds towards the dorsal midline NT closure is completed as the neural folds fuse at the dorsal midline (not shown).

changes. Cells in the midline and lateral neural plate become wedge shaped by constriction along their apical surfaces, thus forming the medial hinge point (MHP) and the dorsal lateral hinge points (DLHPs), respectively. Extrinsic forces contribute to folding of the neural plate around these hinge points and come from the surrounding tissues such as the non-neural ectoderm. Furthermore, the CE movements that occur in the neural and underlying mesodermal tissues drive axial elongation and this has been hypothesized to provide a force for elevation of the neural folds [11]. These combined movements result in direct apposition of the neural folds in the midline, where fusion of the neural folds requires mediolateral intercalation of cells, apoptosis and differential expression of cell adhesion molecules between the neural and non-neural ectoderms. Failure to close the NT in the head region or the posterior region results in exencephaly or spina bifida, respectively, whereas failure of NT closure along the entire anterior–posterior axis results in craniorachischisis.

A PCP-like pathway is required for NT closure

Although previous experiments demonstrated that a PCP-like pathway controls CE movements in neural tissue, it was unclear whether this was the only type of cell movement that required the activity of a PCP-like pathway during NT closure. Additionally, it was not known whether failure of the NT to close was simply because of the lack of axial extension following inhibition of CE movements in the mesoderm. To address these questions, Wallingford and Harland [12] carefully examined NT closure in *Xenopus* embryos using blastomere-specific injection of a mutant form of *dishevelled* (*Dsh*) that inhibits the PCP pathway. In these embryos, the neural folds elevate at the same rate when the PCP pathway was inhibited as compared with controls. Because elevation of the neural folds was observed in embryos that underwent minimal axial elongation, this suggested that axial elongation was not required for elevation of the neural folds. Cell movements in the dorsal epidermis are also an important extrinsic force for apposition of the neural folds, yet no difference was observed in medial movement of the dorsal epidermis when the PCP pathway was inhibited. Moreover, targeted inhibition of Dsh in the dorsal NT and epidermal ectoderm did not affect NT closure, whereas inhibition of Dsh in the ventral/medial NT and notochord resulted in open-NT embryos. Thus, PCP signaling is not required for formation of the hinge points, fusion of the neural folds or in the medially moving dorsal epidermis. Instead, PCP signaling is required predominantly in the medial neural tissue for proper NT closure.

Although it was known that a PCP pathway is required for CE movements in the neural tissue, it was still not clear that blocking CE movements in the neural tissue was responsible for the observed failure of NT closure. The phenotype of embryos with inhibited PCP signaling was variable, thus the severity of the NTD could be correlated with the extent of axial elongation. PCP-inhibited embryos could be grouped into closed-NT and open-NT embryos. Closed-NT embryos elongated substantially, open-NT embryos elongated negligibly and embryos with smaller regions, where the NT was open, elongated to an intermediate degree. Overall, the main difference between closed-NT and open-NT embryos was the distance separating the forming neural folds. Lastly, when observed in cross-section, defective NT closure could be correlated with inhibition of CE movements in the neural tissue but not the notochord. Taken together, these results suggest that NTDs occurred when PCP signaling was blocked owing to an inhibition of CE movements in the neural tissue, resulting in neural folds that were too far apart to fuse and allow NT closure.

PCP and NTDs in mice?

Although the experimental demonstration of CE movements during NT closure in mouse has not been reported, evidence for the involvement of a similar PCP-like pathway in mice comes from analysis of mouse mutants with defects in NT closure. Aspects of the phenotype exhibited either by the spontaneous mouse mutant *loop–tail* (*Lp*), which has a mutation in Vangl2/Lpp1/Ltap (the *Drosophila* homolog of *strabismus/van gogh*), or compound mouse mutants, lacking both *dishevelled 1* and *2* (*Dvl1* and *Dvl2*), are similar to that of *Xenopus* embryos in which the PCP pathway is inhibited [11,13,14]. Both mutant mouse lines exhibit craniorachischisis where the NT is open from the midbrain to the tail and axial elongation is reduced. Furthermore, *Lp* mice exhibit a wider notochord and

Box 1. Pathways that regulate convergent–extension (CE) movements in *Xenopus* and Zebrafish and neural tube (NT) closure in mouse

