ARTICLE

Embryology of Neural Tube Development

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Neurulation is the process of forming the neural tube, which will become the brain and spinal cord. This article reviews the various cellular processes involved in neurulation and discusses possible roles of folate in this process. © 2005 Wiley-Liss, Inc.

KEY WORDS: spina bifida; anencephaly; neurulation

INTRODUCTION

Neurulation is the process of forming the neural tube, which will become the brain and spinal cord. In humans, it begins in the 3rd week after fertilization and requires that the top layers of the embryonic germ disc elevate as folds and fuse in the midline. The phenomenon is complex, involves numerous cell processes, and is often disrupted, resulting in neural tube defects (NTDs), such as anencephaly and spina bifida. Because of its complexity, most defects are considered multifactorial in origin and, while a great deal is known about the cellular events responsible for neurulation, much less is known about the molecular controls.

Cell and Tissue Interactions

At the end of the 2nd week (post fertilization), the embryo is a bilamminar disc consisting of epiblast cells in the top layer and hypoblast in the bottom layer of the streak, lies the primitive node, a depression containing cells important for organizing the embryonic axes (Fig. 1A,B). During gastrulation, epiblast cells migrate toward and through the streak and node, detach, and form two new layers ventral to the remaining eoiblast (Fig. 2A,B). The first cells through the streak displace the original hypoblast to form endoderm, while cells migrating slightly later create a new middle layer, the mesoderm (Fig. 2B). Cells remaining in the epiblast that do not migrate through the streak or node constitute the ectoderm. Cells passing through the node to migrate cranially in the midline (Fig. 2A) form the prechordal plate and notochord (Fig. 3), and these structures initiate the process of neurulation by inducing formation of the neural plate from overlying ectoderm cells (Fig. 1B). Thus, the neural plate is derived from ectoderm and forms in the central part of this upper layer. The remainder of the ectoderm surrounding the neural plate forms the epidermis.

[Sadler, 2004]. Soon, a groove, the primitive streak, appears in the caudal 3rd of the disc (Fig. 1A), signaling the initiation of gastrulation, the process of forming a trilamminar disc containing three germ layers—ectoderm, mesoderm, and endoderm. At the cranial end

At the end of the second week (post fertilization), the embryo is a bilamminar disc consisting of epiblast cells in the top layer and hypoblast in the bottom layer. Soon, a groove, the primitive streak, appears in the caudal 3rd of the disc signaling the initiation of gastrulation, the process of forming a trilamminar disc containing three germ layers—ectoderm, mesoderm, and endoderm.

Induction of the neural plate is due to an inhibition of epidermis formation by signals eminating from the primitive node, not by an activation of neural development. Thus, the default state of the original ectodermal germ layer is neural, not epidermal. The signal itself involves suppression of bone morphogenetic protein (Bmps) and *Wnt* signaling pathways [Harland, 2000]. As a result of these signals, cells destined to form the neural plate elongate in an apical-basal direction to form a thickened region of ectoderm called a placode [Schoenwolf and Powers, 1987; Schoenwolf, 1988]. This neural placode is broader at the cranial end and narrows caudally (Fig. 1B).

Once the plate is formed, it undergoes elongation by convergent extension, a process in which laterally placed cells move toward and are intercalated into the midline [Schoenwolf and Alvarez, 1989; Keller et al., 1992, 2000].

