PRENATAL DIAGNOSIS
Prenat Diagn 2009; **29**: 303–311.
Published online 10 February 2009 in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/pd.2206

REVIEW

Development of the vertebrate central nervous system: formation of the neural tube

Nicholas D. E. Greene* and Andrew J. Copp

Neural Development Unit, UCL Institute of Child Health, London, UK

The developmental process of neurulation involves a series of coordinated morphological events, which result in conversion of the flat neural plate into the neural tube, the primordium of the entire central nervous system (CNS). Failure of neurulation results in neural tube defects (NTDs), severe abnormalities of the CNS, which are among the commonest of congenital malformations in humans. In order to gain insight into the embryological basis of NTDs, such as spina bifida and anencephaly, it is necessary to understand the morphogenetic processes and molecular mechanisms underlying neural tube closure. The mouse is the most extensively studied mammalian experimental model for studies of neurulation, while considerable insight into underlying developmental mechanisms has also arisen from studies in other model systems, particularly birds and amphibians. We describe the process of neural tube formation, discuss the cellular mechanisms involved and highlight recent findings that provide links between molecular signaling pathways and morphogenetic tissue movements. Copyright © 2009 John Wiley & Sons, Ltd.

KEY WORDS: neurulation; neural tube defects; embryology; hinge points; spina bifida; anencephaly

OVERVIEW OF VERTEBRATE NEURULATION

The brain and the spinal cord are derived from the neural tube, a structure, which is formed by a coordinated sequence of morphogenetic steps during embryogenesis. The embryonic precursor of the neural tube is the neural plate, or neuroepithelium, a thickened region of ectoderm on the dorsal surface of the early embryo. The neural plate is subsequently converted into a tube by a two-stage process. Primary neurulation gives rise to the neural tube that will later develop into the brain and most of the spinal cord. In higher vertebrates, this process involves shaping and folding of the neuroepithelium, with formation of neural folds that undergo fusion in the midline to generate the tube (Copp *et al*., 2003b). Following adhesion and fusion, the neural fold apices remodel to create two continuous epithelial layers: the surface ectoderm on the outside and the inner neural tube (Colas and Schoenwolf, 2001). Following completion of primary neurulation, the neural tube at lower levels of the future spine, caudal to the mid-sacral region, is formed by the process of secondary neurulation (Schoenwolf, 1984; Copp and Brook, 1989). Unlike primary neurulation, which involves fusion of neural folds, secondary neurulation involves condensation of a population of mesenchymal cells in the tail bud, to form an epithelial rod. Canalization of this epithelium creates the secondary neural tube, the lumen of which is continuous with that of the primary neural tube (Schoenwolf, 1984). Secondary neurulation is a well recognized feature of human development and leads to formation of the neural tube in the caudal sacral and coccygeal regions.

In this review, we will focus on primary neurulation, failure of which results in 'open' neural tube defects (NTDs), including spina bifida and anencephaly.

DISCONTINUOUS INITIATION OF NEURAL TUBE CLOSURE IN MOUSE AND HUMAN EMBRYOS

In mammals and birds, unlike amphibia, primary neural tube closure is initiated at several discrete points along the rostro–caudal axis, such that closure is a discontinuous process (Figure 1) (Copp *et al*., 1990; Van Straaten *et al*., 1996). In the mouse, initial closure of the neural folds (termed closure 1) occurs at the hindbrain/cervical boundary, at embryonic day (E)8. Closure then progresses in a rostral direction to form the neural tube in the future brain, and in a caudal direction into the future spinal cord (Copp *et al*., 2003b). Further sites of initiation occur at E9 in the cranial region, at the forebrain/midbrain boundary (closure 2) and at the rostral end of the future forebrain (closure 3) (Copp, 2005). The open regions of neural folds between the sites of initial closure are referred to as 'neuropores'—the hindbrain neuropore (between the sites of closure 1 and 2) and anterior neuropore (between closure 2 and 3). As closure proceeds bidirectionally from the sites of closures 1 and 2, and in a caudal direction from the site of closure 3, the neuropores shorten and eventually close, resulting in an intact tube (at E9.5). Spinal neurulation continues by caudal progression of closure from the site of closure 1 and is completed by closure of the posterior neuropore on E10, which marks the end of primary neurulation.

*Correspondence to: Nicholas D. E. Greene, Neural Development Unit, UCL Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, UK. E-mail: n.greene@ich.ucl.ac.uk

Received: 4 September 2008
Revised: 5 November 2008
Accepted: 2 December 2008
Published online: 10 February 2009

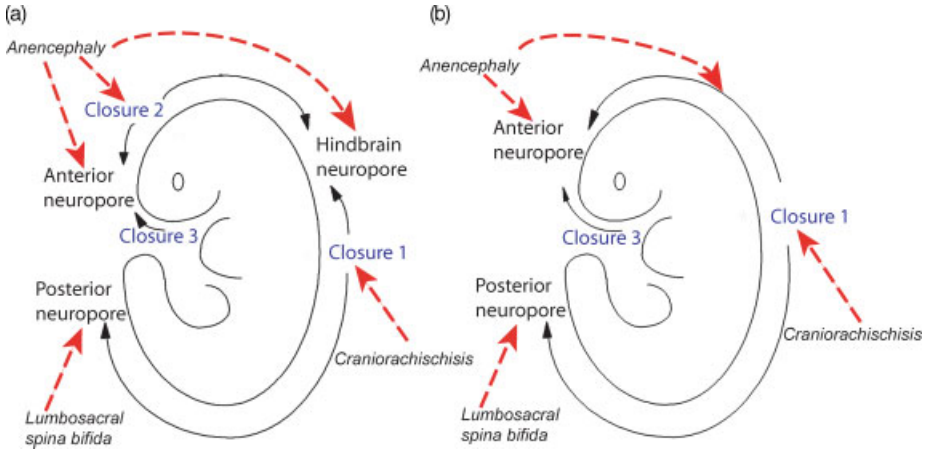

Figure 1—Schematic diagram of neural tube closure and the affected events leading to NTDs (indicated by red arrows). Neural tube closure is initiated by closure 1 at the hindbrain/cervical boundary in mouse (a) and spreads caudally and rostrally from this site (black arrows). Closure 1 in human (b) may occur in the rhombencephalon, more rostrally than in mouse. Failure of closure 1 results in craniorachischisis. A second site of initial closure (closure 2) occurs at the forebrain/midbrain boundary in most mouse strains (a), although the position of the site of closure 2 may vary between strains. This site may be absent in humans (b). Closure also initiates at the rostral limit of the forebrain (closure 3) in mouse and an equivalent closure occurs in humans. Progress of neurulation from the initial sites of fusion results in shortening and closure of the anterior and hindbrain neuropores, indicated by arrows. Failure of initial closure sites or closure of neuropores results in anencephaly. Neurulation progresses caudally from the site of closure 1 until fusion is finally completed by closure of the posterior neuropore. Open spina bifida results from failure of posterior neuropore closure. Secondary neurulation proceeds from the level of the closed posterior neuropore. Modified from (Copp *et al.*, 1994, 2003b)

