Anat Embryol (1996) 194:65–73


ORIGINAL ARTICLE

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# Regional differences in morphogenesis of the neuroepithelium suggest multiple mechanisms of spinal neurulation in the mouse

Accepted: 15 November 1995

**Abstract** A study of neuroepithelial morphogenesis in the mouse embryo has identified three modes of neural tube formation that occur consecutively as neurulation progresses along the spinal region. The three modes of neurulation differ in the extent to which the neuroepithelium exhibits formation of 'hinge points', i.e. localised bending owing to reduction in apical surface area. In Mode 1, bending occurs only in the neuroepithelium overlying the notochord, creating a median hinge point. The neural folds remain straight along both apical and basal surfaces, resulting in a neural tube with a slit-shaped lumen. In Mode 2, the neuroepithelium forms paired dorsolateral hinge points, as well as a median hinge point, whereas the remaining portions of the neuroepithelium do not bend. This produces a neural tube with a diamond-shaped lumen. In Mode 3 neurulation, the entire neuroepithelium exhibits bending, so that the cells specific hinge points are not discernible; the resulting neural tube has a circular lumen. The three modes of neurulation are present in all three strains of mice studied: C57BL/6, CBA/Ca and *curly tail*, a mutant predisposed to neural tube defects. However, *curly tail* embryos exhibit a delay in transition from Mode 2 to Mode 3, preceding faulty closure of the posterior neuropore. This heterogeneity of neurulation morphogenesis in the mouse embryo indicates that the underlying mechanisms may vary along the body axis. Specifically, we suggest that Mode 1 neurulation is driven largely by forces generated extrinsic to the neuroepithelium, in adjacent tissues, whereas Mode 3 neurulation is dependent primarily on forces generated intrinsic to the neuroepithelium. Down the body axis, there is a gradual decrease in the area of ectoderm involved in neural induction and, as neurulation reaches lower spinal levels, the newly induced neural plate exhibits marked indentation from the time of its first appearance. The transition from primary neurulation (neural folding of Mode 3 type) to secondary neurulation (neural tube formation by cavitation) appears to be a smooth continuation of this trend, with loss of contact between the newly induced neuroepithelium and the outside of the embryo.

**Key words** Embryo · Development · Mutant · Neural tube · Neural induction

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## Introduction

Neurulation is the embryonic event responsible for laying down the primordium of the entire central nervous system. Although often considered a relatively uniform process of 'zipping up', there is increasing evidence to suggest that multiple morphogenetic mechanisms may be involved, with variation along the body axis. It has long been known (Criley 1969; Lemire 1969) that the brain and upper spinal cord develop by folding of the neural plate (primary neurulation) whereas the lower spinal cord forms by a different process in which the neural tube develops by cavitation of a condensed mass of cells (secondary neurulation). More recently, primary neurulation has been shown to comprise several distinct phases. Neural tube closure is initiated separately and sequentially at the cervical/hindbrain boundary, the midbrain/forebrain boundary and at the rostral extremity of the forebrain (MacDonald et al. 1989; Golden and Chernoff 1993). Closure spreads between these initiation sites, and caudally from the cervical/hindbrain closure point, to be completed at locations termed neuropores that are sited in the forebrain, hindbrain and upper sacral region. Definition of these initiation and completion sites of neural tube closure has enabled the variation in morphology and axial level of neural tube defects, in both mouse (Copp et

al. 1990) and human (Van Allen et al. 1993), to be understood in terms of the underlying neurulation events.

Further evidence for heterogeneity of neurulation mechanisms comes from genetic analysis of neural tube defects in the mouse that has shown that distinct neurulation events require the function of different genes. Thus, initiation of cervical/hindbrain closure is disturbed in embryos homozygous for the *loop tail* (*Lp*) mutation whereas initiation of midbrain/forebrain closure is unaffected (Copp et al. 1994). Conversely, midbrain/forebrain closure is disturbed by homozygosity for the *splotch* ($Sp^{2H}$) mutation (A.L. Fleming and A.J. Copp, unpublished work) whereas cervical/hindbrain initiation occurs normally. Closure of the posterior neuropore is disturbed in a third mutant, *curly tail* (*ct*), as well as in $Sp^{2H}/Sp^{2H}$ and *Lp*/+ embryos (Copp et al. 1990, 1994). The link between aetiological factors and neurulation events extends also to teratogenic agents. Thus, a wide variety of agents disrupts cranial neural tube closure, yielding exencephaly, whereas relatively few affect closure of the posterior neuropore (Copp et al. 1990). The conclusion from these studies is that the various component events of neurulation have distinct molecular mechanisms.

