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MECHANISMS OF NEURAL TUBE CLOSURE AND DEFECTS

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Neural tube closure (neurulation) is a complex process involving many cell phenomena. Both extrinsic and intrinsic forces are essential to elevate the neural plate into the neural folds and to bring the folds into apposition for closure. Extrinsic forces involve the underlying mesenchyme cells and extracellular matrix, nonneural ectoderm, the gut tube and notochord, and cell surface glycoproteins. Intrinsic forces involve cytoskeletal elements and microtubles, region-specific variations in cell cycle times, positioning of daughter cells during cell division, and rearrangement of neuroepithelial cells. Closure itself begins in the cervical region and proceeds in rostral and caudal directions. In the mouse, multiple additional closure sites occur in the cranial region, but in humans there appears to be only one additional site, at the rostralmost tip of the forebrain. Differences in closure exist in cranial vs. caudal regions, and these variations may play a role in the types of neural tube defects that occur. In this regard, virtually all of the cell phenomena involved in closure present targets for insults leading to abnormalities, although specific mechanisms responsible for neural tube defects have not been well-defined. It is also not clear what the role of folic acid is in normal neural tube closure, although the vitamin may be important for DNA synthesis and/or methylation of macromolecules, such as DNA and protein. Until more is learned about the regulation of neural tube closure at the genetic and cellular levels, understanding how defects occur and developing methods for their prevention will be limited.

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Key Words: neurulation; neural folds; cytoskeleton; extracellular matrix; neural tube; surface coats

Neural tube closure or neurulation is one of the most complex morphogenetic events that occurs during embryogenesis. It involves virtually every aspect of cell biology, from the extracellular matrix, to the cytoskeleton, to cell proliferation, to cell-cell contacts, and so on. Because of this complexity, it is also one of the processes that is easily disrupted, resulting in congenital malformations known as neural tube defects. Virtually any of the aforementioned cellular phenomena is a target for genetic or environmental influences, making neural tube defects truly a multifactorial problem.

Neurulation begins early in gestation, approximately day 17 postfertilization in humans. It is initiated by signals from the primitive node (the organizer) and notochord that cause the overlying epiblast to become thickened, to form the flat neural plate [Sasai and DeRobertis, 1997]. By day 19, the plate has lengthened and the edges in the cranial region begin to elevate on either side, forming the neural groove in the midline (Fig. 1A). Soon the edges of the folds begin to elevate further, rolling into a tube to meet each other and fuse (Figs. 1B, 2, 3). Fusion begin at the level of the fifth somite and proceeds in cranial and caudal directions. The cranial and caudal openings of the tube, created by the initiation of fusion, are known as neuropores (Figs. 2B, 3B). Closure of the cranial neuropore occurs on day 25, followed by closure of the caudal neuropore on approximately day 27 (Fig. 4). Thus, the entire neurulation process requires 10 days and occurs during weeks 3 and 4 postfertilization. Neural tube defects, such as anencephaly and spina bifida, are induced during this time and most are due to inhibition of closure. Closure is dependent upon a coordinated combination of forces generated in cells and tissues outside of the neural plate (extrinsic forces), and upon phenomena within cells of the neural plate itself (intrinsic forces) [Smith and Schoenwolf, 1997].

CLOSURE: EXTRINSIC FORCES

Initial elevation of the neural plate is produced by the underlying mesenchyme. Both cell proliferation and production of extracellular matrices in the mesenchyme result in elevation of the folds (Fig. 5) [Solursh and Morriss, 1977]. Initially, the matrix is predominantly hyaluronic acid, but with time it becomes enriched in chondroitin sulfates and other molecules. Once the plate has elevated and folding begins, the matrix is important for support. Disruption of the matrix during initial stages of neural plate elevation results in neural tube closure defects, such as anencephaly and, potentially, spina bifida [Morriss-Kay and Crutch, 1982; Schoenwolf and Fisher, 1983].

Once neural plate elevation has been initiated, nonneural ectoderm (Fig. 5B) becomes important in folding and closure and provides the main extrinsic force for these events [Alvarez and Schoenwolf, 1992; Moury and Schoenwolf, 1995]. In fact, once the neural plate has been elevated by the underlying mesenchyme in preparation for folding, the mesenchyme, matrix, and endoderm can be removed and closure will occur [Moury and Schoenwolf, 1995]. Thus, the neuroepithelium, together with nonneural ectoderm, is capable of completing closure. Forces generated by nonneural ectoderm are created by changes in cell shape, from a low cuboidal configuration to a

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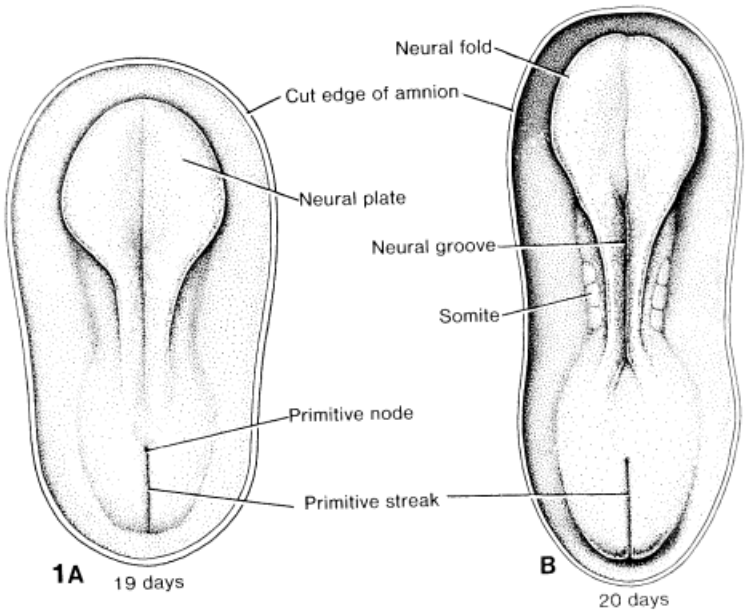