In the human embryo, neural plate bending begins at about 17–18 days after fertilization. Initiation of neural tube closure occurs, as in mice, by a discontinuous process (Figure 1), and analogous events to closure 1 and 3 have been described (O'Rahilly and Müller, 1994, 2002). The site of initial closure (equivalent to mouse closure 1) may occur at a slightly more rostral level in humans than in mouse, being located in the rhombencephalon as opposed to the rhombencephalon/cervical boundary (O'Rahilly and Müller, 2002). Closure at the extreme rostral end of the neural plate (closure 3) appears to occur in humans as in the mouse (O'Rahilly and Müller, 2002). However, the existence of an event equivalent to closure 2 is more controversial, having been proposed in some studies (Van Allen *et al.*, 1993; Golden and Chernoff, 1995; Seller, 1995b) but not others (O'Rahilly and Müller, 2002). The presence of closure 2 has been inferred from observation of late stage anencephalic fetuses (Van Allen *et al.*, 1993; Seller, 1995b), whereas direct analysis of early human embryos has suggested that a closure 2 event either occurs at a more caudal position than in mice, in the hindbrain (Nakatsu *et al.*, 2000), or not at all (O'Rahilly and Müller, 2002). Therefore, there may be variability in the position or occurrence of closure 2 in human neurulation. Closure in the cranial region is completed on day 25 and closure of the posterior neuropore, which completes primary neurulation, at 26–28 days postfertilization.

DIFFERING MECHANISMS OF CLOSURE IN THE CRANIAL AND SPINAL REGIONS

Although the principles of neurulation are conserved throughout primary neurulation, involving elevation and fusion of neural folds, the detailed mechanism appears to differ markedly with axial level and developmental stage. Thus, in the cranial region of the mouse embryo bending of the neural folds during closure is quite different from that in the spinal region. As they initially elevate, about a midline hinge point, the neural folds assume a biconvex appearance in the midbrain with the tips of the folds facing away from the midline. The folds then switch orientation to assume a biconcave shape in which the tips approach each other in the midline, allowing fusion to occur (Morriss-Kay, 1981; Morriss-Kay *et al.*, 1994).

Spinal neurulation contrasts with cranial closure in lacking a biconvex phase of neural fold elevation. Instead, the spinal neural folds exhibit a stereotypical pattern of bending with hinge points at two locations: the median hinge point (MHP) overlying the notochord, which creates the midline 'neural groove' with its V-shaped cross-section, and paired dorsolateral hinge points (DLHPs), which create longitudinal furrows that bring the neural fold tips toward each other in the dorsal midline. Different combinations of bending points are utilized as closure progresses down the spinal neuraxis (Figure 2). In the early stages of spinal neurulation, at E8.5, the neural plate bends solely at the MHP, whereas by early E9.5, as closure progresses to the thoracic level, bending occurs at DLHPs in addition to the MHP. At E10, when the low spinal neural tube is forming, MHP bending is lost and the neural plate bends solely at DLHPs (Shum and Copp, 1996).

FAILURE OF NEURULATION RESULTS IN NEURAL TUBE DEFECTS

Open NTDs (including open spina bifida and anencephaly in mammals) result from failure of the neural

DOI: 10.1002/pd

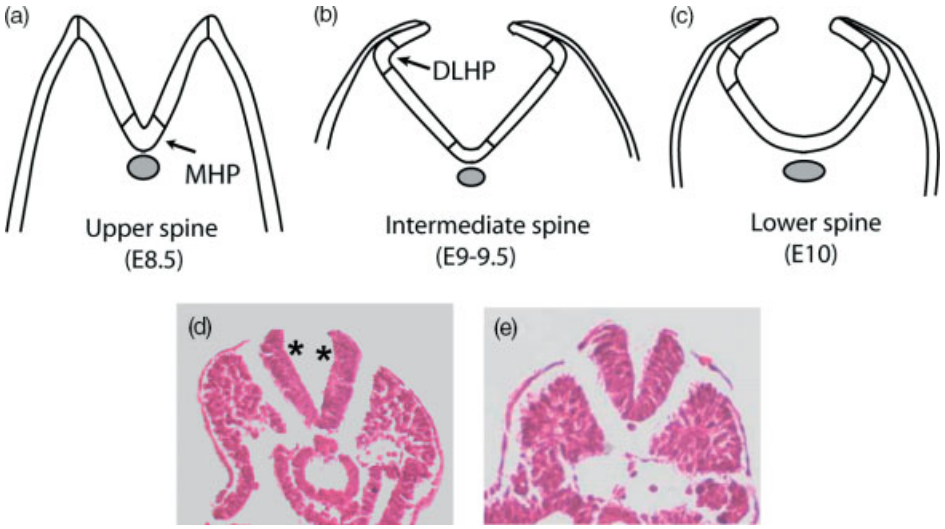

Figure 2—Differing morphology of spinal neurulation, as seen in schematic transverse section. The posterior neuropore progresses along the body axis in a caudal direction during the developmental stages that encompass neural tube closure (E8.5–10.5). The morphology varies such that neuroepithelial bending occurs only at the median hinge point (MHP) at upper spinal levels (b). At intermediate spinal levels (c), closure is characterized by the presence of median and dorsolateral hinge points (DLHP). In the lower spine at the final stages of posterior neuropore closure (c), dorsolateral hinge points are present but there is no median hinge point. In transverse sections through the posterior neuropore at E9.0, DLHPs are evident in wild-type embryos (asterisks in d), but are absent in the neural folds of a homozygous mutant *Zic2*$^{Ku/Ku}$ embryo (e), thereby preventing spinal neural tube closure. Modified from (Copp *et al*., 2003b; Ybot-Gonzalez *et al*., 2007a)

folds to appose and fuse during primary neurulation. These defects are unique to higher vertebrates (including amphibians, birds and mammals), in which primary neurulation involves folding and fusion of neural folds. Moreover, they occur only at the levels of the body axis subject to primary neurulation. Exposure of the unfused neuroepithelium to the amniotic environment has pathological consequences including neuronal damage and degeneration. Open NTDs can occur at different levels of the body axis, both in humans and in mutant mouse strains (Figure 1), and this variation in axial level reflects the occurrence of multiple closure sites.