Despite this advance in our ability to relate specific genes to particular neurulation events, evidence is lacking with regard to the cellular and morphogenetic basis of regional variations in neurulation mechanisms in mammalian embryos. An important contribution to our understanding of the morphological process of neurulation has come from studies in the chick that show that bending of the neural plate involves elevation of the lateral parts of the neural plate around a fixed median locus and convergence of the neural folds towards the dorsal midline (Schoenwolf and Smith 1990). These processes correlate with the formation of hinge points, where cells become predominantly wedge-shaped, located medially above the notochord (the single median hinge point, MHP) and dorsolaterally within each neural fold (paired dorsolateral hinge points, DLHP). Although it has been well documented that there are regional variations in the brain with regard to the pattern of neurulation, resulting in the different shapes of the forebrain, midbrain and hindbrain (Morriss-Kay 1981), neurulation has not been studied systematically in the spinal region of mammalian embryos. For instance, it is not known whether regional differences exist in the presence of the MHP and DLHP. Therefore, in the present study, we made a systematic examination of the morphogenesis of primary and secondary neurulation along the spinal region of two normal mouse strains and of a mutant strain, *curly tail* (*ct*), that is genetically predisposed to spinal neural tube defects as a result of delayed closure of the posterior neuropore. Our morphological studies yield evidence of regional differences in morphogenetic mechanisms along the spinal region that provides a starting point for molecular analysis of the processes involved.

## Materials and methods

Three mouse strains were used in the study. The inbred strains CBA/Ca (abbreviated to CBA) and C57BL/6 (abbreviated to C57) provided a source of non-mutant embryos and the mutant *curly tail* (*ct*) strain provided embryos genetically predisposed to spinal neural tube defects in 40–60% of cases. *Curly tail* is an autosomal recessive mutation, maintained as a randomly-bred colony of homozygous individuals on a genetic background derived from the CBA/Gr and GFF inbred strains (Grüneberg 1954). Our recent studies suggest that the GFF strain, although no longer available for study, was closely related to C57BL/6 (Neumann et al. 1994). Thus, the non-mutant strains chosen were the most appropriate controls for comparison with the *curly tail* mutant strain. Timed matings were performed for each strain, and embryos were removed from the uterus of pregnant females at various times between 8.5 and 10.5 days of gestation (noon on the day of finding a copulation plug was designated as 0.5 days of gestation). All extraembryonic membranes, including the yolk sac and amnion, were removed and somites were counted. The caudal portion of the embryo was removed, washed twice in ice-cold phosphate-buffered saline and then fixed in Bouin's fluid. After dehydration, tissues were embedded in fibrowax (BDH). Serial 6 μm transverse sections were prepared throughout the posterior neuropore region; sections were stained with haematoxylin and eosin and analysed by light microscopy. Photomicrographs were taken on a Zeiss Axiophot microscope.

