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Review

Strategies of vertebrate neurulation and a re-evaluation of teleost neural tube formation

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Abstract

The vertebrate neural tube develops by two distinct mechanisms. Anteriorly, in the brain and future trunk (cervicothoracic) region, 'primary neurulation' occurs, where an epithelial sheet rolls or bends into a tube. Posteriorly, in the future lumbar and tail region, the neural tube forms by 'secondary neurulation', where a mesenchymal cell population condenses to form a solid rod that undergoes transformation to an epithelial tube. Teleost neurulation has been described as different from that of other vertebrates. This is principally because the teleost trunk neural tube initially forms a solid rod (the neural keel) that later develops a lumen. This process has also been termed secondary neurulation. However, this description is not accurate since the teleost neural tube derives from an epithelial sheet that folds. This best fits the description of primary neurulation. It has also been suggested that teleost neurulation is primitive, however, both primary and secondary neurulation are found in groups with a more ancient origin than the teleosts. The similarity between neurulation in teleosts and other vertebrates indicates that this group includes viable models (such as the zebrafish) for understanding human neural tube development.

Keywords: Vertebrate neural tube; Primary neurulation; Secondary neurulation; Nervous system; Neural keel; Teleost; Zebrafish

1. Introduction

Neurulation is defined as the set of morphogenetic movements that result in formation of the neural tube, the future brain and spinal cord. Abnormalities in this process can lead to neural tube defects with devastating effects on nervous system function. In the brain and thoracic region of the spinal cord, neurulation involves a complex set of cell movements that include epithelial columnarization, migration, intercalation, and convergent extension (Colas and Schoenwolf, 2001; Davidson and Keller, 1999). Multiple genes have been implicated in particular steps of neural tube closure and neural tube abnormalities (Colas and Schoenwolf, 2001; Holmberg et al., 2000), however, the genetic basis for neurulation is not well understood. In this report, we consider whether the zebrafish is a good model for determining the genetic basis of neurulation. Not only are extensive genetic and molecular tools available in zebrafish, but also development is rapid and the nervous system is easy to see. Despite these advantages, zebrafish neurulation has been described as different from the processes that occur in amphibia and amniotes. By comparing the mechanism of zebrafish neurulation with that of amphibians, chick, and mouse, we conclude that zebrafish form their neural tube in a similar manner to other vertebrates.

2. The neural tube forms by joining two distinct tubes that form by different mechanisms

The vertebrate neural tube forms from two tubes that develop independently, by distinct morphogenetic and molecular processes. An anterior (or primary) tube extends from the brain to the cervicothoracic region, and a more posterior tube develops later in the lumbar and tail region (Fig. 1A). The anterior tube forms via 'primary neurulation' from an epithelial cell sheet (the neural plate) (Colas and Schoenwolf, 2001). In contrast, the posterior tube forms from the tail bud via 'secondary neurulation' in which there is a transformation of a solid rod of mesenchymal cells to an epithelial tube (Criley, 1969; Griffith et al., 1992). Key to the distinction between primary and secondary neurulation is the definition of epithelial and mesenchymal cell populations. We define epithelial tissues as an organized and contiguous sheet of cells held together by junctional