Cranial NTDs (exencephaly/anencephaly)

Failure of cranial neural tube closure results in cranial NTDs, in which the neural folds remain open and exposed to the environment, and the neuroepithelium characteristically appears to protrude from the developing brain, a phenotype known as exencephaly (Figure 3). The skull vault does not form over the open region and subsequent degeneration of the exposed neural tissue gives rise to the typical appearance of anencephaly that is observed at birth (Wood and Smith, 1984). Human anencephaly can be subdivided into those cases affecting predominantly the rostral brain and skull (meroacrania) and those also affecting posterior brain and skull (holoacrania) (Seller, 1995a), but there is no evidence to suggest that these NTD subtypes equate with failure of precise events of cranial neurulation. In mice, incomplete closure of the cranial neural tube may be caused by failure of one of the initiation events (closure 2 or 3) or by a defect in the subsequent 'zippering' and closure of the anterior or hindbrain neuropores following successful completion of closure 2 and 3 (Fleming and Copp, 2000; Martinez-Barbera *et al*., 2002; Copp, 2005). By analogy, given that closure 2 seems unlikely to be a normal feature of human cranial neurulation (O'Rahilly and Müller, 2002), anencephaly in humans could result from failure of progression of closure rostrally from the prospective hindbrain or caudally from the future forebrain. In both mice and humans, it seems likely that association of split face with anencephaly reflects failure of closure 3.

Spinal NTDs (open spina bifida)

Impairment of spinal neurulation can delay closure of the posterior neuropore or in severe cases prevent closure altogether, resulting in open spina bifida. Although, the developmental origin of open spina bifida (spina bifida aperta) is a failure of closure of the spinal neural folds, the clinical appearance may vary. The major forms are myelomeningocele (meningomyelocele, spina bifida cystica), in which the neural tissue is contained within a meninges-covered sac, and myelocele, in which neural tissue is exposed directly to the amniotic fluid.

The term spina bifida refers to the defects in the vertebral arches that obligatorily accompany open lesions. During normal development, the vertebral arches form from the adjacent sclerotomal component of the axial mesoderm, which migrates to surround the closed neural tube and then differentiates to form the axial skeleton. Where the neural folds remain open, the sclerotome is unable to cover the neuroepithelium and skeletogenesis occurs abnormally, leaving the midline exposed.

In most cases, open spina bifida is accompanied by 'tethering', in which the spinal cord is attached to adjacent tissues and becomes damaged as the vertebral column elongates. Tethering may also contribute to

DOI: 10.1002/pd

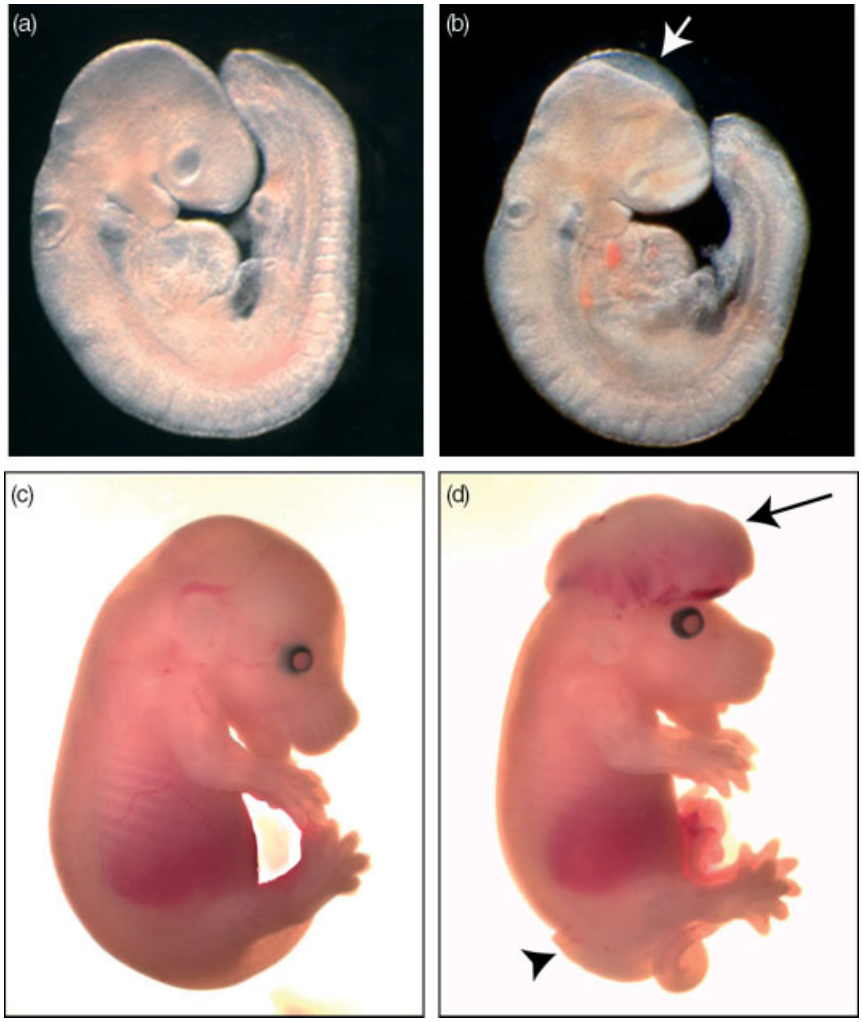

Figure 3—Phenotypic appearance of NTDs in the mouse. In wild-type embryos (a, c) closure of the cranial neural tube is complete by E9.5 (a) and failure of closure (arrow in b) leaves a region of open neural folds (covering the midbrain in the *Pax3* mutant example shown), which is the first stage of exencephaly. At later stages (E15.5 shown), exencephaly appears as a protrusion of nervous tissue in the cranial region (arrow in d), the same fetus also has open spina bifida (arrowhead in d)

damage to closed spinal cord above the open lesion (Stiefel *et al*., 2003). In addition, tethering probably contributes to the development of hydrocephalus, which frequently accompanies open spina bifida. One possible explanation for this association is that attachment of the spinal cord to the growing vertebral column generates traction on the upper cord which causes herniation of the cerebellar vermis (Chiari II malformation) through the foramen magnum and blocks circulation of cerebrospinal fluid (Golden, 2004; Harding and Copp, 2008). However, it has alternatively been suggested that the Chiari II malformation is a separate, coexisting lesion, perhaps reflecting a defect in the posterior skull fossa. Further studies are needed to resolve this issue.

Craniorachischisis

In addition to isolated lesions affecting the cranial or spinal neural tube, NTDs also encompass severe defects, termed craniorachischisis, in which the entire neural tube from the midbrain to the low spine remains open. In the mouse, several genetic mutants that develop craniorachischisis have been identified and analysis of the affected embryos indicates that the fundamental neurulation defect is failure of closure 1, the initial closure event (Copp *et al*., 1994). Given the close resemblance of craniorachischisis in mice to the corresponding human defects, it appears likely that the developmental basis is shared. In mouse mutants that exhibit craniorachischisis, such as the *loop-tail* mouse, closures 2 and 3 occur normally so that the forebrain and rostral midbrain are generally unaffected (Greene *et al*., 1998; Murdoch *et al*., 2003). The relatively normal appearance of the forebrain in many human cases of craniorachischisis suggests that closure 2 (if it exists) and closure 3 also occur normally in these individuals.

