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Distinct Apical and Basolateral Mechanisms Drive Planar Cell Polarity-Dependent Convergent Extension of the Mouse Neural Plate

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SUMMARY

The mechanisms of tissue convergence and extension (CE) driving axial elongation in mammalian embryos, and in particular, the cellular behaviors underlying CE in the epithelial neural tissue, have not been identified. Here we show that mouse neural cells undergo mediolaterally biased cell intercalation and exhibit both apical boundary rearrangement and polarized basolateral protrusive activity. Planar polarization and coordination of these two cell behaviors are essential for neural CE, as shown by failure of mediolateral intercalation in embryos mutant for two proteins associated with planar cell polarity signaling: Vangl2 and Ptk7. Embryos with mutations in *Ptk7* fail to polarize cell behaviors within the plane of the tissue, whereas *Vangl2* mutant embryos maintain tissue polarity and basal protrusive activity but are deficient in apical neighbor exchange. Neuroepithelial cells in both mutants fail to apically constrict, leading to craniorachischisis. These results reveal a cooperative mechanism for cell rearrangement during epithelial morphogenesis.

INTRODUCTION

Neurulation is the process by which the prospective central nervous system is formed. In amniote embryos, this process begins with the formation of the neural plate, a flat sheet of neural ectoderm that undergoes a series of complex shape changes that result in a narrow and elongated structure with a medial neural groove and lateral neural folds (Schoenwolf, 1991). Many of these tissue-level changes are facilitated by changes in cell shape: cells of the lateral neural plate increase their apical-basal height, whereas cells at the midline become wedge shaped, thus creating a hinge point (Schoenwolf, 1985; Schoenwolf and Franks, 1984). The neural folds must then come together medially and fuse to close the neural tube. Neural fold apposition is largely facilitated by the hinge points and the surface ectoderm (Alvarez and Schoenwolf, 1992; Hackett et al., 1997), and as the folds elevate to meet medially, the neural plate also narrows significantly (Jacobson and Gordon, 1976; Schoenwolf, 1985). This narrowing (convergence) may serve to bring the neural folds closer together (Wallingford and Harland, 2002), and it is associated with a concomitant extension of the tissue that contributes to elongation of the neural plate (Jacobson and Gordon, 1976; Schoenwolf, 1985). Although much is understood about the cellular mechanisms that result in the formation of the neural groove and hinge points (Schoenwolf and Powers, 1987; Shum and Copp, 1996; Smith and Schoenwolf, 1987; Smith et al., 1994), much less is known about the process of neural convergence and extension (CE) in amniote embryos.

In amphibian embryos, mediolateral cell intercalation drives CE of the neural plate (Jacobson, 1994; Keller et al., 2000) and is accomplished by polarized protrusive activity and intercalation of deep neural cells (Davidson and Keller, 1999; Elul and Keller, 2000; Elul et al., 1997). Similar cell behaviors underlie CE of the neural keel in zebrafish embryos (Harrington et al., 2010; Warga and Kimmel, 1990), as well as mesoderm intercalation in frogs, fish, and mice (Glickman et al., 2003; Heisenberg et al., 2000; Keller et al., 2000; Shih and Keller, 1992; Yen et al., 2009; Yin et al., 2008). In all of these examples, the polarity of intercalation is determined by planar cell polarity (PCP) signaling (Ciruna et al., 2006; Goto and Keller, 2002; Jessen et al., 2002; Wallingford and Harland, 2001; Yen et al., 2009).

PCP signaling is one mechanism that links the processes of neural CE and neural tube closure, and both fail when PCP signaling is perturbed. In *Xenopus* embryos, loss of PCP signaling leads to failure of neural CE and an open neural tube (Goto and Keller, 2002; Wallingford and Harland, 2002). Defects in PCP signaling are also associated with neural tube closure defects in mice. Indeed, all mouse models of craniorachischisis, a failure of nearly the entire length of the neural tube to close, result from homozygous mutations in PCP components (Curtin et al., 2003; Gerrelli and Copp, 1997; Greene et al., 1998; Hamblet et al., 2002; Kibar et al., 2001; Lu et al., 2004; Murdoch et al., 2003; Wang et al., 2006b). These include *Looptail* (*Lp*) mice, which carry a point mutation in *Vangl2*, a homolog of the *Drosophila* PCP gene *Van Gogh/Strabismus* (Kibar et al., 2001; Murdoch et al., 2001). This mutation prevents delivery of the Vangl2 protein to the plasma membrane (Merte et al., 2010) and may also act in a dominant-negative manner by affecting distribution of other proteins, such as Vangl1 and Pk2 (Song et al., 2010; Yin et al., 2012). PCP phenotypes are also found in mice mutant for *Ptk7*, a gene that is involved in planar polarity, but whose *Drosophila* homolog is not a core member of the PCP pathway (Lu et al., 2004; Peradziryi et al., 2011). How these and other PCP genes regulate neural tube morphogenesis in amniotes is largely unknown. Unlike the neural plate of *Xenopus*

and the neural keel of the zebrafish, in which radial and mediolateral intercalation of deep mesenchymal cells drive CE (Davidson and Keller, 1999; Elul et al., 1997; Harrington et al., 2010), the neural plate of amniote embryos is a single-layered pseudostratified epithelium. We hypothesize that the cellular mechanisms driving elongation of an epithelial tissue likely differ significantly from those of a mesenchymal cell population.