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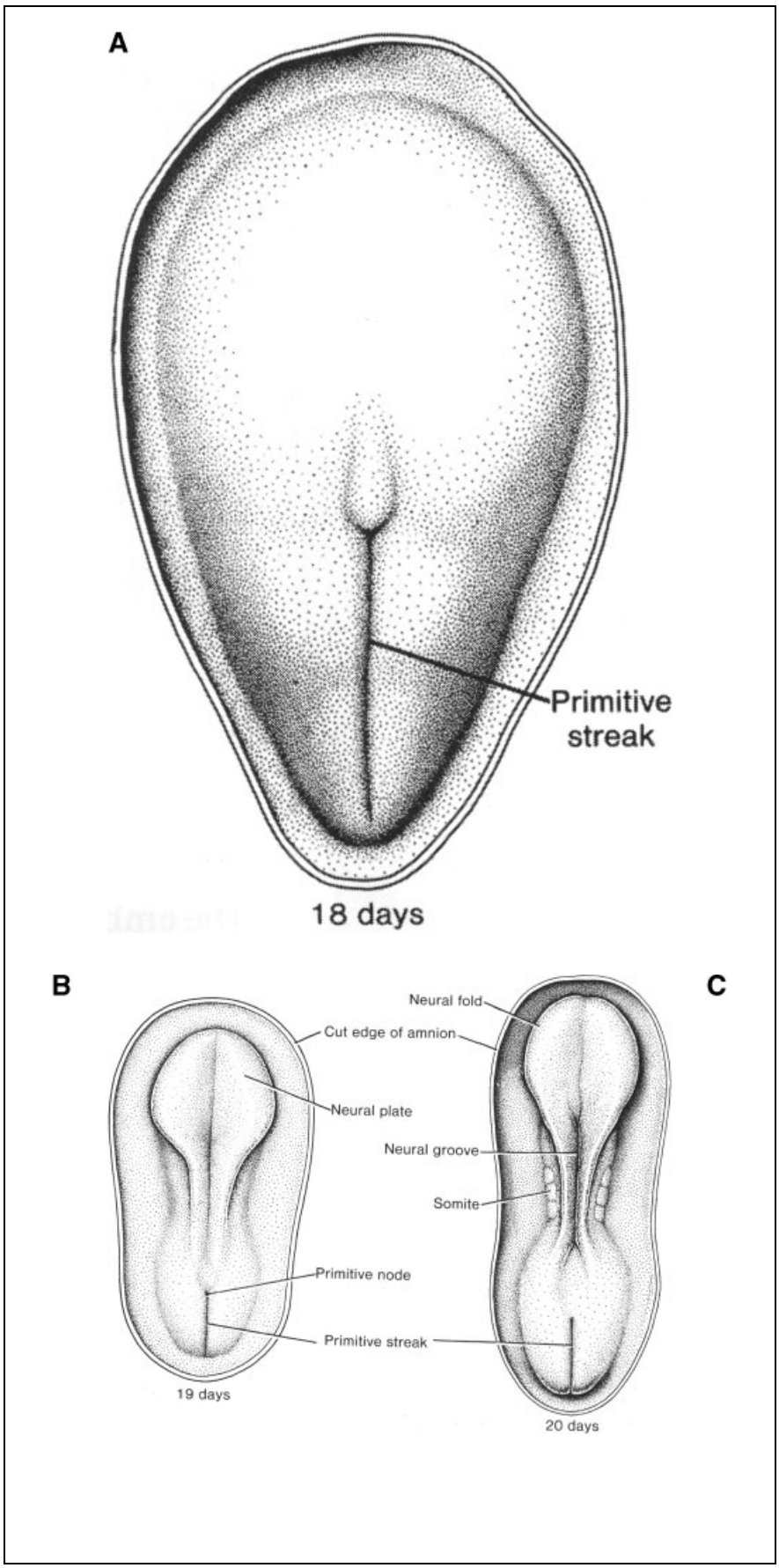

Similar cell movements occur in the underlying mesoderm as the entire body axis lengthens. Recent evidence suggests that *Dishevelled* (*DSH*), a member of the non-canonical *Wnt* signaling pathway is required for the process of convergent extension and that misregulation of this gene causes NTDs in animal models [Wallingford and Harland, 2001, 2002]. In addition to convergent extension, placement of daughter cells within the neural plate that arise from cell division, also causes

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Figure 1. Dorsal views of embryos showing the early stages of gastrulation and neurulation at various days after fertilization. **A**: The primitive streak, consisting of a narrow groove, forms in the caudal part of the embryo. At the streak's cranial end is an elevation, the primitive node surrounding a depression, the primitive pit. It is through the node and streak that epiblast cells migrate to form the three germ layers of the embryo by the process of gastrulation. **B**: The neural plate, a thickening of overlying ectoderm to form a placode, is induced at the cranial end of the embryo and signals the initiation of neurulation. At the caudal end of the embryo, the primitive streak remains involved in the process of gastrulation. Thus, gastrulation and neurulation continue simultaneously in the human embryo. **C**: The neural plate has now elevated to form the neural folds creating a neural groove in the midline. Pairs of somites, representing collections of underlying mesoderm that will form the vertebrae, appear on either side of the neural groove. This mesoderm helps support the elevating neural folds.