Secondary neurulation defects

Although most cases of open spina bifida arise due to failure of primary neurulation, abnormalities in secondary neurulation can lead to closed forms of spina

DOI: 10.1002/pd

bifida in which the separation of the neural tube from surrounding tissues in the tail bud is abnormal. This commonly leads to tethering of the spinal cord, which may cause neuronal damage at the site of the defect, due to differential rates of elongation of the spinal cord and vertebral column as the body axis lengthens during development.

Spina bifida defects not resulting from failure of closure (spina bifida occulta)

In contrast to open lesions that result from failure of neural fold fusion, another group of spina bifida malformations result from defects in development of the axial mesoderm such that the vertebral arches form abnormally, despite the completion of neural tube closure or secondary neural tube formation (depending on axial level). Spina bifida occulta comprises 'closed' defects in which vertebral arches are malformed but the lesion is skin-covered. Defects usually occur at the low lumbar and sacral regions and may be accompanied by tethering of the spinal cord. Defects of the axial mesoderm that forms the vertebrae or cranium may also give rise to encephalocele or meningocele in which the closed neural tube herniates through the affected region of skull or vertebral column, respectively (Harding and Copp, 2008).

CELLULAR AND MOLECULAR BASIS OF NEURULATION

A large amount of information has accumulated on the developmental mechanisms underlying neural tube closure (Colas and Schoenwolf, 2001; Copp *et al*., 2003b; Copp, 2005). In addition, insights into the molecular requirements for neurulation are provided by the more than 150 genetic mutants that exhibit NTDs in mice. Among mouse genetic mutants, around 90% exhibit cranial defects and 25% spina bifida, with some displaying a combination of the defects (Harris and Juriloff, 2007). Two points arise: first, although functional redundancy is likely in neurulation as in most complex biological processes, there are many 'weak points' where loss of a single gene product prevents completion of neural tube closure. Second, the cranial region is much more susceptible to genetic insult than the spinal region, an observation which parallels similar conclusions in relation to the production of NTDs by teratogenic agents (Copp *et al*., 1990). This sensitivity may relate to the complex bending of the cranial neural folds and the physical constraints on closure imposed by the inherent flexure of the cranial region. In humans, anencephaly does not appear to be more common than spina bifida, possibly suggesting that cranial neurulation is more robust than in mice. Alternatively, the genetic factors found to cause cranial NTDs in mice may be uncommon in the human population compared to factors that predispose to spinal NTDs.

A key aim for developmental biologists is to understand how function of the many genes required for neurulation lead ultimately to the complex morphological tissue movements required for neural tube closure. A full understanding of the neurulation process will therefore require integration of data regarding gene and protein function, their effects on cellular properties and the consequent mechanical outcomes (Copp *et al*., 2003b; Chen and Brodland, 2008). A detailed description of each of the potential mechanisms underlying neural tube closure or development of NTDs is beyond the scope of this review. However, we will highlight some of the long-standing hypotheses and recent advances toward linking molecular pathways and morphological outcomes.

Shaping of the neuroepithelium

Shaping of the initially disc-shaped neural plate is required to ensure formation of a tube rather than a spherical vesicle (Schoenwolf and Smith, 1990a). Prior to and during closure, the neural plate lengthens and narrows with concomitant formation of neural folds at the lateral edges. Several processes may contribute to this process: (1) the rostro–caudal axis elongates as a result of growth of the caudal region of the embryo, that continues throughout the period of neural tube closure; (2) apicobasal elongation of cells as the neural plate forms may contribute to narrowing (Colas and Schoenwolf, 2001) and (3) convergent extension cell movements contribute to narrowing and lengthening of the neural plate (Keller, 2002) and are essential for initiation of mammalian neural tube closure (Copp *et al*., 2003a; Ueno and Greene, 2003).

First described in amphibia, convergent extension is driven by lateral-to-medial displacement of cells in the presumptive mesoderm and neural plate. Cell intercalation in the midline leads to medial–lateral narrowing (convergence) and rostro–caudal lengthening (extension) of the body axis (Jacobson and Gordon, 1976; Keller *et al*., 1992). At the molecular level, convergent extension depends on a noncanonical Wnt signaling pathway, termed the planar cell polarity (PCP) pathway (Mlodzik, 2002; Zohn *et al*., 2003). This pathway shares components of the Wnt signaling pathway, such as frizzled membrane receptors, but does not involve downstream signaling via stabilization of beta-catenin. Specific inhibition of PCP signaling in the frog *Xenopus laevis*, by functional disruption of the key signaling molecule dishevelled (Dvl), resulted in inhibition of convergent extension and gave rise to short, broad embryos in which the neural folds remained apart and did not close (Wallingford and Harland, 2001, 2002). These findings provided a mechanistic link between the PCP pathway, convergent extension and neurulation. Experimental disturbance of other components of the PCP pathway including strabismus and prickle also inhibits convergent extension (Darken *et al*., 2002; Goto and Keller, 2002; Takeuchi *et al*., 2003). At the cellular level, PCP signaling is thought to control polarized cellular motility, in particular, through regulation of formation

DOI: 10.1002/pd

of stable mediolaterally oriented actin-rich lamellipodia, which provide cell–cell and cell-matrix traction (Wallingford *et al*., 2000; Keller *et al*., 2008).

In mice, loss of function of the PCP pathway gene *Vangl2* (the homolog of Drosophila strabismus/Van gogh) in the *loop-tail* mutant (Kibar *et al*., 2001; Murdoch *et al*., 2001) or two of the three disheveled genes (*Dvl-1* and *-2*) (Hamblet *et al*., 2002), also suppresses convergent extension cell movements resulting in a broad neural plate and failure of initiation of closure (closure 1), leading to craniorachischisis (Greene *et al*., 1998; Wang *et al*., 2006b; Ybot-Gonzalez *et al*., 2007b). Other genes encoding PCP components have also been found to cause craniorachischisis when mutant or knocked out in the mouse. These include *Celsr1* (the homolog of *Drosophila flamingo/starry night*) in *Crash* mice (Curtin *et al*., 2003), *Scrb1* (in the *Circletail* mouse) (Murdoch *et al*., 2003), the tyrosine kinase *Ptk7* (Lu *et al*., 2004) and *frizzled-3* and *frizzled-6* (craniorachischisis in double knockout) (Wang *et al*., 2006a). PCP pathway genes that play a role in convergent extension thus represent good candidates as the causative genes for NTDs, particularly craniorachischisis, in humans.

CRANIAL NEURULATION—A ROLE FOR MESENCHYME EXPANSION AND THE ACTIN CYTOSKELETON?