(a) In *Xenopus*, CE movements in the mesoderm and/or neural tissue phenotypes are observed when components of a planar cell polarity (PCP)-like pathway are experimentally deregulated by overexpression or blocked using dominant-negative constructs or morpholino antisense oligonucleotides. The *Xenopus* PCP-like pathway regulating CE movements involves Wnt11 activation of Frizzled 8 or 7 (Fz8/7) that activates dishevelled (Dsh). Dsh either activates Rac, leading to activation of c-Jun N-terminal kinase (JNK), or associates with Daam1 a GDP–GTP exchange factor for the Rho GTP-binding protein, leading to activation of Rho [23]. Glypican4 [24], Prickle (Pk) [25] and Stbm (a homolog of *Drosophila strabismus* (*Stbm*)/*van gogh* (*Vang*) are also required for CE movements and/or NT closure. (b) In Zebrafish, mutant fish lines have uncovered a role for the following genes regulating CE movements during Zebrafish gastrulation: *silberblick* (*Slb*) has a mutation in *Wnt11*, *trilobite* (*Tri*) has a mutation in a homolog of *Stbm/Vang*, and *knypek* (*Kny*) has a mutation in *glypican*. Fz, Dsh, Prickle (Pk) [26] and Rho kinase 2 (ROK2) were shown to be necessary for gastrulation by morpholino or dominant-negative experiments. (c) In mouse, disruption or deletion of homologs of fly PCP genes results in craniorachischisis, thus defining a PCP-like pathway regulating NT closure. In mouse, the following mutations result in craniorachischisis: (1) Target deletion of *dishevelled 1* and *2* (*Dvl1* and *Dvl2*); (2) the mutant mouse line *loop–tail* (*Lp*) that has mutations in *Vangl2* (formerly known as *Lpp1* or *Ltap*), a homolog of *Drosophila Stbm*/*Vang* [11,14]; (3) the allelic *spin cycle* (*Scy*) and *Crash* (*Crsh*) mouse mutant lines, which have mutations in *Celsrl*, a homolog of *Drosophila flamingo*/*starry night* [19]; and (4) *circletail* (*Crc*), which has a mutation in a homolog of *Drosophila Scribble* (*Scrb1*) [16]. In figure I, the core PCP genes that mediate PCP pathways common to ommatidia, bristle and hair orientation are colored blue. Other components that are implicated in tissue-specific readouts (e.g. only in the orientation of the ommatidia) are in red. Components that appear to be specific for vertebrate pathways are in green. Data are reviewed in (Refs [3–5]) unless otherwise cited.

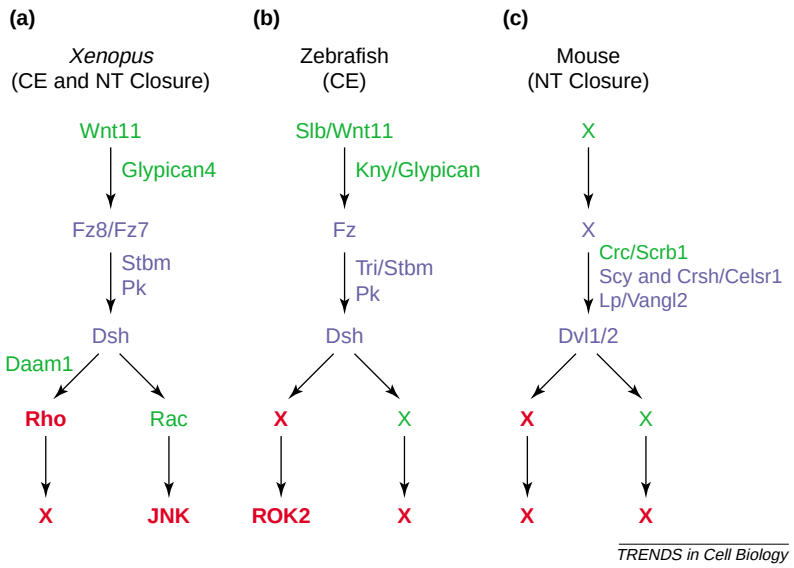

Fig. I.

floorplate, whereas this has not been examined in *Dvl1* and *Dvl2* compound homozygous mice [15]. Although the failure of CE movements to occur properly has not been demonstrated in *Dvl1/2* or *Lp* mutant embryos, the phenotype common to that observed in *Xenopus* – of a widened floorplate and notochord and reduced axial elongation – strongly suggests that these PCP components will also be required for CE-type movements in mice. Live-imaging of cell movements during closure of wild-type and mutant mouse NTs, similar to that done in the Wallingford and Harland study, will help to address these issues.