Fig. 1. Drawings of neurulating human embryos. **A:** By 19 days postfertilization the neural plate is well-formed in the cranial region, and the folds are beginning to elevate. **B:** By 20 days postfertilization somites have started to form along the sides of the neural folds, which are about to begin fusion around the neural groove in the cervical region. Note that the primitive node and streak are present and that cells ingress at these areas to form the three germ layers (ectoderm, mesoderm, and endoderm), a process called gastrulation. Thus, while the cranial end of the embryo is neurulating, the caudal end is still gastrulating to make the cells necessary for forming more caudal regions (from Langman's Medical Embryology, 7th ed., Williams and Wilkins, Baltimore).

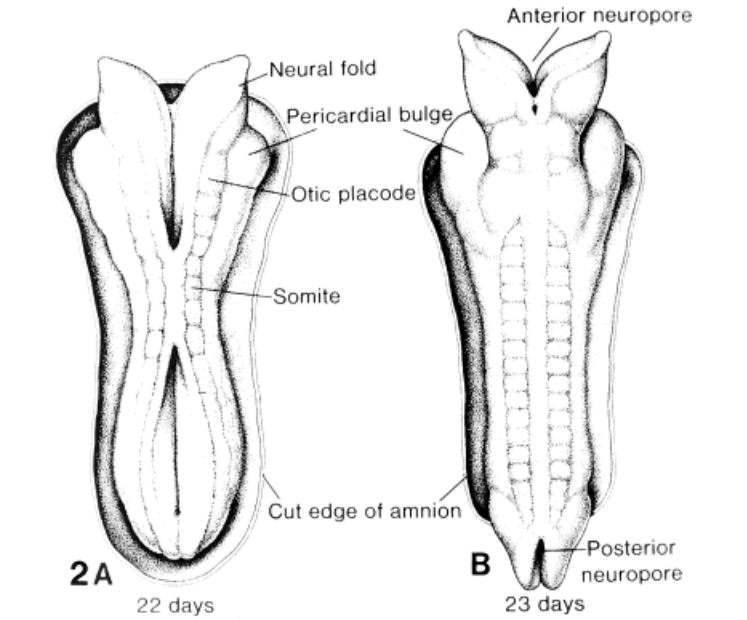

Fig. 2. Drawings of neurulating human embryos. **A:** By 22 days postfertilization, closure of the neural folds has been initiated and is proceeding in cranial and caudal directions. **B:** By 23 days postfertilization, closure has continued and the cranial and caudal neural folds are larger than those in the caudal region (from Langman's Medical Embryology, 7th ed., Williams and Wilkins, Baltimore).

squamous morphology. This change produces an expansion laterally that pushes the folds toward the midline [Schoenwolf and Alvarez, 1991]. Repositioning of the ectoderm cells in a caudomedial direction and a pattern of cell division that occurs rostrocaudally assist in lengthening the ectoderm and the neural plate [Schoenwolf and Alvarez, 1991; Sausedo et al., 1997].

Despite the fact that closure of the neural tube can occur when only the neural and nonneural ectoderm are left intact, it appears that the underlying gut endoderm and/or notochord also play a role in normal lengthening and closure. Evidence for this hypothesis is derived from studies in *curly tail* and *Splotch* mice that often have spina bifida. In *curly tail* affected animals, cell proliferation in the hindgut endoderm and notochord is decreased, causing an ''overgrowth'' of the neural tube. In turn, this overgrowth causes an abnormal flexion in the caudal neural folds that prevents their closure [Copp et al., 1988]. In *Splotch* mutants, the posterior neuropore is enlarged due to delayed closure produced by abnormalities in the notochord and mesoderm [Yang and Trasler, 1991].

Another extrinsic factor involves the synthesis of cell surface glycoproteins that are essential for closure. Synthesis begins in the nonneural and neural ectoderm immediately prior to fusion (Fig. 6) and provides the ''glue'' for maintaining initial contact between the folds [Sadler, 1978]. These ''surface coats'' may also provide recognition sites for cell-cell adhesion to occur between opposing folds. Similar coats are produced in other regions of epithelial fusion, such as the palate, and inhibition of their synthesis has been shown to inhibit fusion of the palatal shelves [Green and Pratt, 1977].