The mechanisms controlling the complex bending of the neural folds during cranial neurulation are poorly understood, but there is evidence for roles of the underlying cranial mesenchyme and the actin cytoskeleton. Elevation of the cranial neural folds is preceded by an increase in extracellular space, owing to accumulation of highly hydrated extracellular matrix molecules, and increased proliferation rate in the subjacent cranial mesenchyme (Solursh and Morriss, 1977; Morriss and Solursh, 1978a,b; Tuckett and Morriss-Kay, 1986). It is hypothesized that expansion of the mesenchyme supports the elevation of the convex neural folds. In support of this idea, treatment of rat embryos in culture with hyaluronidase that digests extracellular matrix hyaluronan, results in collapse of the cranial mesenchyme and causes delay of cranial neural tube closure (Morriss-Kay *et al*., 1986). A cellular deficit in the cranial mesenchyme, due to reduced proliferation, is also associated with cranial NTDs in *Twist* knockout mice (Chen and Behringer, 1995). In contrast to the cranial region, spinal neurulation does not depend on integrity of adjacent mesoderm, since closure continues even when the paraxial mesoderm is removed from the spinal region of mouse embryos (Ybot-Gonzalez *et al*., 2002).

A long-standing question in neurulation studies relates to the potential role of the cytoskeleton in formation and movement of the neural folds (Nagele and Lee, 1980; Sadler *et al*., 1982; Schoenwolf *et al*., 1988). Actin microfilaments, and actin-associated proteins such as MARCKS, are localized circumferentially at the apical region of cells in the neural folds such that the future lumen is bordered by actin (Sadler *et al*., 1982; Ybot-Gonzalez and Copp, 1999; Zolessi and Arruti, 2001). An obvious implication of this asymmetric distribution in the neuroepithelium is that constriction of apically located microfilaments could reduce the apical surface area of neuroepithelial cells and contribute to bending and closure of the neural folds (see below). Indeed, experimental disruption of the actin cytoskeleton by actin-disassembling drugs such as cytochalasins causes exencephaly in cultured rodent embryos indicating a role in cranial neural tube closure (Morriss-Kay and Tuckett, 1985; Matsuda and Keino, 1994; Ybot-Gonzalez and Copp, 1999). At higher doses than those required to induce exencephaly, cytochalasin D also inhibits closure 1 (Ybot-Gonzalez and Copp, 1999). These observations suggest that elevation or apposition of the neural folds at the initial closure site and in the future brain depends on the integrity of the actin microfilaments. The occurrence of exencephaly in several mouse mutants in which cytoskeletal function is disturbed provides further evidence for a critical role in cranial closure (Copp *et al*., 2003b; Harris and Juriloff, 2007). However, whether the contraction of actin microfilaments plays an active role in neural fold elevation, or rather acts to stabilize this dynamic process, remains unclear.

There appears to be differential requirement for the actin cytoskeleton with axial level, since spinal neurulation appears resistant to cytochalasin D. Closure continues in treated embryos, although toxicity of this agent precludes analysis of closure throughout the entire period of spinal neurulation (Ybot-Gonzalez and Copp, 1999). Interestingly, although most mice with knockout mutations in cytoskeletal genes exhibit only cranial NTDs (Copp, 2005), mice with inactivation of *Shroom3* (Hildebrand and Soriano, 1999) and *MARCKS-related protein* (MacMARCKS) (Wu *et al*., 1996) develop spina bifida in some homozygotes, in addition to the more prevalent phenotype of exencephaly. Thus, regulation of the actin–myosin cytoskeleton may be necessary to support the entire closing neuroepithelium, but appears more critical in cranial than in spinal neurulation.

MOLECULAR REGULATION OF NEURAL PLATE BENDING

The differential usage of MHPs and DLHPs at varying axial levels (Figure 2) means there is a switch from midline-to-dorsolateral bending as neurulation proceeds. DLHPs appear essential for neural tube closure in the low spine, since mice, lacking function of the Zic2 transcription factor exhibit a normal MHP but fail to develop DLHPs (Ybot-Gonzalez *et al*., 2007a) and develop extensive spina bifida (Nagai *et al*., 2000; Elms *et al*., 2003). In contrast, formation of a defined midline bend appears dispensable for closure. During the final period of spinal neurulation, the posterior neuropore does not have a defined MHP and closure is mediated entirely by DLHPs. Indeed, embryos lacking a notochord do not exhibit a MHP, but form ectopic DLHPs along the entire neuraxis, and are able to complete neural tube closure (Ybot-Gonzalez *et al*., 2002). Therefore,

DOI: 10.1002/pd

spinal neural tube closure continues in the absence of the MHP by a 'default' mechanism involving DLHPs only. The lack of requirement for a MHP in neural tube closure is supported by the fact that spinal NTDs do not arise in mouse embryos lacking sonic hedgehog (Shh), HNF3β (Fox a2), Gli2 or Gli 1/2 (Ang and Rossant, 1994; Weinstein *et al*., 1994; Chiang *et al*., 1996; Ding *et al*., 1998; Matise *et al*., 1998; Park *et al*., 2000), all of which fail to form a floor plate. In these targeted mouse mutants, the lumen of the neural tube is circular suggesting that closure occurs by a DLHP-dependent mechanism.

The defined bending of the neuroepithelium at the MHP and DLHP is regulated by mutually antagonistic dorsal and ventral signals external to the neural folds. MHP bending is stimulated by factors secreted by the notochord (Smith and Schoenwolf, 1989; Davidson *et al*., 1999), including sonic hedgehog (Shh), whereas DLHP formation is simultaneously inhibited by Shh (Ybot-Gonzalez *et al*., 2002). Hence, in embryos that lack a notochord, DLHP formation is de-repressed along the whole body axis. Shh blocks DLHP formation via inhibition of Noggin, an antagonist of bone morphogenetic protein (BMP) signaling. BMP2 is secreted from the dorsal-most surface ectoderm and, like Shh, inhibits dorsolateral bending whereas, Noggin, secreted from the tips of the neural folds, overcomes BMP2-mediated inhibition and enables formation of DLHPs (Ybot-Gonzalez *et al*., 2007a). In the upper spine, Shh is secreted strongly from the notochord and suppresses Noggin-mediated DLHP formation whereas, in the low spine, notochordal Shh production is greatly diminished; Noggin is de-repressed, and DLHP formation occurs (Ybot-Gonzalez *et al*., 2007a).

CELLULAR BASIS OF BENDING AT MHP AND DLHPS

At neural plate stages, the neuroepithelium is a pseudostratified epithelium, of single cell thickness. One factor that may contribute to bending of the neuroepithelium at the hinge points is the local adoption of a wedge cell shape, where cells have a wide basal (nonlumenal) pole (Schoenwolf and Smith, 1990a,b). One possibility is that contraction of circumferentially arranged actin-containing microfilaments may lead to cell wedging at hinge points (Schoenwolf and Smith, 1990b). However, actin microfilaments are not concentrated at the hinge points but rather show an even distribution throughout the neuroepithelium. The MHP and the DLHPs are also resistant to cytochalasins (Schoenwolf *et al*., 1988; Ybot-Gonzalez and Copp, 1999). Moreover, the MHP in the mouse is associated with diminished apico–basal cell height, whereas in *Xenopus* apical constriction driven by the Shroom3 protein, is associated with increase in apico–basal height, a microtubule-dependent process (Lee *et al*., 2007). It seems most likely that microfilaments stabilize the shape of the neural folds or newly formed neural tube, rather than driving the wedging of cells at hinge points (Schoenwolf and Smith, 1990b; Colas and Schoenwolf, 2001).