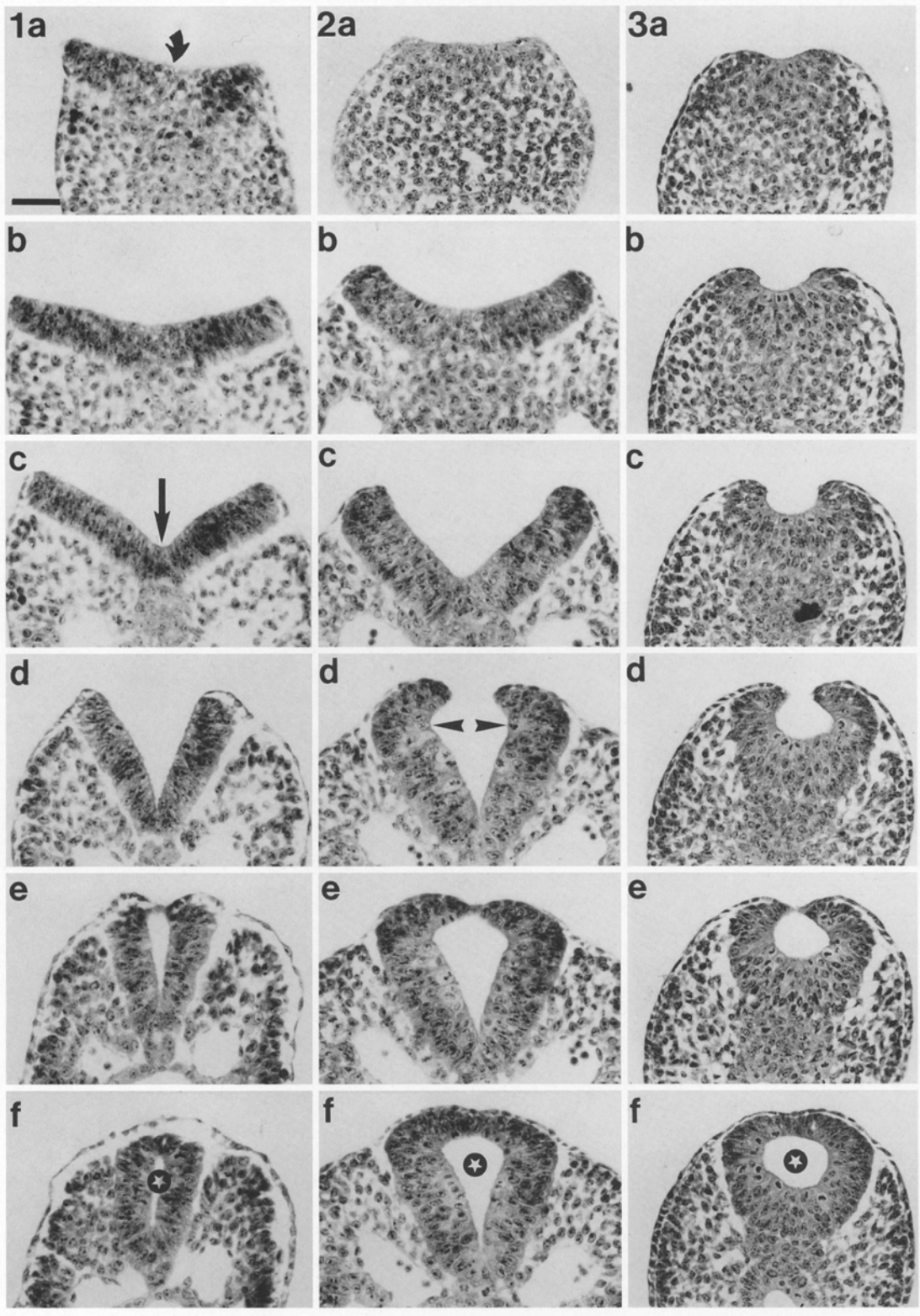
## Results

### Three modes of primary neurulation in the spinal region

Primary neurulation in the spinal region of the mouse embryo proceeds in a cranio-caudal direction. Embryos between the 8 and 30 somite stages contain a posterior neuropore that exhibits all stages of the neurulation process. Thus, the neural plate has just begun to form at the caudal extremity of the neuropore, neural fold elevation is in progress at more cranial levels, while the cranial limit of the neuropore consists of newly closed neural tube. Therefore, by analysing serial sections of the posterior neuropore region in a caudal to cranial direction at a particular developmental stage, the effect is similar to following the sequential changes of neuroepithelial folding at a particular level of the body axis. Embryos were examined between the 8 and 31 somite stages, and three modes of neurulation were identified. Each occurs at a particular axial level and result in the formation of a neural tube with a lumen of characteristic shape.

#### *Mode 1*

Neural induction is underway at the caudal end of the posterior neuropore, resulting in a relatively flat neural plate that covers the whole of the dorsal surface of the embryo (Fig. 1a). Moving in a cranial direction, the lateral aspects of the neural plate begin to elevate relative to the medial region (Fig. 1b). A MHP can be identified at the apex of a V-shaped neural groove (Fig. 1c). More cranially, the sides of the neural plate continue to elevate but, characteristically, remain straight along both apical and basal surfaces (Fig. 1d). At the cranial limit of the posterior neuropore, the tips of the neural folds meet in




**Figs. 1–3** Serial transverse sections presented in a caudal to cranial sequence (*a* to *f*) through the posterior neuropore of CBA embryos to show the three modes of neurulation visible along the spinal region. In all three embryos, the posterior neuropore lies in the presomitic mesoderm. *Bar* 50 μm

**Fig. 1** Embryo with 12 somites demonstrating Mode 1 neurulation. Note the initially flat neural plate (*curved arrow* in *a*), median hinge point (*straight arrow* in *c*), straight sides of neural plate during elevation, and slit-like lumen of the neural tube (*asterisk* in *f*)