Epithelial intercalation has been best characterized in non-neural epithelia, where a variety of cellular mechanisms have been found to drive CE. Cells in the *Drosophila* germband, for example, rearrange via oriented neighbor exchange and the formation/resolution of multicellular rosette structures (Bertet et al., 2004; Blankenship et al., 2006; Zallen and Blankenship, 2008). In contrast, the epidermal cells of the *Caenorhabditis elegans* embryo rearrange via large basolateral protrusions that invade between adjacent cells (Williams-Masson et al., 1998). The sea urchin archenteron and the notochord of the ascidian embryo also elongate via intercalation driven by polarized protrusive activity (Hardin, 1989; Munro and Odell, 2002). *Drosophila* imaginal discs elongate and evaginate by a combination of several mechanisms, including cell intercalation, cell shape change, and cell division (Condic et al., 1991; Fristrom, 1976; Taylor and Adler, 2008). Epithelial tubules in the mouse postnatal kidney elongate by polarized cell divisions (Carroll and Das, 2011; Fischer et al., 2006), whereas embryonic kidney tubules elongate by cell intercalations around the circumference of the tube (Karner et al., 2009), driven, at least in part, by polarized formation and resolution of epithelial rosettes (Lienkamp et al., 2012).

Thus, there are several mechanisms that can facilitate elongation of epithelial sheets and tubes, some of which may be conserved in the neural plate of amniote embryos. Indeed, the neural plate of the chick embryo is believed to elongate through a combination of several of these mechanisms, including cell intercalation, cell shape changes, and oriented division (Sausedo et al., 1997; Schoenwolf, 1991; Schoenwolf and Alvarez, 1989; Schoenwolf and Powers, 1987; Schoenwolf and Yuan, 1995). Mediolateral cell boundary shortening in the chick forebrain neural plate drives both narrowing of the neural plate and apical constriction of neural epithelial cells, but it does not appear to result in appreciable cell intercalation (Nishimura et al., 2012). Although it has been demonstrated that the mouse neural tube undergoes CE (Ybot-Gonzalez et al., 2007), the underlying cellular mechanism(s) remains unknown.

Here we have identified the cellular mechanisms of neural plate elongation in mouse embryos by direct observation of live, whole embryos using time-lapse confocal microscopy. We have found that the neural epithelium undergoes mediolateral cell intercalation and exhibits both apical boundary rearrangement and mediolaterally biased basolateral protrusive activity. Both of these mechanisms contribute, perhaps equally, to mediolateral cell intercalation. We also demonstrate that *Ptk7* and *Vangl2*, two regulators of planar cell polarity, regulate neural cell intercalation and CE. *Ptk7* mutant embryos fail to polarize intercalation events within the plane of the tissue, affecting both apical and basal cell behaviors, whereas *Vangl2 Lp* mutant embryos maintain tissue polarity but are deficient in apical neighbor exchange, thus affecting only apical cell behavior. Observation of these distinct cell behavior phenotypes has allowed us to functionally separate mechanisms in both the apical and basal domains of intercalating epithelial cells.

RESULTS

The Mouse Neural Plate Undergoes Convergent Extension

Eight-hour time-lapse confocal movies were made of e8.0 *mT/mG:ZP3* cre embryos in which every cell expresses membrane-targeted EGFP (mG). These time-lapse series focus on the ventral neural plate beginning at approximately the two- to four-somite stage (see Movie S1 available online). To quantify the normal progress of neural CE, we measured tissue shape changes by using distortion diagrams. Diagrams overlying wild-type (WT) neural plates undergo substantial elongation and modest narrowing (Figures 1A and 1A′) that is indicative of CE. The extent of CE was determined by measuring the change in average anterior-posterior (AP) length and mediolateral (ML) width of distortion diagrams over time. WT neural plates elongate by an average of 22.3% and narrow by an average of 7.7%, resulting in a 35.4% average increase in overall AP/ML ratio, or CE index (Figures 1G and 1H).

Mouse neural tissue is highly proliferative, and oriented division may contribute to the overall elongation and shaping of the neural tube (Sausedo et al., 1997). We measured the orientation of both the division plane and final position of daughter cells relative to the AP axis in dividing cells observed within four WT time-lapse movies. No bias in the orientation of either was observed (Figure S1). It is conceivable, however, that oriented cell divisions may play a more substantial role in neural elongation at later stages of development. Because our analysis encompasses neural plate morphogenesis only at early somite stages, we cannot exclude this possibility. Regardless of their orientations, in the mouse, cell cycles include growth and increase the volume of the tissue. The amount of convergence observed (7.7%) is relatively modest compared with the amount of extension (22.3%), suggesting that elongation of the neural plate likely occurs by a combination of increased tissue volume and convergence, with the increase in volume being channeled into extension.

Neural CE Is Disrupted in Embryos Mutant for Vangl2 and Ptk7

Embryos homozygous for mutations in *Vangl2* or *Ptk7* exhibit dramatic defects in axial elongation. Both are born with severely shortened AP body axes and exhibit craniorachischisis, a failure of the neural tube to close posterior to the midbrain (Greene et al., 1998; Lu et al., 2004). To determine how neural CE is affected by mutations in these genes, 8 hr time-lapse sequences were made of homozygous mutant embryos (Movie S1), and overall tissue distortions were analyzed. The CE index of $Vangl2^{Lp/Lp}$ mutant (*Lp* mutant) neural plates was 20.7% on average, compared with 35.4% in WT embryos (Figures 1C, 1C′, and 1G), consistent with the reduction of CE reported previously in *Lp* mutant embryos (Wang et al., 2006a; Ybot-Gonzalez et al., 2007). The CE index of $Ptk7^{XST87/XST87}$ mutant (*Ptk7* mutant) neural plates was only 6.7% (Figures 1E, 1E′, and 1G), a significantly smaller value than that of WT. A similar reduction in CE is detected when tissue distortion is measured