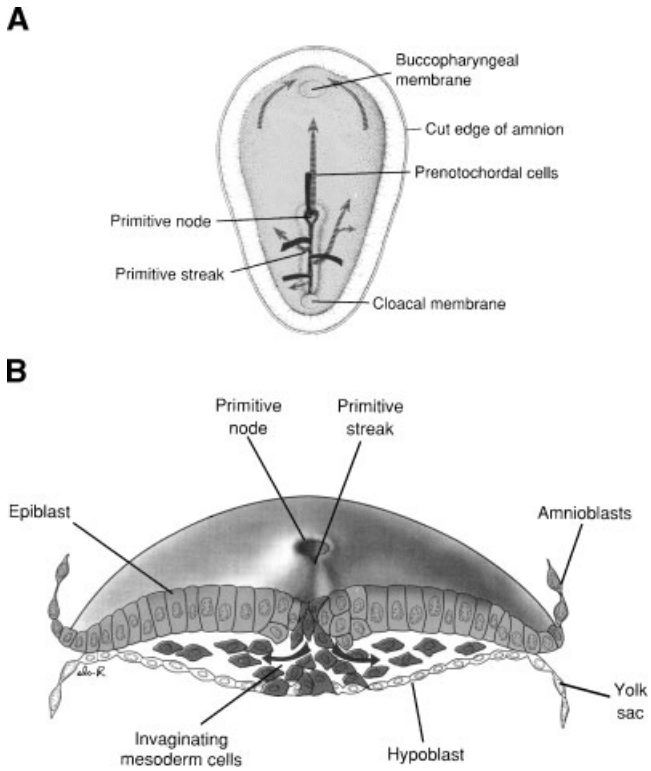

Figure 2. Schematic representation of the process of gastrulation. **A**: Arrows indicate the direction of migration of epiblast cells toward and through the primitive node and streak. Cells that migrate through the node and move directly cranially form the prechordal plate and notochord in the midline, **B**: Cross section through the primitive streak showing epiblast cells detaching and migrating to form a middle layer (the mesoderm) between the original epiblast and hypoblast. A new lower layer (the endoderm) is also formed as the ingressing cells displace those of the hypoblast. Cells remaining in the epiblast that do not migrate through the streak form the ectoderm. Thus, three germ layers are created.

lengthening in a craniocaudal direction. In this case, half of the cell division planes are positioned to place daughter cells in the longitudinal axis of the plate [Sausedo et al., 1997].

Once the neural plate is induced, its lateral borders elevate into the neural folds (Fig. 1C) and these folds move toward the midline to fuse. Initial elevation of the folds involves proliferation of the underlying mesoderm and production of hyaluronic acid by this tissue [Solursh and Morriss, 1977; Morriss-Kay and Crutch, 1982]. After elevation has occurred, bending of the neural folds proceeds in two steps: furrowing and folding [Colas and Schoenwolf, 2001]. Furrowing requires formation of three hinge points in the neural tissue: a median hinge point (MHP), overlying the prechordal plate and notochord and extending the entire length of the neural tube; and paired lateral hinge points (LHPs) along the sides of the folds in the cranial region (LHPs do not form in the spinal cord region of the neural tube; Fig. 4). Folding occurs around these hinge points with that involving the MHP resulting in elevation of the folds and that around the LHPs producing convergence of the folds [Schoenwolf and Franks, 1984; Colas and Schoenwolf, 2001]. The molecular signal for inducing the MHP is *Sonic Hedgehog*, secreted by the prechordal plate and notochord [Smith and Schoenwolf, 1989; Jessell and Sanes, 2000], but it is not known what signals the LHPs.