**Fig. 2** Embryo with 20 somites demonstrating Mode 2 neurulation. Note the reduction in area of induced neuroepithelium (*a*) and paired dorsolateral hinge points (*arrowheads* in *d*) and diamond-shaped lumen of the neural tube (*asterisk* in *f*)

**Fig. 3** Embryo with 29 somites demonstrating Mode 3 neurulation. Note the generalised wedging of the neuroepithelium and the round lumen of the neural tube (*asterisk* in *f*)

**Table 1** Summary of the three modes of spinal neurulation (*MHP* median hinge point, *DLHP* dorsolateral hinge point)

| Mode | Neural plate | MHP | DLHP | Wedging of all neuroepithelial cells? | Neural lumen shape |
|---|---|---|---|---|---|
| 1 | Flat, occupies entire dorsal surface | Yes | No | No | Slit-like |
| 2 | Slightly concave, occupies medial part of dorsal surface | Yes | Yes | No | Diamond |
| 3 | Grooved, at midline of dorsal surface | No | No | Yes | Round |

**Table 2** Distribution of modes of neurulation among embryos of the three mouse strains, according to somite stage

| Somite No. | Mode 1 | | | Mode 2 | | | Mode 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | C57 | CBA | *curly tail* | C57 | CBA | *curly tail* | C57 | CBA | *curly tail* |
| 8 | ** | * | | | | | | | |
| 9 | * | * | * | | | | | | |
| 10 | * | | | | | | | | |
| 11 | ** | | | | | | | | |
| 12 | * | ** | ** | | | | | | |
| 13 | ** | ** | | | | | | | |
| 14 | **** | ** | * | | | | | | |
| 15 | **** | ** | ** | | | | | * | |
| 16 | ** | | ** | | | | | | |
| 17 | *** | | * | | *** | ** | | | |
| 18 | ** | | | ** | *** | **** | | * | |
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| 20 | | | | ** | ** | ** | | | * |
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| 24 | | | | ***** | *++ | ** | | * | |
| 25 | | | | **++ | *+++ | *** | | * | |
| 26 | | | | +++++ | ++++ | **+ | | ** | |
| 27 | | | | + | | ***++ | | *** | |
| 28 | | | | | | **++ | * | | |
| 29 | | | | | | *++++ | **C | **CC | ** |
| 30 | | | | | | +++ | *CC | **CCC | * |
| 31 | | | | | | * | CCCCC | CCCC | |

* Represents a single embryo
\+ Represents a single embryo in late Mode 2
C Represents a single embryo with closed posterior neuropore