Interestingly, the spontaneous mouse mutant *circletail* (*Crc*) also exhibits craniorachischisis and a decrease in axial elongation [16]. *Crc* mice have a mutation in a homolog of the *Drosophila scribble* (*Scrb1*) gene, a gene that has not been implicated in PCP but regulates apical–basal polarity in flies [16,17]. Intriguingly, *Crc* and *Lp* genetically interact as compound heterozygous mutants exhibit craniorachischisis, suggesting that these genes act in the same or parallel pathways [16]. Lastly, both *Lp* and *Crc* mutant mice exhibit improper orientation of hair cell stereocilia bundles within the cochlea of the inner ear, an example of a PCP phenotype in mammals [18]. This finding suggests that both *Lp* and *Crc* regulate a PCP pathway in mammals to orient stereocilia. Alternatively, proper apical–basal polarity might be required for the establishment of PCP within an epithelium. Recently, mutations in *Celsr1* the mouse homolog of the *Drosophila* PCP gene *flamingo*/*starry night* were identified in the allelic *spin cycle* (*Scy*) and *crash* (*Crsh*) mutant mouse lines [19]. Both *Scy* and *Crsh* mutant mice exhibit craniorachischisis and a disorganization of stereocilia bundles, further suggesting that mammalian PCP-like pathways can regulate neural tube closure and stereocilia orientation.

The observation that a small percentage (2–3%) of *Dvl2* mutant embryos exhibit exencephaly and spinabifida

whereas compound mutants lacking both *Dvl1* and *Dvl2*exhibit craniorachischisis, raises the interesting possibility that milder NTDs might result from partial inhibition of PCP pathways. This possibility is supported by a re-examination of the phenotypes of mice mutant for homologs of genes that can regulate PCP pathways in flies. For example, deletion of *p190 RhoGAP*, a negative regulator of Rho activity, results in exencephaly in approximately one-third of mutant embryos [20]. These embryos exhibited a slightly widened floorplate, reminiscent of other vertebrate PCP phenotypes, and an abnormal morphology of the roof plate in the spinal cord, suggesting a mild neural closure defect in more caudal regions. The testing of genetic interactions between *p190 RhoGAP* and other potential PCP genes might help to determine whether the NTD in these mice arises because of defects in a PCP-like pathway.

Redundancy or early lethality might hamper the elucidation of the role of other genes involved in PCP-like pathways regulating NT closure in mouse. For instance, targeted mutations of genes that control CE movements in other vertebrates cause early embryonic lethality, thus limiting an analysis of their role in NT closure. For example, *Rac1* homozygous mutant mouse embryos exhibit defects in mesoderm migration and they arrest growth before NT closure [21]. Tissue- and developmental-stage specific deletion of genes in mice or the generation of hypomorphic alleles will allow further examination of the contribution of other genes that regulate PCP-like pathways required for NT closure.

Concluding remarks – PCP and NTDs in humans?

In humans, NTDs arise in 1 in 1000 live births, with craniorachischisis accounting for ~10% of these NTDs [22]. To date, there are over 60 mutant mouse lines with NTDs [22]. The majority of these lines exhibit exencephaly, spinabifida or both. Disruption of a wide variety of genes results in NTDs in mice, including genes involved in: patterning of the NT along the dorsal–ventral or anterior–posterior axis; the organization of the cytoskeleton or extracellular matrix; and the regulation of cell death or proliferation.

Interestingly, the only mutant mouse lines that exhibits craniorachischisis carry mutations in genes whose *Drosophila* homologs control epithelial polarity. Experiments by Wallingford and Harland suggest that in *Xenopus* these NTDs result from a defect in CE in the medial neural tissue resulting in neural folds too far apart to close. Future studies will confirm whether CE also plays a similar role in NT closure in mice. Additionally, evidence in mice suggests that mild defects in PCP-like pathways can also result in exencephaly and spinabifida. These observations raise the distinct possibility that mutations in genes that regulate epithelial polarity, or PCP-like pathways in particular, might also be responsible for these types of NTDs in humans.

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