INTRINSIC FORCES

Cell shape changes in the neuroepithelial cells are an important force in modifying the configuration of the neural plate. Cytoskeletal elements, such as microtubules and microfilaments, together with changes in cell cycle times, contribute greatly to elevation and closure of the neural folds [Karfunkel, 1974; Smith and Schoenwolf, 1997]. Microtubes are important for increasing cell height and transforming the neural plate epithelium from cuboidal to columnar [Schoenwolf and Powers, 1987]. Actin microfilaments (Fig. 7), actin-binding proteins, including spectrin, and myosin create contractile forces that aid in elevation of the neural plate and in the subsequent shaping and bending of the neural folds [Karfunkel, 1974; Nagele and Lee, 1980; Sadler et al., 1982, 1986]. During plate elevation in the cranial region, actin filaments are located in the basal aspects of neural epithelial cells, presumably to assist in creating the biconvex appearance of these structures prior to fold formation (Fig. 5B). Once folding begins and the edges of the neural

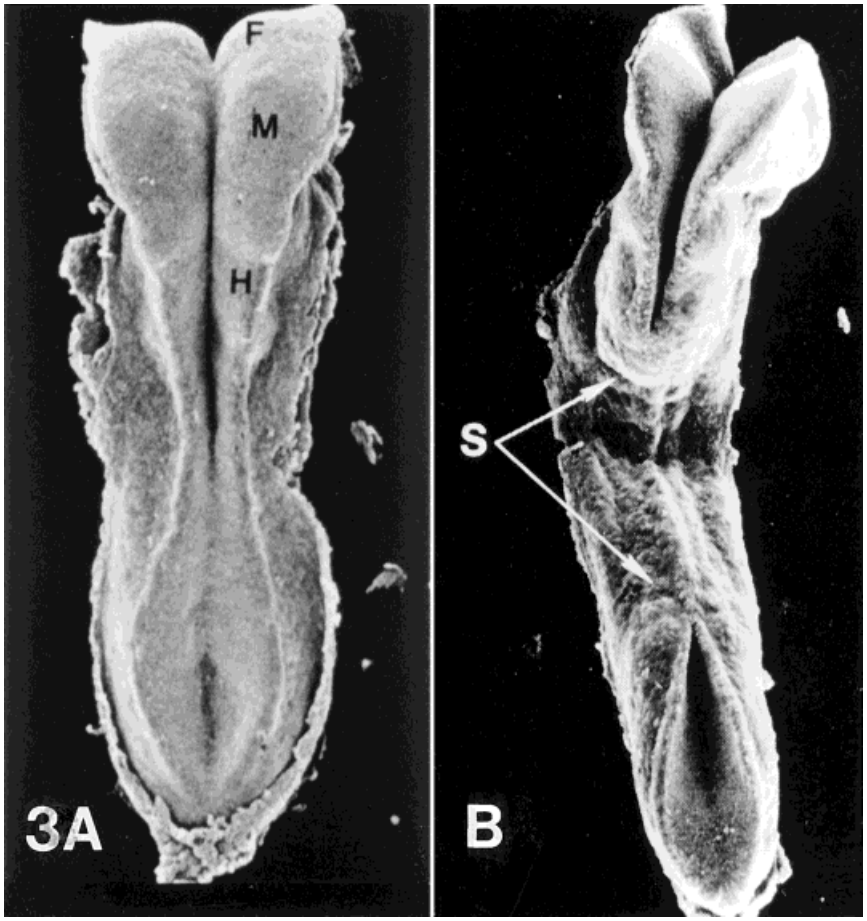

Fig. 3. Scanning electron micrographs of neurulation-stage mouse embryos. **A:** Stage comparable to 20-day human embryo (Fig. 1B), showing enlargement of the cranial neural folds on either side of the neural groove and the presence of the primitive streak at the caudal end of the embryo. By this stage, which is prior to closure, cranial neural folds have initiated differentiation into fore (F), mid (M) and hindbrain (H) areas. **B:** Stage comparable to 22 days in the human, showing that closure of the neural tube has been initiated and is proceeding in cranial and caudal directions. The cranial and caudal openings of the tube form the anterior and posterior neuropores, respectively. The primitive streak is visible at the caudal end of the embryo, and somites (S) are forming along the neural tube (from Langman's Medical Embryology, 7th ed., Williams and Wilkins, Baltimore).

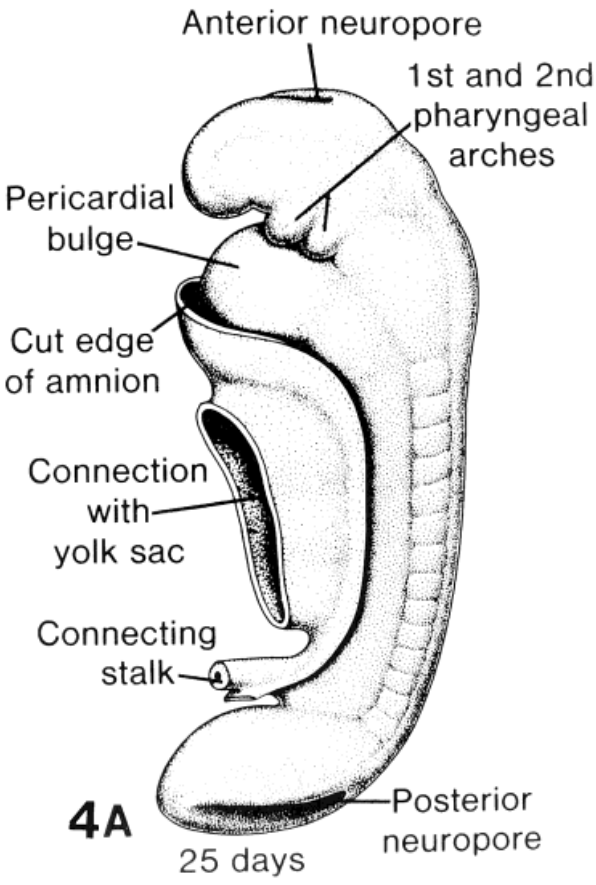

Fig. 4. Drawing of a human embryo at 25 days postfertilization. Closure of the anterior neuropore will be completed on this day, with closure of the posterior neurospore occurring on day 27. By this time, the embryo has folded ventrally into a "C" shape and numerous other structures have initiated their development, including the heart and cranial facial region (pharyngeal arches) (from Langman's Medical Embryology, 7th ed., Williams and Wilkins, Baltimore).