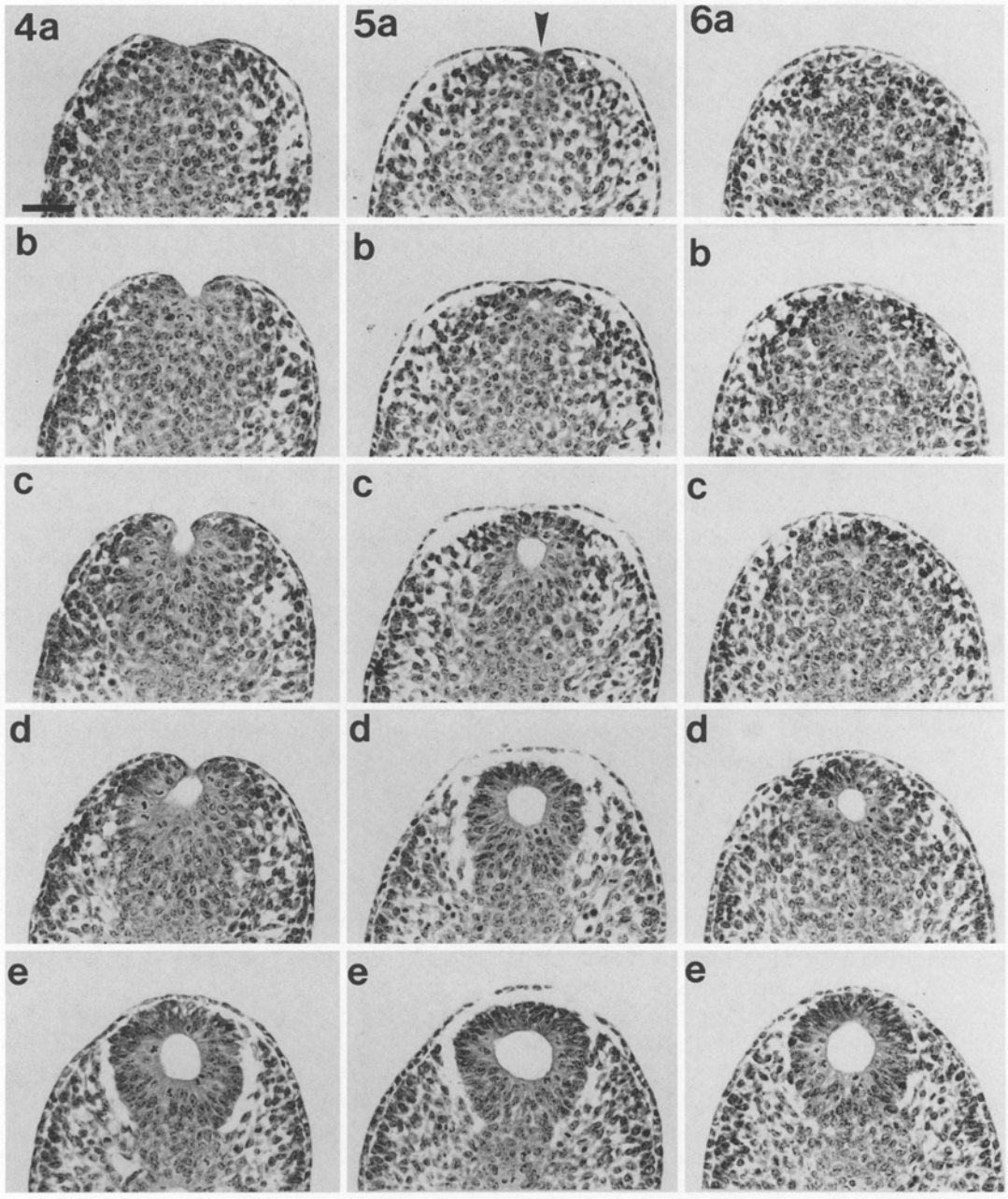
the dorsal midline (Fig. 1e). Fusion of the surface ectoderm on the two sides then takes place to form a continuous layer while neuroepithelial fusion forms the roof plate of the neural tube. The neural lumen, so formed, is a narrow space of uniform width along the dorso-ventral axis (Fig. 1f).

*Mode 2*

Induction of the dorsal ectoderm to become neuroepithelium is restricted to a smaller area of the dorsal embryonic surface than in Mode 1 (compare Figs. 1a and 2a). As in Mode 1, a MHP develops as the lateral aspects of the neural plate elevate (Fig. 2b, c), but when the neural folds are elevated approximately half-way, two furrows, the DLHP, form on the apical surface of the neural folds, which appear to bring about convergence of the tips of the neural folds towards the dorsal midline (Fig. 2d). Fusion of the neural folds produces a neural tube with a diamond-shaped lumen (Fig. 2e, f). Mode 2 neurulation varies in appearance depending on the precise level along the future spinal cord. Moving in a caudal direction, the area of induced neuroepithelium becomes smaller and more concave, and DLHP formation occurs earlier in the process of neural fold elevation (referred to subsequently as 'late Mode 2').

*Mode 3*

Induction of neuroepithelium is now reduced to a very small area at the dorsal midline of the ectoderm (Fig. 3a) and the neuroepithelium is concave from the time of its




**Figs. 4–6** Serial transverse sections presented in a caudal to cranial sequence (*a* to *e*) through the caudal region of C57 embryos at the stage of transition from primary (Mode 3) to secondary neurulation. *Bar* 50 μm

**Fig. 4** Embryo with 30 somites demonstrating the completion of primary neurulation. The newly induced neural plate is indented from the time of its first appearance (*a*) and contact between the neural groove and the outside is rapidly lost (*d*)

**Fig. 5** Embryo with 31 somites in which secondary neurulation has just begun. The appearance is very similar to **Fig. 4**, with a persistent indentation of the dorsal surface ectoderm (*arrowhead* in *a*), even though the connection between the neural lumen and the outside has been lost

**Fig. 6** Embryo with 34 somites in which secondary neurulation is well underway. The dorsal indentation at the caudal end is no longer visible (compare **5a** with **6a**). The neural lumen is round, similar to that formed in Mode 3 primary neurulation (compare **Figs. 4e** and **6e**)

first appearance (Fig. 3a, b). A neural groove with a round base is formed and gradually deepens (Fig. 3b, c). Noticeably, the neuroepithelium and the underlying mesoderm are not clearly demarcated from each other, unlike Mode 1 and 2 neurulation (compare Figs. 1c, 2c and 3c). The concave curvature increases progressively until the tips of the neural folds approach one another (Fig. 3d) and meet in the dorsal midline (Fig. 3e). No distinct MHP nor DLHP can be observed since all cells of the neuroepithelium appear to be participating in its general curvature. The lumen of the Mode 3 neural tube is circular (Fig. 3f).