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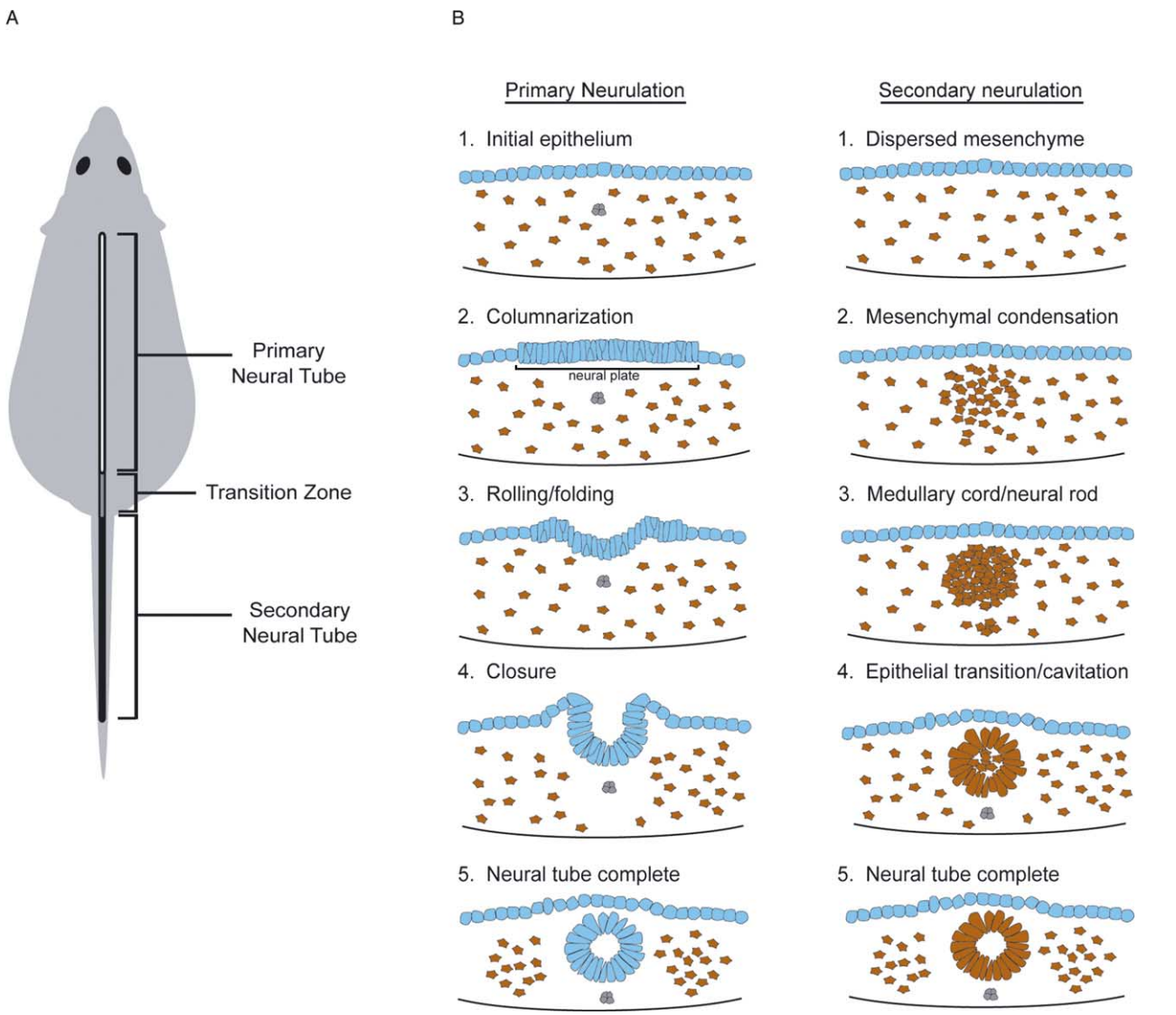

Fig. 1. The neural tube forms by two different mechanisms along the anteroposterior axis. (A) Level of the neural tube at which primary and secondary neurulation occur. In a transition zone near the junction of primary and secondary tubes, a mix of the mechanisms may be present. (B) Comparison of primary and secondary neurulation. (a) Primary neurulation involves columnarization of an existing epithelium, and then rolling or folding the epithelium (blue). (b) secondary neurulation is characterized by condensation of mesenchyme (brown) to form a rod, which then undergoes an epithelial transition to form the neural tube.

complexes, while we define mesenchymal tissue as a loosely associated group of cells.

For both the primary and secondary neural tubes, closure of the tube does not occur synchronously along the anteroposterior axis, but generally progresses from anterior to posterior. Further, the precise position to which the anterior tube extends is variable in different organisms, and a region of transition between anterior and posterior neural tubes is often apparent. For example, in chick, there is an overlap region where primary neurulation occurs more dorsally and secondary neurulation more ventrally within the same neural tube. Thus, in this region, there are two neural tubes that eventually coalesce into a single tube (Schoenwolf and Delongo, 1980). As will be discussed in the following sections, primary and secondary neurulation involve distinct morphogenetic and molecular mechanisms.

3. Primary neurulation

The key characteristic of primary neurulation is that it occurs from a preexisting epithelial substrate, which folds, rolls, or bends into a tube. The term ‘primary’ refers to the tissues involved in this process as derived from the three germ layers of ‘primary body development’ (Holmdahl, 1932; Nakao and Ishizawa, 1984). Neurulation of the anterior neural tube has been carefully described in frog, chick, mouse, and rabbit (Davidson and Keller, 1999; Morriss-Kay et al., 1994; Peeters et al., 1998;