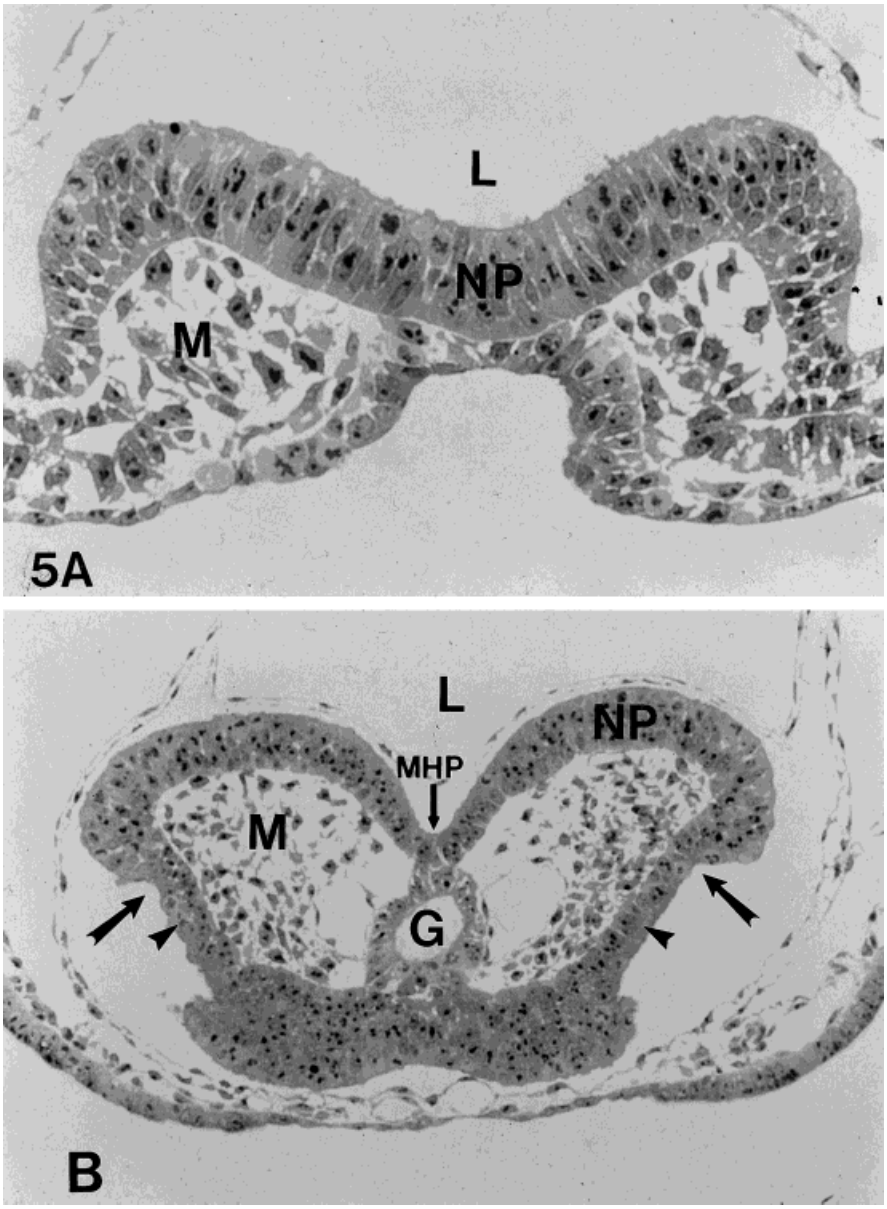

Fig. 5. Histological cross sections through the cranial neural folds of mouse embryos at various stages of neurulation. **A:** Initial stages of neural plate (NP) elevation produced by the underlying mesenchyme (M) and by actin microfilaments in the base of the neuroepithelial cells. L, prospective lumen. **B:** Maximal elevation of the neural plate (NP) produced by the underlying mesenchyme (M) and actin microfilaments in the basal regions of the neuroepithelial cells. From this stage on, the tips of the neural folds at the neural-nonneural ectoderm border (arrows) will elevate and close around the lumen (L). By now, a median hinge point (MHP) has formed at the floor of the neural groove. Note that the nonneural ectoderm cells (arrowheads) appear cuboidal at this stage. Later they will become squamous as they assist in pushing the folds toward the midline (see Fig. 8). G, gut tube.

plate begin to elevate toward the midline, these microfilaments are repositioned to the apical regions of the neuroepithelium [Sadler et al., 1982]. Here they contract and create a purse-string effect that narrows the apical area of the cell. In combination in all cells, this reduction in apical area assists in bending and elevating the neural folds. Disruption of the actin microfilaments with cytochalosins causes severe neural tube defects in chicks and rodents [Lee and Kalmus, 1976; Morriss-Kay, 1981].