An alternative explanation for cell wedging at hinge points is based on the observation that cell cycle-dependent variations in the apico–basal position of nuclei within the neuroepithelium can dictate cell shape (Schoenwolf and Smith, 1990b). Local disturbance of interkinetic nuclear migration leads to accumulation of nuclei basally, in S-phase of the cell cycle, with consequent adoption of a wedge shape. Indeed, the MHP is enriched for such cells, unlike nonbending neuroepithelial regions (Schoenwolf and Franks, 1984). A requirement for cell cycle regulation and interkinetic nuclear migration in formation of the DLHP has not been demonstrated to date.

ADHESION OF APPOSED NEURAL FOLDS IS ESSENTIAL FOR CLOSURE

Once the tips of the neural folds have achieved apposition at the dorsal midline, fusion must occur at the contact points in order to complete neural tube formation. Cellular protrusions, resembling filopodia, have been detected on the cells at the tips of the neural folds (Geelen and Langman, 1979). These appear to interdigitate as the bilateral folds come into apposition, providing an initial contact that facilitates subsequent cell adhesion. NTDs would be expected to result from loss of function of the key molecules that mediate adhesion. Thus, mice that lack the cell surface Eph ligand, ephrinA5 exhibit exencephaly as do mice that lack the EphA7 receptor, a potential ligand for ephrinA5 (Holmberg *et al*., 2000).

ADDITIONAL CELLULAR DISTURBANCES THAT MAY BE IMPLICATED IN CAUSATION OF NTDS

In addition to defects of shaping, bending or adhesion of the neural folds, other cellular disturbances have also been proposed to potentially play a role in NTDs, mainly on the basis of outcome of pharmacological or genetic manipulation of the mouse embryo. These include excessive or insufficient apoptosis and premature neurogenesis in the neural folds (Ishibashi *et al*., 1995; Weil *et al*., 1997; Copp, 2005). Failure of emigration of neural crest cells has also been proposed as a potential cause of NTDs in the cranial region (Morriss-Kay *et al*., 1994), where unlike the spinal neural tube, neural crest cells migrate prior to closure of the neural folds (Morriss-Kay and Tan, 1987). The evidence in mammalian embryos is that nonneural tissues are unlikely to provide the motive force for spinal neurulation. However, abnormalities in nonneural tissues can have deleterious effects, even though these tissues do not 'actively' drive neurulation. For example, reduced proliferation in the hindgut of *curly tail* (*grainyhead-like-3* mutant) mice, leads to a growth imbalance between dorsal and ventral tissues that causes excessive curvature of the caudal region, which suppresses closure of the posterior neuropore (Van Straaten and Copp, 2001; Gustavsson *et al*., 2007, 2008).

DOI: 10.1002/pd

SUMMARY

Formation of the neural tube depends on a series of coordinated morphological events that results in conversion of the flat neural plate into the neural tube. The details of the neurulation process vary along the body axis in terms of timing, morphology and sensitivity to genetic or environmental insult. Failure of neurulation results in NTDs that may occur at cranial and/or spinal levels, and constitute a group of severe birth defects in humans. Evidence from careful observation as well as genetic or experimental manipulations of model organisms has contributed to an increasing understanding both of the normal processes of neurulation and the mechanisms underlying the development of NTDs. Going forward, a challenge will be to integrate findings on molecular events, with their cellular outcomes and ultimately understand how this translates into the mechanical forces that drive folding and closure of the neural folds.

REFERENCES

Ang S-L, Rossant J. 1994. *HNF-3β* is essential for node and notochord formation in mouse development. *Cell* **78**: 561–574.

Chen Z-F, Behringer RR. 1995. *twist* is required in head mesenchyme for cranial neural tube morphogenesis. *Genes Dev* **9**: 686–699.

Chen X, Brodland GW. 2008. Multi-scale finite element modeling allows the mechanics of amphibian neurulation to be elucidated. *Phys. Biol.* **5**: 015003 (15 pp).

Chiang C, Litingtung Y, Lee E, *et al*. 1996. Cyclopia and defective axial patterning in mice lacking *Sonic hedgehog* gene function. *Nature* **383**: 407–413.

Colas JF, Schoenwolf GC. 2001. Towards a cellular and molecular understanding of neurulation. *Dev Dyn* **221**: 117–145.

Copp AJ. 2005. Neurulation in the cranial region—normal and abnormal. *J Anat* **207**: 623–635.

Copp AJ, Brook FA. 1989. Does lumbosacral spina bifida arise by failure of neural folding or by defective canalisation? *J Med Genet* **26**: 160–166.

Copp AJ, Brook FA, Estibeiro JP, Shum ASW, Cockroft DL. 1990. The embryonic development of mammalian neural tube defects. *Prog Neurobiol* **35**: 363–403.

Copp AJ, Checiu I, Henson JN. 1994. Developmental basis of severe neural tube defects in the *loop-tail* (*Lp*) mutant mouse: Use of microsatellite DNA markers to identify embryonic genotype. *Dev Biol* **165**: 20–29.

Copp AJ, Greene NDE, Murdoch JN. 2003a. Dishevelled: linking convergent extension with neural tube closure. *Trends Neurosci* **26**: 453–455.

Copp AJ, Greene NDE, Murdoch JN. 2003b. The genetic basis of mammalian neurulation. *Nat Rev Genet* **4**: 784–793.

Curtin JA, Quint E, Tsipouri V, *et al*. 2003. Mutation of *Celsr1* disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse. *Curr Biol* **13**: 1–20.

Darken RS, Scola AM, Rakeman AS, Das G, Mlodzik M, Wilson PA. 2002. The planar polarity gene strabismus regulates convergent extension movements in Xenopus. *EMBO J* **21**: 976–985.

Davidson BP, Kinder SJ, Steiner K, Schoenwolf GC, Tam PPL. 1999. Impact of node ablation on the morphogenesis of the body axis and the lateral asymmetry of the mouse embryo during early organogenesis. *Dev Biol* **211**: 11–26.

Ding Q, Motoyama J, Gasca S, *et al*. 1998. Diminished Sonic hedgehog signaling and lack of floor plate differentiation in Gli2 mutant mice. *Development* **125**: 2533–2543.

Elms P, Siggers P, Napper D, Greenfield A, Arkell R. 2003. Zic2 is required for neural crest formation and hindbrain patterning during mouse development. *Dev Biol* **264**: 391–406.

Fleming A, Copp AJ. 2000. A genetic risk factor for mouse neural tube defects: defining the embryonic basis. *Hum Mol Genet* **9**: 575–581.

Geelen JAG, Langman J. 1979. Ultrastructural observations on closure of the neural tube in the mouse. *Anat Embryol* **156**: 73–88.

Golden JA. 2004. Chiari malformations. *Pathology & Genetics. Developmental Neuropathology*. ISN Neuropath Press: Basel; 90–94.