### Comparison of the mode of neurulation in three mouse strains

Table 1 summarises the morphological features that characterise the three modes of neurulation found in the spinal region. With these characteristics in mind, serial sections were examined through embryos with 8–31 somites, taken from the C57, CBA and *curly tail* strains. Prior to the 22 somite stage, embryos of all three strains behave very similarly (Table 2): Mode 1 neurulation occurs until the 17–18 somite stage when there is an abrupt transition to Mode 2 neurulation. The strains vary, however, in the stage of onset of 'late Mode 2' neurulation, in which DLHP formation is visible as soon as the neural folds begin to elevate. This occurs from the 22 somite stage in CBA embryos, but not until the 25 somite stage in C57 and 26–27 somite stage in *curly tail*. By the 27–28 somite stage, almost all C57 and CBA embryos have switched to Mode 3 neurulation whereas *curly tail* embryos persist in Mode 2, apart from a few embryos with fairly normal-sized neuropores that exhibit Mode 3 neurulation. From the 29 somite stage onwards, most C57 and CBA embryos have closed neuropores whereas all *curly tail* embryos had persistently open neuropores up to the 31 somite stage. Closure in *curly tail* embryos is known to occur mainly between the 33 and 40 somite stages (Van Straaten et al. 1992) although a proportion fail to close their neuropores altogether, progressing to the development of spinal bifida.

### Transition from primary to secondary neurulation

The transition from Mode 3 primary neurulation to secondary neurulation is clearly seen in a series of three C57 embryos with 30, 31 and 34 somites respectively (Figs. 4, 5 and 6). Just prior to completion of primary neurulation (Fig. 4), a small depression is visible at the dorsal midline of the surface ectoderm (Fig. 4a). Examining serial sections in a caudal to cranial direction, the depression gradually gets deeper (Fig. 4b) and a small round groove is formed (Fig. 4c). Within a short distance, the neural groove closes dorsally (Fig. 4d) and a neural tube with a circular neural lumen can be seen (Fig. 4e). This represents the final stage in posterior neuropore closure.

Figure 5 shows a 31 somite stage C57 embryo, in which secondary neurulation has just begun, immediately following posterior neuropore closure. At the caudal end, a slight depression, similar to that seen in primary neurulation (e.g. Fig. 4a), is found at the dorsal midline of the surface ectoderm (Fig. 5a). Moving cranially, a small cavity appears just beneath the dorsal midline of the surface ectoderm (Fig. 5b) and resembles that formed in primary neurulation (Fig. 4b, c) except that the surface ectoderm is intact and there is no connection to the outside. The cavity gradually enlarges at more cranial levels (Fig. 5c, d) forming a neural tube with a round neural lumen (Fig. 5e). The neural tube at this cranial level was probably derived from Mode 3 primary neurulation.

The caudal end of a 34-somite stage embryo is shown in Fig. 6 to illustrate a more advanced stage in a secondary neurulation. The caudal end of the embryo consists of mesenchymal-like cells enclosed within a smooth surface ectoderm, with no dorsal depression (Fig. 6a). Moving in a cranial direction, an organised group of cells with distinct apicobasal orientation becomes visible in the dorsal part of the mesenchyme (Fig. 6b). A small cavity forms in the centre of these cells (Fig. 6c), gradually enlarges (Fig. 6d) and forms the round lumen of the secondary neural tube (Fig. 6e).

## Discussion

In this study, we made a systematic analysis of the pattern of neuroepithelial morphogenesis during neurulation in the spinal region of the mouse embryo. Three distinct modes of primary neurulation have been identified, which occur consecutively in three different strains of mice. These three patterns of morphogenesis differ in both the shape and area of neural induction, and in the extent to which they involve hinge points (MHP and DLHP). In Mode 1 there is a MHP but no DLHP, in Mode 2 both MHP and DLHP are present and in Mode 3 the neuroepithelium exhibits generalised curvature and specific hinge points cannot be discerned. The finding of all three modes of neurulation in all three strains of mice argues that this pattern of morphogenesis may be of general relevance in mouse neurulation.