Cell shape changes are also caused by alterations in cell cycle times in different regions of the neural tube [Schoenwolf and Smith, 1990; Smith and Schoenwolf, 1997]. Neural epithelial cells exhibit a phenomenon known as interkinetic nuclear migration, in which the nucleus moves from the base of the cell to the apices, where it divides at the lumen of the neural tube. Since mitotic nuclei are larger than interphase nuclei, the position of mitotic figures in the cell dictates the widest region. Thus, if a group of neighboring cells all had increased cell cycle times, such that mitotic figures appeared at the lumen with less frequency, then their nuclei would remain in the basal region for longer periods of time. Since the position of the nucleus dictates which region of the cell has the greatest diameter, these cells would tend to be wider at the base and narrower at the top, i.e., wedge-shaped. Indeed, this phenomenon occurs in cells in the midline of the neural groove, to create the median hinge point (MHP). It also occurs at dorsolateral hinge points (DHLP) along the lateral walls of the cranial neural folds, to assist with creation of wedge-shaped cells, and in turn bending of the folds toward the midline in these regions (Fig. 8) [Schoenwolf and Smith, 1990; Smith and Schoenwolf, 1997]. In the meantime, cells that do not lie within these regions undergo an increase in cell height and decrease in cell diameter, primarily through the extension of microtubules.

In addition to creating wedge-shaped cells at critical points of bending, cell proliferation also serves to lengthen the neural plate. Lengthening occurs because mitotic spindles of neuroepithelial cells are oriented in a rostrocaudal plane, resulting in cell division in these directions and positioning of daughter cells along the longitudinal axis [Sausedo et al., 1997].

Finally, neuroepithelial cells undergo two rounds of rearrangement during neurulation, whereby they reposition themselves along the longitudinal axis [Schoenwolf and Alvarez, 1989]. As a result, the width of the neural plate decreases, and the length increases. However, in the forebrain, which becomes quite large, lateral expansion also occurs and appears to be mediated by a rostral migration of cells from the midbrain and/or rostral hindbrain regions [Morriss-Kay and Tuckett, 1987].

CLOSURE SITES

Controversy has arisen about sites of neural tube closure and whether or not closure is initiated in the cervical region and then proceeds in an uninterrupted zipper-like fashion in rostro-caudal directions. Clearly, in the mouse, zippering occurs longitudinally in a caudal direction from the cervical region to closure of the posterior neuropore, and there are no ''secondary'' sites of closure (Fig. 3). However, multiple initiation sites of