Golden JA, Chernoff GF. 1995. Multiple sites of anterior neural tube closure in humans: Evidence from anterior neural tube defects (anencephaly). *Pediatrics* **95**: 506–510.

Goto T, Keller R. 2002. The planar cell polarity gene Strabismus regulates convergence and extension and neural fold closure in Xenopus. *Dev Biol* **247**: 165–181.

Greene NDE, Gerrelli D, Van Straaten HWM, Copp AJ. 1998. Abnormalities of floor plate, notochord and somite differentiation in the *loop-tail* (*Lp*) mouse: a model of severe neural tube defects. *Mech Dev* **73**: 59–72.

Gustavsson P, Copp AJ, Greene ND. 2008. Grainyhead genes and mammalian neural tube closure. *Birth Defects Res. Part A : Clin Mol Teratol* **82**: 728–735.

Gustavsson P, Greene ND, Lad D, *et al*. 2007. Increased expression of Grainyhead-like-3 rescues spina bifida in a folate-resistant mouse model. *Hum Mol Genet* **16**: 2640–2646.

Hamblet NS, Lijam N, Ruiz-Lozano P, *et al*. 2002. Dishevelled 2 is essential for cardiac outflow tract development, somite segmentation and neural tube closure. *Development* **129**: 5827–5838.

Harding BN, Copp AJ. 2008. Congenital malformations. In *Greenfield's Neuropathology* (8th edn), Love S, Louis D, Ellison D (eds). Hodder Arnold: London.

Harris MJ, Juriloff DM. 2007. Mouse mutants with neural tube closure defects and their role in understanding human neural tube defects. *Birth Defects Res A Clin Mol Teratol* **79**: 187–210.

Hildebrand JD, Soriano P. 1999. Shroom, a PDZ domain-containing actin-binding protein, is required for neural tube morphogenesis in mice. *Cell* **99**: 485–497.

Holmberg J, Clarke DL, Frisén J. 2000. Regulation of repulsion versus adhesion by different splice forms of an Eph receptor. *Nature* **408**: 203–206.

Ishibashi M, Ang S-L, Shiota K, Nakanishi S, Kageyama R, Guillemot F. 1995. Targeted disruption of mammalian *hairy* and *Enhancer of split* homolog-1 (*HES-1*) leads to up-regulation of neural helix-loop-helix factors, premature neurogenesis, and severe neural tube defects. *Genes Dev* **9**: 3136–3148.

Jacobson AG, Gordon R. 1976. Changes in the shape of the developing vertebrate nervous system analyzed experimentally, mathematically and by computer simulation. *J Exp Zool* **197**: 191–246.

Keller R. 2002. Shaping the vertebrate body plan by polarized embryonic cell movements. *Science* **298**: 1950–1954.

Keller R, Shih J, Sater AK, Moreno C. 1992. Planar induction of convergence and extension of the neural plate by the organizer of *Xenopus*. *Dev Dyn* **193**: 218–234.

Keller R, Shook D, Skoglund P. 2008. The forces that shape embryos: physical aspects of convergent extension by cell intercalation. *Phys Biol* **5**: 015007 (23 pp).

Kibar Z, Vogan KJ, Groulx N, Justice MJ, Underhill DA, Gros P. 2001. *Ltap*, a mammalian homolog of *Drosophila Strabismus/Van Gogh*, is altered in the mouse neural tube mutant Loop-tail. *Nat Genet* **28**: 251–255.

Lee C, Scherr HM, Wallingford JB. 2007. Shroom family proteins regulate gamma-tubulin distribution and microtubule architecture during epithelial cell shape change. *Development* **134**: 1431–1441.

Lu X, Borchers AG, Jolicoeur C, Rayburn H, Baker JC, Tessier-Lavigne M. 2004. PTK7/CCK-4 is a novel regulator of planar cell polarity in vertebrates. *Nature* **430**: 93–98.

Martinez-Barbera JP, Rodriguez TA, Greene NDE, *et al*. 2002. Folic acid prevents exencephaly in *Cited2* deficient mice. *Hum Mol Genet* **11**: 283–293.

Matise MP, Epstein DJ, Park HL, Platt KA, Joyner AL. 1998. Gli2 is required for induction of floor plate and adjacent cells, but not most ventral neurons in the mouse central nervous system. *Development* **125**: 2759–2770.

Matsuda M, Keino H. 1994. An open cephalic neural tube reproducibly induced by cytochalasin D in rat embryos *in vitro*. *Zoolog Sci* **11**: 547–553.

Mlodzik M. 2002. Planar cell polarization: do the same mechanisms regulate Drosophila tissue polarity and vertebrate gastrulation? *Trends Genet* **18**: 564–571.