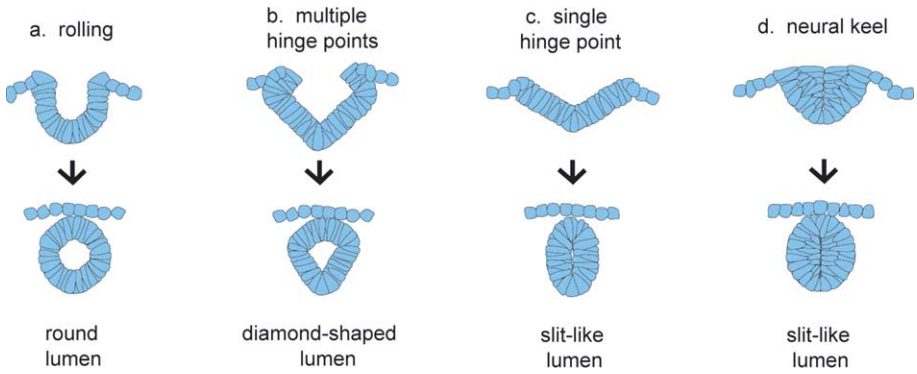

Fig. 2. Variations of primary neurulation. The neural tube is shown at the open stage (top row) and after initial closure (bottom row) (see also Fig. 1). The initial flat neuroepithelium may roll smoothly into a tube (a), bend sharply at one (b) or more (c) hinge points, or form a solid rod of cells (d). See text for details.

Smith and Schoenwolf, 1991), and while slightly different in each of these animals, the basic steps which are specific to primary neurulation are conserved in all these species (Fig. 2). These include columnarization of the ectoderm to form the neural plate, thickening of the edges of the neural plate to form the neural folds, convergent extension of the neural plate that assists bending to form the neural groove and also elongates the future neural tube, and closure of the groove to form the neural tube (Fig. 1B) (Colas and Schoenwolf, 2001).

While primary neurulation always starts with an epithelial substrate and ends with a tube, some of the steps involved can be quite variable between vertebrates and within one species at different levels of the anterior neural tube (Fig. 2). This variability is apparent at the point when an 'open' neural tube has formed, that is, one that is partially rolled up but where the folds have not yet fused. In order to form the open neural tube, there can be a smooth rolling of the epithelium (Fig. 2a). This occurs in the brain of the frog *Xenopus* and also in part of the mouse spinal cord. Another strategy to form the open neural tube is a bending of the epithelium at defined 'hinge points', where cells become wedge shaped (Fig. 2b,c). Even in a single species it has been shown that the neural plate folds or rolls differently depending on anteroposterior location and developmental stage (Shum and Copp, 1996). For example, in mouse, at the 12 somite stage in the region of the posterior neuropore, one median acute bend or 'hinge point' is apparent, with the two sides of the neural plate elevating and simply folding up, leaving a slit-like lumen. Later, at the 20 somite stage, in the posterior neuropore region, there are two additional dorsolateral hinge points. Finally, at the 29 somite stage, the neuroepithelium at the posterior neuropore region rolls into a round tube, with no obvious hinge points (Shum and Copp, 1996). Similarly, in the chick, along the length of the anterior neural tube, a median hinge point is located in the ventral midline (overlying the notochord), while in the brain region, two additional dorsolateral hinge points develop. As a result of the different movements involved in tube closure, the appearance of the initial neural tube cavity can be quite variable (Fig. 2). The lumen of the chick brain is large and diamond shaped, whereas in the spinal cord region, it is slit-like (Smith and Schoenwolf, 1991). In *Xenopus*, the lumen of the spinal cord is initially occluded, and subsequently opens from ventral to dorsal (Davidson and Keller, 1999). Finally, the open neural tube may not have a medial opening but may be a solid rod of cells, the neural 'keel' (Fig. 2d). This is apparent in *Xenopus* (Davidson and Keller, 1999), and, as will be discussed below, in the teleosts. Despite these variations, the underlying morphogenetic movements involved in the reshaping of the neuroepithelium are thought to be conserved.

A large number of candidate genes and molecules have been implicated in defects of primary neurulation and include those that regulate cell adhesion and cytoskeletal dynamics (see reviews Copp et al., 2003; Colas and Schoenwolf, 2001). Although it is not yet clear whether these candidate genes directly affect the neural tube or act more indirectly, recent studies have begun to address the molecular mechanisms involved. For example, the actin binding protein Shroom regulates apical constriction and is required for hingepoint formation during neural tube closure in *Xenopus* (Haigo et al., 2003). The adhesion signaling molecule p190 RhoGAP may also play a role in neural fold closure, for its loss disrupts the apical constriction of neuroepithelial cells (Brouns et al., 2000). The non-canonical Wnt pathway plays a key role in the convergent extension movements of the neural plate (Wallingford and Harland, 2002; Wiggan and Hamel, 2002). Mutation of the cell membrane signaling molecule ephrin A5 prevents the fusion of the neural folds at the dorsal midline, but does not effect their elevation or apposition (Holmberg et al., 2000). Moreover, a wide variety of teratogenic agents disrupt cranial but not cervicothoracic primary neural tube closure, showing that primary neurulation may not be regulated uniformly along the neural tube (Copp et al., 1990; Shum and Copp, 1996).