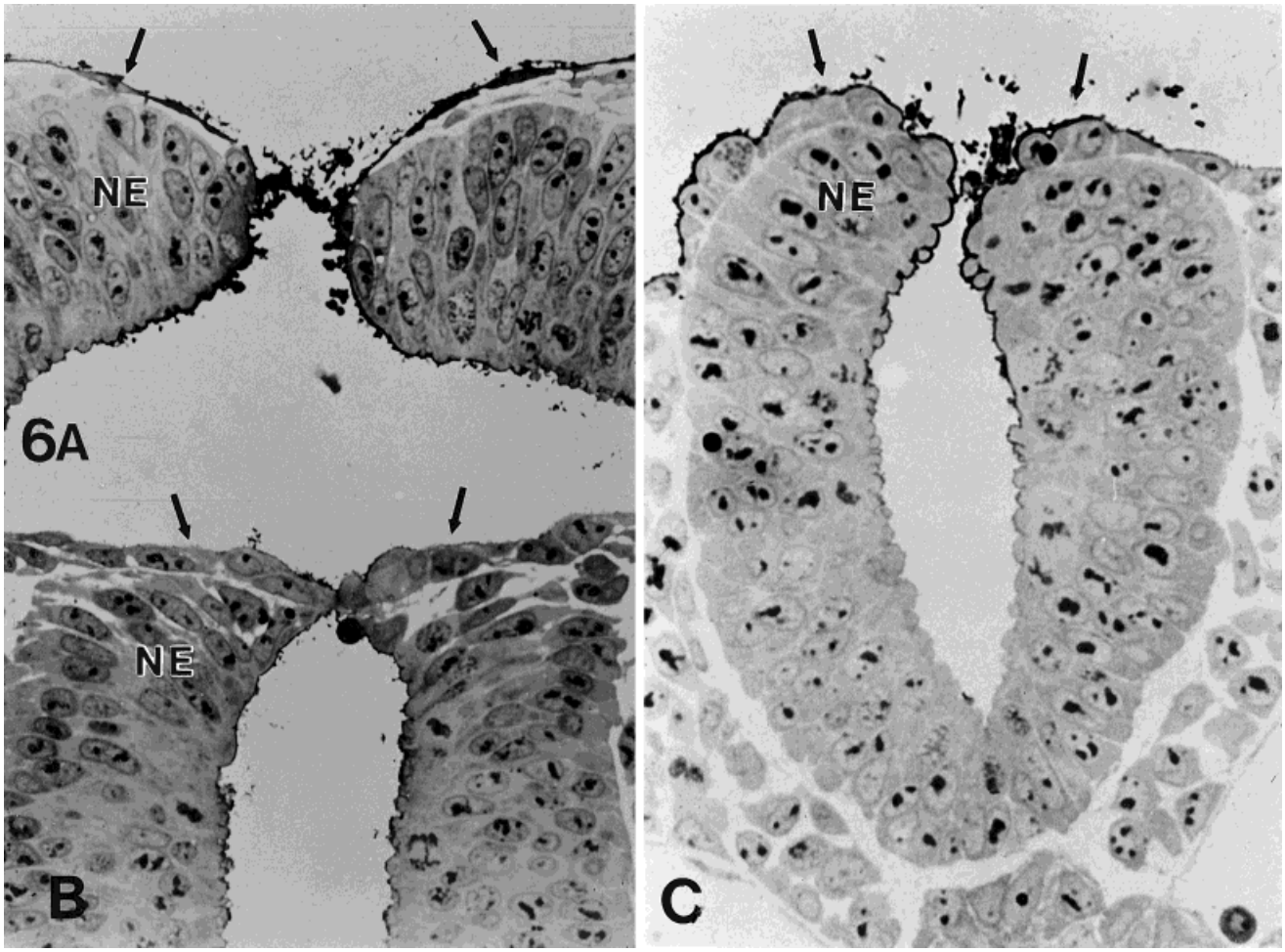

Fig. 6. Ruthenium red-stained histological sections through the midbrain **(A)**, hindbrain **(B)**, and spinal cord **(C)** at the time of neural fold apposition in mouse embryos. Glycoprotein surface coats provide the "glue" to hold the folds together initially and also provide a means for cell-cell recognition. As the folds make contact, surface coat material is reduced. Note that overlying ectoderm cells (arrows) make initial contact in cranial neural folds, whereas neuroepithelial cells (NE) are the first to approximate each other in the spinal cord region.

closure occur in the cranial region [Geelen and Langman, 1977; Juriloff et al., 1991]. Site 1 is located in the cervical region and moves in cranial-caudal directions; site 2 occurs at the forebrain-midbrain junction and also moves bidirectionally; site 3 is initiated at the rostral extreme of the forebrain and moves caudally to meet closure site 2; and site 4 occurs in the hindbrain and completes closure between sites 1 and 2. Thus, closure of the cranial neural folds is complex, with multiple sites, and has been associated with exencephaly in genetically abnormal strains of mice-[Gunn et al., 1995].

Based on the description of closure in the mouse and on the location of neural tube defects, Van Allen et al. [1993] hypothesized that similar sites occurred in humans. Thus, inhibition of the zippers in various closure sites in both cranial and caudal regions of the neural tube was proposed as a mechanism to account for the various types of defects. In fact, an additional closure site at the caudal end of the neural tube was postulated to occur in humans, based on the frequency of defects in this region [Van Allen et al., 1993]. However, neural tube morphology and closure in humans may be more similar to those in chicks than in rodents. Based on observations from the Carnegie collection, O'Rahilly and Muller [1989] concluded that humans have only two initation sites for closure: the cervical site and the rostral forebrain site. Models on display in the collection support the claim that the human neural tube is indeed more tube-like as in the chick, and that multiple closure sites do not exist. Preliminary studies of neurulating human embryos from Paris by Vekemans and Sulik also appear to confirm the chick-like appearance of the human embryo (personal communication).

Whether or not multiple closure sites and zippers exist, closure itself and the shape of the neural folds is vary in different regions. For example, cranial folds are larger and surround a larger lumen than caudal folds forming the spinal cord (contrast Figs. 6C and 8). Also, mechanisms of closure in different regions vary. Thus, in cranial folds, overlying ectoderm makes initial contact (Fig. 6A,B), whereas in spinal cord regions neuroepithelial cells meet first and overlying ectoderm closes later (Fig. 6C) [Sadler, 1978]. Therefore, defects in different regions may depend on different mechanisms of closure at a particular site. They may also be due to the timing of the teratogenic insult and the degree of closure that has already occurred. One caveat resulting from these notable differences in closure between species is that rodents may not be the best model for understanding the origins of neural tube defects in humans. The fact that it is very difficult to produce spina bifida in rodents, while being relatively easy to cause exencephaly, whereas in humans the two occur with approximately equal fre-