Morriss GM, Solursh M. 1978a. Regional differences in mesenchymal cell morphology and glycosaminoglycans in early neural-fold stage rat embryos. *J Embryol Exp Morphol* **46**: 37–52.
Morriss GM, Solursh M. 1978b. The role of primary mesenchyme in normal and abnormal morphogenesis of mammalian neural folds. *Zoon* **6**: 33–38.
Morriss-Kay GM. 1981. Growth and development of pattern in the cranial neural epithelium of rat embryos during neurulation. *J Embryol Exp Morphol* **65**(Suppl.): 225–241.
Morriss-Kay G, Tan S-S. 1987. Mapping cranial neural crest cell migration pathways in mammalian embryos. *Trends Genet* **3**: 257–261.
Morriss-Kay GM, Tuckett F. 1985. The role of microfilaments in cranial neurulation in rat embryos: effects of short-term exposure to cytochalasin D. *J Embryol Exp Morphol* **88**: 333–348.
Morriss-Kay GM, Tuckett F, Solursh M. 1986. The effects of Streptomyces hyaluronidase on tissue organization and cell cycle time in rat embryos. *J Embryol Exp Morphol* **98**: 59–70.
Morriss-Kay G, Wood H, Chen W-H. 1994. Normal neurulation in mammals. *Ciba Found Symp* **181**: 51–63.
Murdoch JN, Doudney K, Paternotte C, Copp AJ, Stanier P. 2001. Severe neural tube defects in the *loop-tail* mouse result from mutation of *Lpp1*, a novel gene involved in floor plate specification. *Hum Mol Genet* **10**: 2593–2601.
Murdoch JN, Henderson DJ, Doudney K, *et al*. 2003. Disruption of *scribble* (*Scrb1*) causes severe neural tube defects in the *circletail* mouse. *Hum Mol Genet* **12**: 87–98.
Nagai T, Aruga J, Minowa O, *et al*. 2000. Zic2 regulates the kinetics of neurulation. *Proc Natl Acad Sci U S A* **97**: 1618–1623.
Nagele RG, Lee H. 1980. Studies on the mechanisms of neurulation in the chick: microfilament-mediated changes in cell shape during uplifting of neural folds. *J Exp Zool* **213**: 391–398.
Nakatsu T, Uwabe C, Shiota K. 2000. Neural tube closure in humans initiates at multiple sites: evidence from human embryos and implications for the pathogenesis of neural tube defects. *Anat Embryol* **201**: 455–466.
O'Rahilly R, Müller F. 1994. Neurulation in human embryos. In *Neural Tube Defects (Ciba Foundation Symposium 181)*, Bock G, Marsh J (eds). John Wiley and Sons: Chichester; 70–89.
O'Rahilly R, Müller F. 2002. The two sites of fusion of the neural folds and the two neuropores in the human embryo. *Teratology* **65**: 162–170.
Park HL, Bai C, Platt KA, *et al*. 2000. Mouse *Gli1* mutants are viable but have defects in SHH signaling in combination with a *Gli2* mutation. *Development* **127**: 1593–1605.
Sadler TW, Greenberg D, Coughlin P, Lessard JL. 1982. Actin distribution patterns in the mouse neural tube during neurulation. *Science* **215**: 172–174.
Schoenwolf GC. 1984. Histological and ultrastructural studies of secondary neurulation of mouse embryos. *Am J Anat* **169**: 361–374.
Schoenwolf GC, Folsom D, Moe A. 1988. A reexamination of the role of microfilaments in neurulation in the chick embryo. *Anat Rec* **220**: 87–102.
Schoenwolf GC, Franks MV. 1984. Quantitative analyses of changes in cell shapes during bending of the avian neural plate. *Dev Biol* **105**: 257–272.
Schoenwolf GC, Smith JL. 1990a. Epithelial cell wedging: a fundamental cell behavior contributing to hinge point formation during epithelial morphogenesis. *Semin Dev Biol* **1**: 325–334.
Schoenwolf GC, Smith JL. 1990b. Mechanisms of neurulation: Traditional viewpoint and recent advances. *Development* **109**: 243–270.
Seller MJ. 1995a. Sex, neural tube defects, and multisite closure of the human neural tube. *Am J Med Genet* **58**: 332–336.
Seller MJ. 1995b. Further evidence for an intermittent pattern of neural tube closure in humans. *J Med Genet* **32**: 205–207.
Shum ASW, Copp AJ. 1996. Regional differences in morphogenesis of the neuroepithelium suggest multiple mechanisms of spinal neurulation in the mouse. *Anat Embryol* **194**: 65–73.
Smith JL, Schoenwolf GC. 1989. Notochordal induction of cell wedging in the chick neural plate and its role in neural tube formation. *J Exp Zool* **250**: 49–62.
Solursh M, Morriss GM. 1977. Glycosaminoglycan synthesis in rat embryos during the formation of the primary mesenchyme and neural folds. *Dev Biol* **57**: 75–86.
Stiefel D, Shibata T, Meuli M, Duffy P, Copp AJ. 2003. Tethering of the spinal cord in mouse fetuses and neonates with spina bifida. *J Neurosurg (Spine)* **99**: 206–213.
Takeuchi M, Nakabayashi J, Sakaguchi T, *et al*. 2003. The prickle-related gene in vertebrates is essential for gastrulation cell movements. *Curr Biol* **13**: 674–679.
Tuckett F, Morriss-Kay GM. 1986. The distribution of fibronectin, laminin and entactin in the neurulating rat embryo studied by indirect immunofluorescence. *J Embryol Exp Morphol* **94**: 95–112.
Ueno N, Greene NDE. 2003. Planar cell polarity genes and neural tube closure. *Birth Defects Res (Part C)* **69**: 318–324.
Van Allen MI, Kalousek DK, Chernoff GF, *et al*. 1993. Evidence for multi-site closure of the neural tube in humans. *Am J Med Genet* **47**: 723–743.
Van Straaten HWM, Copp AJ. 2001. Curly tail: a 50-year history of the mouse spina bifida model. *Anat Embryol* **203**: 225–237.
Van Straaten HWM, Jansen HCJP, Peeters MCE, Copp AJ, Hekking JWM. 1996. Neural tube closure in the chick embryo is multiphasic. *Dev Dyn* **207**: 309–318.
Wallingford JB, Harland RM. 2001. *Xenopus* Dishevelled signaling regulates both neural and mesodermal convergent extension: parallel forces elongating the body axis. *Development* **128**: 2581–2592.
Wallingford JB, Harland RM. 2002. Neural tube closure requires Dishevelled-dependent convergent extension of the midline. *Development* **129**: 5815–5825.
Wallingford JB, Rowning BA, Vogeli KM, Rothbächer U, Fraser SE, Harland RM. 2000. Dishevelled controls cell polarity during *Xenopus* gastrulation. *Nature* **405**: 81–85.
Wang Y, Guo N, Nathans J. 2006a. The role of Frizzled3 and Frizzled6 in neural tube closure and in the planar polarity of inner-ear sensory hair cells. *J Neurosci* **26**: 2147–2156.
Wang J, Hamblet NS, Mark S, *et al*. 2006b. Dishevelled genes mediate a conserved mammalian PCP pathway to regulate convergent extension during neurulation. *Development* **133**: 1767–1778.
Weil M, Jacobson MD, Raff MC. 1997. Is programmed cell death required for neural tube closure. *Curr Biol* **7**: 281–284.
Weinstein DC, Ruiz A, Altaba I, *et al*. 1994. The winged-helix transcription factor *HNF-3β* is required for notochord development in the mouse embryo. *Cell* **78**: 575–588.
Wood LR, Smith MT. 1984. Generation of anencephaly: 1. Aberrant neurulation and 2. Conversion of exencephaly to anencephaly. *J Neuropathol Exp Neurol* **43**: 620–633.
Wu M, Chen DF, Sasaoka T, Tonegawa S. 1996. Neural tube defects and abnormal brain development in F52-deficient mice. *Proc Natl Acad Sci U S A* **93**: 2110–2115.
Ybot-Gonzalez P, Cogram P, Gerrelli D, Copp AJ. 2002. Sonic hedgehog and the molecular regulation of neural tube closure. *Development* **129**: 2507–2517.
Ybot-Gonzalez P, Copp AJ. 1999. Bending of the neural plate during mouse spinal neurulation is independent of actin microfilaments. *Dev Dyn* **215**: 273–283.
Ybot-Gonzalez P, Gaston-Massuet C, Girdler G, *et al*. 2007a. Neural plate morphogenesis during mouse neurulation is regulated by antagonism of BMP signalling. *Development* **134**: 3203–3211.
Ybot-Gonzalez P, Savery D, Gerrelli D, *et al*. 2007b. Convergent extension, planar-cell-polarity signalling and initiation of mouse neural tube closure. *Development* **134**: 789–799.
Zohn IE, Chesnutt CR, Niswander L. 2003. Cell polarity pathways converge and extend to regulate neural tube closure. *Trends Cell Biol* **13**: 451–454.
Zolessi FR, Arruti C. 2001. Apical accumulation of MARCKS in neural plate cells during neurulation in the chick embryo. *BMC Dev Biol* **1**: 1–7.

DOI: 10.1002/pd