4. Secondary neurulation

In contrast to primary neurulation, the term secondary neurulation refers to the 'secondary body development' that occurs in vertebrates, in which the posterior or tail region of

the organism develops from tissue of the undifferentiated tail bud after more anterior regions have developed (Griffith et al., 1992; Holmdahl, 1932; Nakao and Ishizawa, 1984; Schoenwolf and Delongo, 1980). The unifying concept underlying secondary neurulation is that the posterior neural tube derives from a mesenchymal population of cells, rather than an epithelial population as in primary neurulation. These mesenchymal cells coalesce into a rod (the medullary cord) that transforms to an epithelium (the presumptive neuroepithelium) and a lumen develops to form a tube. Thus, during secondary neurulation, there is no epithelial neural plate intermediary equivalent to the case in primary neurulation (Fig. 1B).

As in primary neurulation, there are a number of variations to secondary neurulation in different species or in the same species at different stages (Fig. 3). In mouse, after medullary cord formation, the secondary neural tube forms by two mechanisms. In day 9.5–10 embryos, the entire mesenchymal region undergoing neurulation becomes epithelial, with later appearance of a lumen (Fig. 3a). In older embryos (day 11–12), only the dorsal part of the medullary cord becomes an epithelium initially—the medullary 'plate' (Fig. 3b). Mesenchymal cells are recruited from the edges of the plate and added to the epithelium to eventually form a tube (Schoenwolf, 1984). In chick, formation of the medullary cord is followed by a separation into two cell populations: the central cells remain mesenchymal, while the peripheral cells become epithelial (Fig. 3c). Cavitation occurs at the boundaries between these two populations, where small cavities of varied size and shape form and coalesce into a single, central lumen (Catala et al., 1995; Schoenwolf and Delongo, 1980).

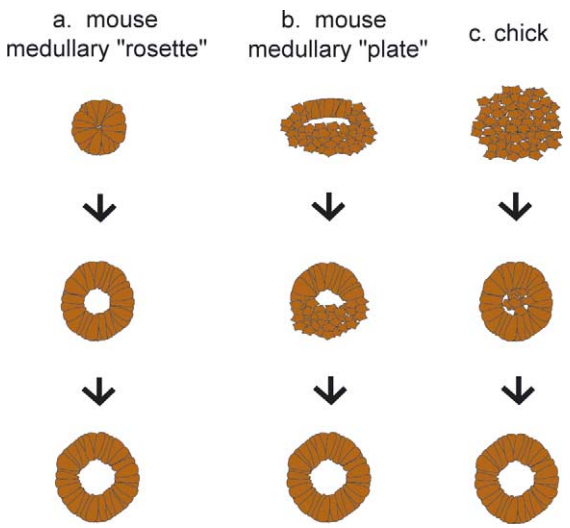

Fig. 3. Variations of secondary neurulation. The neural tube is shown subsequent to condensation of the starting mesenchyme into the medullary cord (see also Fig. 1). In one variation (a) a solid mass of epithelium (the medullary rosette) expands to form a lumen. (b) A medullary plate comprises an epithelium abutting mesenchyme with a space between. The epithelium expands to replace the mesenchyme with concomitant lumen expansion. (c) The medullary cord may form an epithelium at its edges while mesenchyme remains centrally. A lumen forms by expansion of spaces between the epithelium and mesenchyme and by loss of the mesenchyme. See text for details.

It is likely that the molecular mechanisms underlying secondary neurulation are distinct from those involved in primary neurulation, however, few candidate genes implicated in secondary neurulation have been identified. One example of a candidate gene is N-CAM. Whereas the form of N-CAM with a low sialic acid content has been implicated in primary neurulation, the highly sialated form has been suggested to be important for the mesenchymal to epithelial transition of secondary neurulation (Griffith et al., 1992; Sunshine et al., 1987). The secreted protein, midkine, has been implicated in the mesenchymal to epithelial transition during formation of the chick secondary neural tube (Griffith, 1997). Mesenchymal to epithelial transitions also occur during formation of other hollow structures. For example, in the early mouse embryo, this process transforms the solid embryonic ectoderm into the columnar epithelium surrounding the proamniotic cavity. BMP signaling plays a role in this conversion process (Coucouvanis and Martin, 1995, 1999) and it is plausible that the molecular mechanisms underlying formation of a proamniotic cavity and secondary neural tube are conserved.