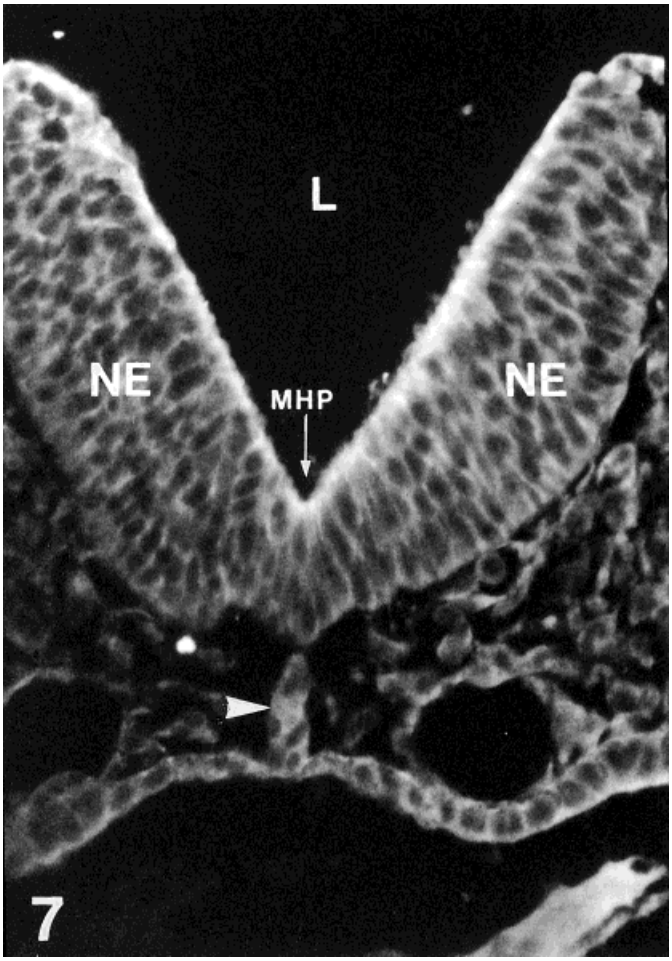

Fig. 7. Cross section through the caudal neural folds of a mouse embryo stained with anti-actin antibodies. The heaviest staining is at the apices of neuroepithelial cells (NE), where actin and other elements of the cytoskeleton are concentrated. These elements provide contractile forces that create a purse-string affect to assist in elevating and closing the neural folds. The notochord (arrowhead) lies immediately ventral to the median hinge point (MHP). L, neural lumen.

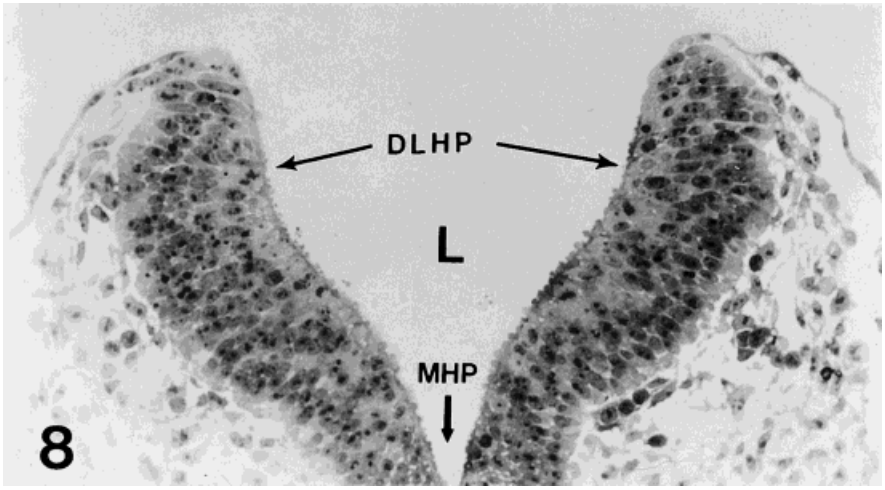

Fig. 8. Histological section through the hindbrain region of a mouse embryo. The median hinge point is just below the frame (MHP). Dorsolateral hinge points (DLHP) are forming at both sides of the neural folds. Cells in these regions will become wedge-shaped, with their apices at the narrow end to assist in bending of the folds toward the midline. Note that the nonneural ectoderm now has a squamous morphology as it assists in pushing the neural folds toward the midline (contrast with Fig. 5B). L, lumen.

quency, also supports the contention that other models should be developed.

Yet another complicating factor in humans is the role of folic acid in the closure process and how the vitamin interacts with the multiple cellular processes essential for neurulation. Although folate is known to reduce the incidence of neural tube defects by 50–70% [Medical Research Council Vitamin Research Group, 1991; Czeizel and Dudas, 1992; Wald, 1993], its mechanism of action has not been defined. The vitamin is important in two prominent pathways that could impact on neural tube closure: de novo synthesis of nucleic acids for cell proliferation, and methylation of macromolecules [Scott et al., 1994; Christensen and Rosenblatt, 1995]. As already discussed, cell proliferation is essential for shaping the neural folds and represents a major intrinsic factor for this process. On the other hand, methylation of macromolecules, such as DNA and cytoskeletal proteins, may also be important in producing closure. Methylation of DNA is one mechanism regulating gene expression [Razin and Kafri, 1994], whereas methylation of proteins may regulate their function [Klein, 1997]. An answer to folate's mechanism(s) of action is important because it may result in even more effective prevention strategies. For example, the methylation pathway involves methionine, which is synthesized from folic acid and homocysteine, and which has been shown to be effective in preventing neural tube defects and promoting successful pregnancies in women and monkeys with poor reproductive histories [Klein, 1997]. Thus, methionine may be another key factor in producing normal neural tube development.

In summary, it should be noted that neurulation is a complicated phenomenon involving multiple cells and processes. How these processes are orchestrated to produce normal development is not known. Furthermore, the specific genes regulating these events; the role of folic acid, methionine, and other micronutrients in promoting normal cellular behavior; and the types of animal models that should be employed to investigate the process remain to be determined. ■

REFERENCES

Alvarez IS, Schoenwolf GC. Expansion of surface epithelium provides the major extrinsic force for bending the neural plate. J Exp Zool 1992;261:340–348.

Christensen B, Rosenblatt DS. Effects of folate deficiency on embryonic development. Baillieres Clin Haematol 1995;8:617–637.