Shaping of the hinge points and neural folds requires microfilaments, microtubules, and changes in mitotic rates that, together, create apical constriction and basal expansion of the neural cells. Microfilaments containing actin and myosin are anchored by proteins in the apices of neural plate cells [Karfunkel, 1974; Nagele and Lee, 1980; Sadler et al., 1982, 1986; Lee and Nagele, 1985]. During hinge point formation and bending, these proteins are concentrated in the apices of the cells and, presumably, assist with constriction in this region. Disruption of their integrity in animal models results in NTDs, supporting a claim for their role in the process [Lee and Kalmus, 1976; Morriss-Kay, 1981].

Another factor affecting cell shape is mitosis. Neuroepithelial cells during neurulation are rapidly dividing with cell cycle times of 4–6 hr. During mitosis, nuclei in the neural plate travel the length of the cell from base to apex as DNA synthesis and cell division occur, a process called interkinetic nuclear

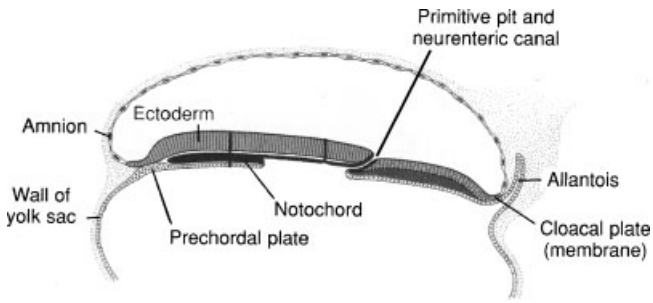

Figure 3. A mid-sagittal section through the embryo at the time of gastrulation. The embryo is shaped like a disc suspended between the amniotic and yolk sac cavities. Cells migrating through the node have formed the notochord and prechordal plate in the midline and these structures will induce the overlying ectoderm to form the neural plate.

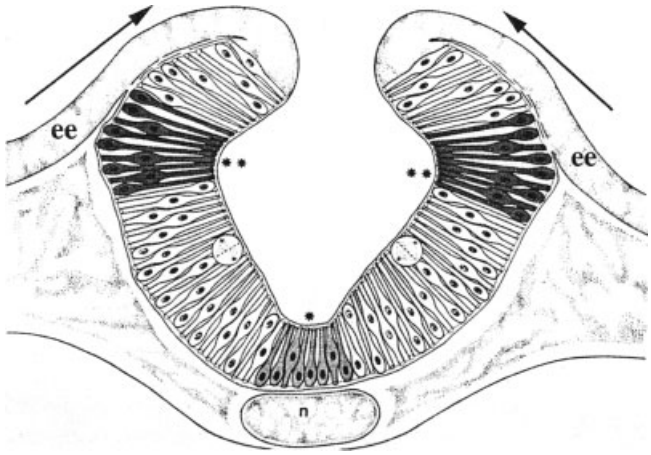

Figure 4. Cross section through the cranial neural folds as they approach each other in the midline. Median (*) and lateral (*) hinge points have formed to assist with the bending process. Overlying ectoderm (ee) creates forces that push the folds together. Notochord (n).

migration. Cell division occurs at the luminal surface where mitotic figures cause an expansion because of their large size. Where hinge points form, the cell cycle lengthens, such that nuclei remain at the base of cells for longer periods of time, thereby widening the base and narrowing the apex of cells located in these regions [Smith and Schoenwolf, 1987, 1988].

In addition to intrinsic forces in neuroepithelial cells themselves, non-neural ectoderm is a major contributor to bending of the neural folds [Sausedo et al., 1997; Colas and Schoenwolf, 2001; Lawson et al., 2001]. Thus, this ectoderm expands medially pushing the folds closer toward the midline (Fig. 4). Ectoderm expansion is mediated by cell flattening, intercalation, and oriented mitosis. During intercalation, ectoderm cells exchange neighbors creating a flow like a liquid in the plane of the tissue, pushing the neural folds [Colas and Schoenwolf, 2001].

Once neural folds meet in the midline, they undergo fusion (Fig. 5A). Cell processes are extended from one fold to the other and cell surface coats, consisting of glycoproteins, are deposited at regions of fusion [Sadler, 1978]. These surface coats act as a glue to hold the folds in place until more permanent cell to cell contacts can be established.