5. Teleost neurulation: background

Descriptions of teleost neurulation have been confusing. This is primarily because in the brain and trunk region, the zebrafish neurectoderm first forms a solid rod, the neural keel, and only later forms a tube with a lumen (Fig. 2d). Initially, the neural keel was thought to be a mass of mesenchymal cells, and neurulation therefore equivalent to the secondary process that occurs in the tailbud of most animal groups (von Kupffer, 1890; Reichenbach et al., 1990). More careful examination using several model systems, including the zebrafish, showed that this is not correct (Geldmacher-Voss et al., 2003; Kingsbury, 1932; Miyayama and Fujimoto, 1977; Reichenbach et al., 1990; Strahle and Blader, 1994). However, teleost neurulation has continued to be termed secondary (Geldmacher-Voss et al., 2003; Handrigan, 2003; Kimmel et al., 1995; Papan and Campos-Ortega, 1994). Given the variations of primary and secondary neurulation in other groups, we re-evaluated the literature and the mechanism of teleost neurulation.

6. Mechanism of zebrafish trunk neurulation

In the zebrafish, which we consider as a teleost model, only the spinal cord (anterior trunk region) has been carefully examined (Fig. 4). Several studies have analyzed zebrafish neurulation using fate mapping, time-course serial sectioning, and confocal time-lapse imaging of neurulation movements in vivo (Geldmacher-Voss et al., 2003;

Kimmel et al., 1994; Papan and Campos-Ortega, 1994; Papan and Campos-Ortega, 1999; Schmitz et al., 1993). We consider the events of zebrafish neurulation in developmental order, and highlight aspects of this process that have led to its designation as different from that of neurulation in other vertebrates.

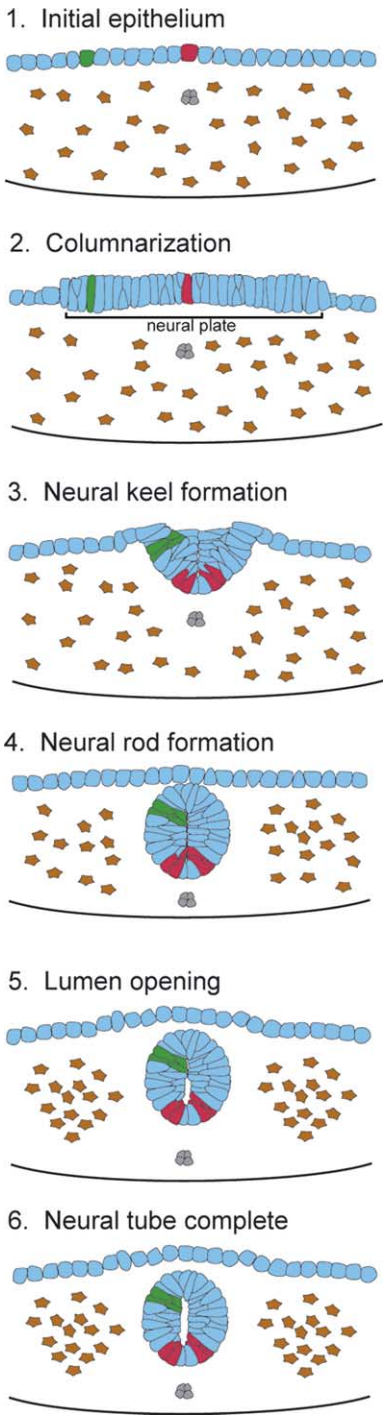

Fig. 4. Zebrafish trunk neurulation. An initial epithelium columnarizes to form the neural plate, which then forms a solid neural keel and solid tube. The midline of the tube becomes distinct and a lumen opens from ventral to dorsal. Neural plate cells lineage labeled prior to neural keel formation (red and green) maintain their relative positions during neural tube formation, indicating that cells form the keel by cryptic rolling or folding. The derivation of the zebrafish neural tube from an epithelium and the cell movements involved are typical of primary neurulation.