Copp AJ, Brook FA, Roberts HJ. A cell type specific abnormality of cell proliferation in

mutant *curly tail* mouse embryos developing spinal neural tube defects. Development 1988;104:285–295.
Czeizel AE, Dudas I. Prevention of the first occurrence of neural tube defects by periconceptional vitamin supplementation. N Engl J Med 1992;327:1832–1835.
Geelen JAG, Langman L. Closure of the neural tube in the cephalic region of the mouse embryo. Anat Rec 1977;189:625–640.
Green RM, Pratt RM. Inhibition by diazo-oxo-norleucine (DON) of rat palatal glycoprotein synthesis and epithelial cell adhesion in vitro. Exp Cell Res 1977;105:27–37.
Gunn TM, Juriloff DM, Harris MJ. Genetically determined absence of an initiation site of cranial neural tube closure is causally related to exencephaly in SELH/Bc mouse embryos. Teratology 1995;52:101–108.
Juriloff DM, Harris MJ, Tom C, et al. Normal mouse strains differ in the site of initiation of closure of the cranial neural tube. Teratology 1991;44:225–233.
Karfunkel P. The mechanisms of neural tube formation. Int Rev Cytol 1974;38:245–271.
Klein NW. The use of whole rat embryo cultures to identify and characterize causes of reproductive failure. Int J Dev Biol 1997;41:267–273.
Lee HY, Kalmus GN. Effects of cytochalasin B on the morphogenesis of explanted early chick embryos. Growth 1976;40:153–162.
Medical Research Council Vitamin Research Group. Prevention of neural tube defects: Results of the medical research council vitamin study. Lancet 1991;338:131–137.
Morriss-Kay GM. Growth and development of pattern in the cranial neuroepithelium of rat embryos during neurulation. J Embryol Exp Morphol 1981;65:225–241.
Morriss-Kay GM, Crutch B. Culture of rat embryos with B-D-xyloside: Evidence for a role for proteoglycans in neurulation. J Anat 1982;134: 491–506.
Morriss-Kay GM, Tuckett F. Fluidity of the neural epithelium during forebrain formation in rat embryos. J Cell Sci 1987;8:433–449.
Moury JD, Schoenwolf GC. Cooperative model of epithelial shaping and bending during avian neurulation: Autonomous movements of the neural plate, autonomous movements of the epidermis, and interactions in the neural plate/epidermis transition zone. Dev Dyn 1995;204:323–337.
Nagele RG, Lee HY. Studies on the mechanism of neurulation in the chick: Microfilament-mediated changes in cell shape during uplifting of neural folds. J Exp Zool 1980;213:391–398.
O'Rahilly R, Muller F. Bidirectional closure of the rostral neurospore in the human embryo. Am J Anat 1989;184:259–268.
Razin A, Kafri T. DNA methylation from embryo to adult. Prog Nucleic Acid Res 1994;48: 53–79.
Sadler TW. Distribution of surface coat material on fusing neural folds of mouse embryos during neurulation. Anat Rec 1978;191:345–350.
Sadler TW, Lessard JL, Greenberg D, et al. Actin distribution patterns in the mouse neural tube during neurulation. Science 1982;215:172–174.
Sadler TW, Burridge K, Yonker J. A potential role for spectrin during neurulation. J Embryol Exp Morphol 1986;94:73–82.
Sasai Y, DeRobertis EM. Ectodermal patterning in vertebrate embryos. Dev Biol 1997;182:5–20.
Sausedo RA, Smith JL, Schoenwolf GC. Role of nonrandomly oriented cell division in shaping and bending of the neural plate. J Comp Neurol 1997;381:473–488.
Schoenwolf GC, Alvarez IS. Roles of neuroepithelial cell rearrangement and division in shaping of the avian neural plate. Development 1989;106:427–439.
Schoenwolf GC, Alvarez IS. Specification of neuroepithelium and surface epithelium in avian transplantation chimeras. Development 1991;112:713–722.
Schoenwolf GC, Fisher M. Analysis of the effects of *Streptomyces* hyaluronidase on formation of the neural tube. J Embryol Exp Morphol 1983;73: 1–15.
Schoenwolf GC, Powers MI. Shaping of the chick neuroepithelium during primary and secondary neurulation: Role of cell elongation. Anat Rec 1987;218:182–195.
Schoenwolf GC, Smith JL. Epithelial cell wedging: A fundamental cell behavior contributing to hinge point formation during epithelial morphogenesis. Semin Dev Biol 1990;1:325–334.
Scott JM, Weir DG, Molloy A, et al. Folic acid metabolism and mechanisms of neural tube defects. Ciba Found Symp 1994;181:180–191.
Solursh M, Morriss GM. Glycosaminoglycan synthesis in rat embryos during formation of the primary mesenchyme and neural folds. Dev Biol 1977;57:75–86.
Smith JL, Schoenwolf GC. Neurulation: Coming to closure. Trends Neurosci 1997;20:510–517.
Van Allen MI, Kalousek DK, Chernoff GF, et al. Evidence for multi-site closure of the neural tube in humans. Am J Med Genet 1993;47: 723–743.
Wald N. Folic acid and the prevention of neural tube defects. Ann NY Acad Sci 1993;678:112–129.
Yang XM, Trasler DG. Abnormalities of neural tube formation in pre-spina bifida splotch-delayed mouse embryos. Teratology 1991;43: 643–657.