### Role of hinge points in primary neurulation

Although the formation of hinge points during neurulation has been described in the chick (Schoenwolf and Smith 1990) and the mouse (the present study), it is not clear what role hinge points play in the neurulation process. The MHP does not appear to be essential for closure of the neural tube, since notochordless chick embryos do not develop a MHP and yet neurulation still occurs (Smith and Schoenwolf 1989), albeit with reduced efficacy. Similarly, in the mouse, inactivation of the gene *HNF*3β yields embryos that lack a notochord and MHP, and yet open neural tubes are not among the defects described for these embryos (Ang and Rossant 1994; Weinstein et al. 1994). Both notochord and floor plate (MHP) are intimately involved in dorso-ventral patterning of the neural tube (Tessier-Lavigne et al. 1988; Van Straaten et al. 1988; Smith and Schoenwolf 1989), but it seems unlikely that either structure is directly involved in closure of the neural tube.

As for the DLHP, they are present in all regions of the chick brain, but are absent from the spinal cord, except at its caudal end, the sinus rhomboidalis. For most of the length of the chick spinal cord, bending is limited to the MHP so that the apical surfaces of the neural folds become closely apposed when the neural tube closes (Schoenwolf and Desmond 1984; Schoenwolf and Smith

1990). This resembles neurulation in the upper spinal region of the mouse (Mode 1), but our study has shown that DLHP are present during spinal neurulation in mouse embryos with more than 16 somites (i.e. from the mid-thoracic level downwards). In the chick, when DLHP formation was inhibited by cytochalasin D, convergence of the neural folds was blocked and neural folds failed to fuse across the midline (Schoenwolf et al. 1988), demonstrating a role of DLHP in neural folds convergence.

## Mechanisms of primary spinal neurulation

The existence of several morphological modes of neurulation throughout the spinal region of the mouse embryo suggests that the underlying mechanisms of neurulation are heterogeneous. Two basic models have been proposed for the mechanism of neurulation in higher vertebrates. In the 'intrinsic model', changes within the neuroepithelium are considered to be primarily responsible for folding of the neural plate and closure of the neural tube. This is usually seen as involving contraction of apically-arranged microfilaments that causes neuroepithelial cells to adopt a wedge-shape, with a reduction in the apical surface area of the neuroepithelium (Karfunkel 1974). Conversely, the 'extrinsic model' views neural folding as resulting primarily from changes outside the neuroepithelium, for instance involving an increase in volume of the mesodermal extracellular matrix (Morriss and Solursh 1978; Morris-Wiman and Brinkley 1990) or expansion of the surface ectoderm (Alvarez and Schoenwolf 1992).

The three modes of mouse spinal neurulation could differ depending on whether intrinsic or extrinsic forces are mainly operating. Thus, in Mode 1 neurulation, intrinsic forces can be predicted to play a negligible role since cell wedging is observed only at the MHP, and we have seen that the MHP is not required for neural tube closure. Mode 1 closure may be primarily dependent on extrinsic forces from lateral tissues, as suggested by Schoenwolf (1988) for the chick. On the other hand, Mode 3 neurulation appears to involve neuroepithelial wedging by the majority of neuroepithelial cells, suggesting that intrinsic forces may predominate at more caudal levels. Indeed, it has been shown that the posterior neuropore of mouse embryos with 20–29 somites can undergo closure in the absence of adjacent tissues (Van Straaten et al. 1993), consistent with this idea. Mode 2 neurulation involves both MHP and DLHP, but other regions of the neuroepithelium do not undergo wedging, and may be dependent on both intrinsic and extrinsic forces. These predictions are testable by use of drugs, such as cytochalasins, that disrupt microfilament function. Mode 3 neurulation should be the most susceptible to such agents whereas Mode 1 neurulation should be resistant. Mode 2 may be intermediate in its sensitivity. Although studies using cytochalasins to inhibit microfilament function have been described in mammalian embryos (Austin et al. 1982; Morriss-Kay and Tuckett 1985; Smedley and Stanisstreet 1986), there has been no analysis of embryos at different stages of spinal neurulation, which would be necessary to test our prediction.