As in other vertebrates, an early step in zebrafish neurulation is formation of the neural plate. It is not clear whether cells in the zebrafish neural plate are connected by tight junctions, as expected for an epithelium. Indeed, the zebrafish neural plate does not show polarized expression of ZO-1, a molecule that can be associated with tight junctions and/or adherens junctions, but is not an unequivocal epithelial marker (Geldmacher-Voss et al., 2003; Aaku-Saraste et al., 1996). Expression of occludin, which does always indicate the presence of tight junctions, and is present in the neuroepithelium of other vertebrate embryos undergoing neurulation (Aaku-Saraste et al., 1996), has not been analyzed in zebrafish. However, morphologically the zebrafish neural plate is clearly an epithelium in the sense of being a cohesive sheet of cells which moves in an ordered manner. In particular, the lateral edges of the neural plate thicken and neural plate cells appear to ‘sink’ and form the neural keel, which is a solid mass or rod of cells (Schmitz et al., 1993). Recent data shows that even at the neural keel stage, the midline is distinct (Geldmacher-Voss et al., 2003), with a pseudostratified columnar epithelium on either side. Subsequently, the lumen of the neural tube opens, beginning ventrally and moving dorsally (Schmitz et al., 1993). The initial epithelial nature of the zebrafish neural plate suggests that zebrafish trunk neurulation occurs by a primary neurulation mode. It has been argued that there is no obvious ‘rolling up’ of the zebrafish neural plate, for there is no obvious elevation of neural folds, and instead the neural plate seems to sink down into the embryo. However, fate mapping indicates that the movements of the neural plate are equivalent in teleosts and other vertebrates, where initial mediolateral cell position corresponds to later dorsoventral position in the neural tube (Fig. 4) (Papan and Campos-Ortega, 1994). Even more convincingly, confocal time-lapse imaging in vivo shows a clear rolling of the neural plate, characteristic of primary neurulation (Geldmacher-Voss et al., 2003). Consistently, in the teleost *Chiclasoma nigrofasciatum*, a dorsal median neural groove is visible once the covering enveloping layer is removed from the embryo (Reichenbach et al., 1990). Thus, both the initial cell type and cell movements of the zebrafish are characteristic of primary neurulation.

Another phenomenon that has suggested teleost neurulation is unusual is the ability of daughter cells to cross the midline of the zebrafish neural keel, prior to lumen formation. Some cells have been shown to cross the midline of the *Xenopus* and chick neural tubes soon after closure (Collazo et al., 1993; Schoenwolf, 2003), although not at the frequency which occurs in zebrafish (Geldmacher-Voss et al., 2003). The ability of cells to cross the midline and the reorganization of the neural keel cells as the lumen opens, raises the question of whether neural keel cells have mesenchymal character that allows them to move relative to one another. Although cells can cross the midline,

the midline is always apparent, emphasizing that the neural keel is not simply a mass of disorganized mesenchymal cells. The distinction between mesenchymal and epithelial cells is fluid as these tissue states are interconvertible. However, rather than being discrete states, epithelial and mesenchymal tissues may form a continuum of cell states, where epithelial cell populations may have some mesenchymal character, and vice versa. This may be particularly true where an epithelium is undergoing cell rearrangement. For example, in the *Xenopus* neural tube during convergent extension, the epithelium transiently consists of bipolar protrusive cells that appear much more 'mesenchymal' than epithelial (Davidson and Keller, 1999). Similarly, in the zebrafish neural keel, the neuroepithelium may comprise cells less tightly associated than some epithelia but much more organized than a typical mesenchymal tissue.

Perhaps the major point of confusion with regard to teleost neurulation is the opening of a lumen in the neural keel after formation of this solid tube. This is the phenomenon that has led to the designation of teleost trunk neurulation as secondary (Kimmel et al., 1995; Papan and Campos-Ortega, 1994). However, this is not an accurate use of the term secondary neurulation, since the initial substrate for the trunk neural tube is epithelial and not mesenchymal. Lumen formation after initial primary neurulation is not a teleost-specific phenomenon, and in both chick and *Xenopus*, the trunk spinal cord is initially occluded, with a lumen developing after neural tube closure. Indeed, the developing *Xenopus* spinal cord appears similar to the zebrafish neural keel (Davidson and Keller, 1999). Although it has not yet been investigated, it is likely that true secondary neurulation occurs in the tailbud region of the zebrafish as in other vertebrates.

In conclusion, assessment of the literature has allowed us to understand the range of variation that is found in primary neurulation. This assessment in combination with experiments in the zebrafish strongly suggests that in the trunk region of zebrafish, the neural tube forms by a primary neurulation mechanism.

7. Evolutionary considerations: is zebrafish neurulation primitive?

The distinction between primary and secondary neurulation is particularly apparent in the stages that bracket formation of the neural tube. For example, prior to neural tube formation, primary neurulation utilizes a flat neural plate whereas secondary neurulation begins with a mesenchymal population of cells, which condense. These early stages may best serve as comparisons for evolutionary conservation. As discussed above, details of the precise movements in different vertebrates show a variety of solutions to the problem of changing the sheet of neural plate cells into a tube, and these details may be less helpful in evolutionary comparisons.

Previous descriptions of zebrafish trunk neurulation have suggested that this process is more primitive than neurulation in 'higher' vertebrates. This does not appear to be true, since organisms from more primitive lineages clearly undergo both primary and secondary neurulation. For example, the sturgeon, from a more ancient lineage than zebrafish, generates the neural tube by a direct folding of neural plate epithelium (Ginsburg and Dettlaff, 1991). The myxinoids (hagfish) and elasmobranches (sharks and rays), from even more primitive lineages, use primary neurulation in the brain and spinal cord region and secondary neurulation in the tail (Nakao and Ishizawa, 1984). Amphioxus and ascidians (both protochordates) roll up an epithelial neural plate (Conklin, 1932; Holland et al., 1996; Swalla, 1993), characteristic of primary neurulation.

Thus, evolutionarily, teleosts are flanked by more ancient and more modern lineages that employ primary neurulation anteriorly and secondary neurulation posteriorly. This is consistent with conclusions that teleost trunk neurulation should be described as primary.

8. Molecular mechanisms of neurulation: where does the zebrafish fit in?

The morphological similarities between primary neurulation in zebrafish and other vertebrates indicate that the underlying molecular mechanisms will also be similar. Conservation of neural patterning mechanisms between zebrafish and other vertebrates (reviewed in Tropepe and Sive, 2003) further supports this hypothesis. However, surprisingly little molecular and genetic analysis of zebrafish neurulation has been reported. Although a comprehensive screen in fish for neurulation mutants has not been carried out, previous large scale screens for embryonic lethal mutants identified many with defects in nervous system development (Jiang et al., 1996; Schier et al., 1996). In Table 1, we list some of the zebrafish mutants (and corresponding genes) with phenotypes consistent with neurulation defects, although most have not been well characterized. Some show clear abnormalities in neurulation, others display neural tube or neural determination defects that may include abnormal neurulation. Several of the mutated genes correspond to those with a neurulation or neural tube defect in mice, supporting the proposal that molecular mechanisms of neurulation are conserved. However, fish and mouse phenotypes may be different or of differing severity, reflecting subtle differences in gene function and/or genetic redundancy between these species. Below, we discuss a few examples of zebrafish mutants and the principles they illustrate.

One of the most striking neurulation mutants in zebrafish corresponds to N-cadherin, a calcium dependent cell adhesion molecule (Lele et al., 2002; Malicki et al., 2003). Several loss-of-function alleles have been isolated (*parachute*, *glass onion*) which show a severe neurulation

Table 1
Selected zebrafish mutants with putative neurulation defects

Mutant	Gene	Function/Phenotype (Ref.)
Trilobite[a]	*vangl2*	**PCP pathway** defects in cell movement (Jessen et al., 2002; Kibar et al., 2001)
Grumpy	*lamb1*	**Adhesion (laminin beta 1)** notochord and brain defects (Parsons et al., 2002)
Heart and soul	*aPKCλ*	**Epithelial Polarity** retina/neural tube defects (Horne-Badovinac et al., 2001; Peterson et al., 2001)
Nagie oko	*nok*	**Epithelial Polarity (MAGUK)** retina/ brain defects (Wei and Malicki, 2002)
Parachute/ Glass onion[a]	*n-cad*	**Cell Adhesion** neural tube/eye defects (Lele et al., 2002; Malicki et al., 2003)
Sleepy	*lamc1*	**Adhesion (laminin gamma 1)** irregular brain shape (Parsons et al., 2002)
Sonic you[a]	*shh*	**Hedgehog** defects in neural patterning (Harris and Juriloff, 1997; Schauerte et al., 1998)
One eyed pinhead	*oep*	**Nodal (EGF-CFC member and a co-receptor for nodal)** cyclopia, mutation in human holoprosencephaly (Zhang et al., 1998)
Masterblind[a]	*axin1*	**Neural specification** reduced eye/forebrain (Heisenberg et al., 2001; Zeng et al., 1997)
Neckless[a]	*aldh1a2*	**Neural specification (retinaldehydrogenase type 2)** defects in neural tube and paraxial mesoderm, (Begemann et al., 2001; Niederreither et al., 1999)
Somitabun[a]	*smad5*	**Neural specification** expansion of neuroectoderm (Chang et al., 1999; Hild et al., 1999)
Lock jaw/ Mont blanc[a]	*tfap2a*	**Neural crest development** (Harris and Juriloff, 1997; Holzschuh et al., 2003; Knight et al., 2003)