## Modes of neurulation and neural tube defects

The *curly tail* mouse strain, in which approximately half of the embryos undergo delayed closure of the posterior neuropore (Grüneberg, 1954), exhibits delay in the transition from Mode 2 to Mode 3 neurulation, which occurs in CBA and C57 embryos around the 25–27 somite stage. However, the first sign of abnormality in *curly tail* embryos can be detected 4–8 h earlier in development, at the 23 somite stage, when the rate of closure of the posterior neuropore starts to decelerate (Van Straaten et al. 1992). Delay of neuropore closure in *curly tail* has been demonstrated to result from a cell proliferation defect that affects the hindgut and notochord, but not the neuroepithelium (Copp et al. 1988), this growth imbalance causes abnormally enhanced ventral curvature of the caudal region that retards closure of the neuropore (Brook et al. 1991). The cell proliferation defect in *curly tail* embryos is related to reduced expression of retinoic acid receptor β specifically in the hindgut endoderm (Chen et al. 1995). Thus, the cause of neural tube defects in *curly tail* is extrinsic to the neuroepithelium, and this point was demonstrated experimentally by the finding that the neuroepithelium of the posterior neuropore of *curly tail* embryos can close in a non-delayed fashion after isolation from adjacent tissues (Van Straaten et al. 1993). Therefore, the delay in transition from Mode 2 to Mode 3 in *curly tail* embryos probably represents an effect of the abnormality in adjacent tissues, perhaps mediated by stretching of the neural plate due to the enhanced curvature of the body axis.

## Transition from primary to secondary neurulation

The gradual change in shape and area of induction of dorsal ectoderm to form neuroepithelium along the future spinal cord provides insight into the way in which the embryo makes the transition from primary to secondary neurulation. Passing down the spinal region, the area of induced neuroepithelium becomes smaller and the newly formed neural plate becomes increasingly indented. In Mode 3 neurulation, the neuroepithelium is indented as a neural groove from the time of its first appearance. It is tempting to suggest, therefore, that secondary neurulation represents a further continuation of the trends towards indentation of the newly-induced neural plate; however, when the posterior neuropore closes, cells beneath the dorsal midline are induced to acquire characteristics of neuroepithelial cells, e.g. apicobasal orientation. According to this argument, spinal neurulation in the mouse should be viewed not as consisting of two distinct events (primary and secondary neurulation)

but, rather, as a gradually evolving process in which a series of transitions (Mode 1 to Mode 2 to Mode 3 to cavitation) occur sequentially as neurulation passes down the body axis.

If the above hypothesis is correct, this would imply that secondary neurulation is not an autonomous event that always occurs at a particular body axial level but, rather, is the phase of spinal neurulation that follows, and is initiated by, closure of the posterior neuropore. There is some evidence to support this view. When the rate of neurulation was experimentally hastened in CBA mouse embryos such that the posterior neuropore closed at the 26 somite stage instead of the usual 31 somite stage, we found that secondary neurulation occurred precociously at a level where primary neurulation usually takes place (A.S.W. Shum and A.J. Copp, unpublished material). Moreover, in *curly tail* embryos where posterior neuropore closure is delayed, formation of a closed neural tube by secondary neurulation begins at a much lower axial level (Copp and Brook 1989). This is in contrast to the finding in the chick embryo that when the posterior neuropore is experimentally prevented from closing, secondary neurulation still occurs normally from its usual level (Costanzo et al. 1982); in other words, secondary neurulation occurs in the chick as an inherent property of a particular level of the body axis. This difference between mouse and chick embryos may be related to the fact that, in the mouse, secondary neurulation begins immediately caudal to the primary neural tube (Schoenwolf 1984) such that the primary and secondary neural tubes join end-to-end whereas, in the chick, secondary neurulation initiates ventral to the primary neural tube and there is an overlap zone where the dorsal portion of the neural tube forms by primary neurulation and the ventral portion forms by secondary neurulation (Schoenwolf 1978; Schoenwolf and De Longo 1980). Moreover, the secondary neural lumen is formed by coalescence of multiple lumens in the chick, whereas in the mouse, only a single neural lumen is formed. Therefore, the mechanism of initiation of secondary neurulation in mouse and chick appear to be quite different.

**Acknowledgements** This work was supported by the Imperial Cancer Research Fund (ASWS, AJC), by UPGC Direct Grant for Research SP93.8 (ASWS), by RGC Earmarked Research Grant CUHK 438/95M (ASWS), by the Wellcome Trust (AJC), and as part of a Multicenter Agreement for Studying Neural Tube Defects in Mutant Mice funded by the National Institutes of Child Health and Human Development, USA, through Cooperative Agreement HD 28882 (AJC). The authors would like to thank Pierre Wong for his technical assistance.

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