[a] Indicates zebrafish mutants carrying mutations in zebrafish orthologs of mouse NTD genes (Juriloff and Harris, 2000).

defect, including disruption in neuroectodermal cell adhesion and convergent extension movements (Lele et al., 2002). Although N-cadherin also is expressed during neurulation in mammals, the mouse null mutant displays undulation of the neural tube but maintains epithelial integrity (Radice et al., 1997). It is likely that in the mouse mutant, other cell adhesion molecules can partially substitute for the loss of N-cadherin. There are over 80 cadherins in mammals (reviewed in Yagi and Takeichi, 2000), including over a dozen in the nervous system (Redies and Takeichi, 1996), and it has been shown that cadherins can substitute for each other in several experiments. For example, E-cadherin can rescue the cardiac phenotype of N-cadherin knockouts in mice (Luo et al., 2001). Disruption of chick N-cadherin shows a moderate phenotype lying between the zebrafish and mouse defects- layering of the neural tissue remains but the epithelial structure is disrupted (Ganzler-Odenthal and Redies, 1998).

The planar cell polarity (PCP) pathway, mediated by non-canonical Wnt signaling and necessary for convergent extension movements during *Xenopus* neurulation, has also been shown to be necessary for zebrafish cell movements. For example, the zebrafish mutant *trilobite* has a mutation in a gene homologous to the mouse *loop-tail*, a PDZ domain containing protein in the PCP (Jessen and Solnica-Krezel, 2004; Jessen et al., 2002; Kibar et al., 2001a,b). While the mouse mutant exhibits a severe neural tube defect in which the neural tube fails to close, the zebrafish mutant displays gastrulation and neuronal migration defects (Jessen et al., 2002). It is possible that the gastrulation defect masks later neurulation defects or that the differences in mouse and fish phenotypes reflect redundancy in PCP family members.

Another class of mutants which display neurulation defects in zebrafish are the apical-basal polarity mutants, such as *nagie oko*, which encodes a MAGUK-family protein involved in maintaining epithelial integrity, and *heart and soul*, an atypical protein kinase C lambda which is involved in adherens junction formation (Horne-Badovinac et al., 2001; Peterson et al., 2001; Wei and Malicki, 2002). Although their neurulation phenotypes are not well studied, it is clear that disruption of the apical-basal polarity of an epithelium would have a striking effect on morphogenesis.

9. Where does one go next? Strategies for using the zebrafish as a neurulation model

The similarities between neurulation in zebrafish and mammals make the zebrafish a viable model system for analyzing this process. How can the zebrafish best be used to study neurulation? A crucial goal is to perform large scale genetic screens to isolate a large set of neurulation mutants. This could be achieved by chemical or insertional screening. Genetic screens in the zebrafish are feasible for two reasons. First is the set of attributes that make zebrafish a good genetic model: short generation time, large clutch size and inexpensive breeding. Second is the ease with which fish embryos can be viewed: the neural plate is visible in the unmanipulated embryo, or where a neurectoderm-specific promoter drives a lineage marker such as GFP. These attributes are not found in mammalian embryos, and while amphibian embryos are easy to view, the current lack of genetic techniques is a deficit. A second goal is to test the set of genes implicated in mammalian or amphibian neurulation, by defining zebrafish mutants in these genes. While gene 'knockout' by homologous recombination in ES cells is not yet available for the zebrafish, PCR-based screening for mutations in specific genes is possible and promising (Wienholds et al., 2002, 2003). Molecular inhibition of gene function by injection of antisense morpholino-based oligonucleotides into zebrafish embryos has also proven effective (Nasevicius and Ekker, 2000). A third goal that is a logical outcome of the others is to examine interactions between genes, using epistasis analysis and other methods. For example, double mutants can be constructed by standard genetic crosses, or by a combination of molecular and genetic techniques, where antisense oligonucleotides are injected into embryos mutant for a single gene. Definition of

a large, perhaps comprehensive, set of neurulation mutants would be a huge step for the field.

10. Concluding remarks

Several lines of evidence indicate that the zebrafish anterior neural tube, in the spinal cord region, neurulates by a mechanism termed 'primary neurulation' similar to that found in other vertebrates. Variations in primary neurulation are found throughout the vertebrates and zebrafish fall into this spectrum of variation. The zebrafish holds tremendous promise for identification of genes which control neurulation.

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