Many of these same events promote closure of the neural folds in prospective spinal cord regions. However, no LHPs appear, presumably because of the smaller lumen that forms in this region versus that formed in the larger brain vessicles. Instead, closure of spinal folds resembles the process of closing an open book lying on a flat surface. The sides elevate with little bending, except at the MHP, until the sides are parallel. At this point, the tips of the folds bulge toward each other to fuse around a narrow lumen. In another difference from cranial fold closure, neurectoderm cells make initial contact between opposing neural folds, whereas overlying ectoderm initiates contact in cranial regions. It is also not clear that microfilaments play a major role in closure of the spinal folds, since their disruption does not result in NTDs in this region [Ybot-Gonzalez and Copp, 1999].

Closure itself first occurs near the junction of the hindbrain and spinal cord at the level of the 5th somite (Fig. 5A). It then proceeds in zipper-like fashion cranially and caudally (Fig. 5B) [O'Rahilly and Muller, 2002].

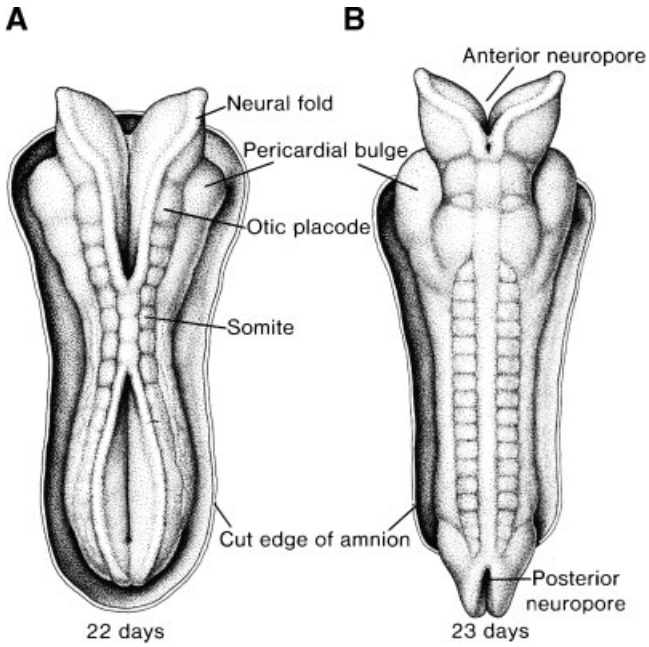

Figure 5. Dorsal views of neurulating embryos at different stages of neural tube closure (postfertilization). **A**: Closure begins at the caudal end of the hindbrain near its junction with the spinal cord. The neural folds then zipper in both directions. **B**: Continued zippering closes the neural tube. However, before the process is complete, a second closure site appears in the forebrain and this site zippers cranially and caudally to meet the advancing closure process that was initiated in the hindbrain. Prior to completion of closure, the open ends of the tube form the anterior (cranial) and posterior (caudal) neuropores.

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After initiation of closure, the open regions of the neural tube are called the anterior (cranial) and posterior (caudal) neuropores (Figs. 5B and 6A). Caudally, the zippering process continues until the posterior neuropore is closed, but in the cranial region a second site of closure appears in the forebrain [O'Rahilly and Muller, 2002]. Zippering then proceeds bidirectionally from this point to meet the advancing zippering process approaching from the hindbrain as well as cranially to close the most rostral part of the forebrain. The cranial neuropore is closed on day 25, while the caudal neuropore completes closure on day 28 (Fig. 6A,B). Once closure is initiated at a site, neurectoderm cells reorganize to form the roof of the neural tube, while overlying epidermal cells form the ectodermal layer of the skin.

The neurulation process just described is called primary neurulation, and it is responsible for establishing the brain and spinal cord regions down to the lowest sacral levels (probably S4-5). From this level caudalward, secondary neurulation forms the remainder of the cord. In this phenomenon, the neural tube forms from mesoderm cells that coalesce and then epithelialize [Schoenwolf, 1979]. These epithelial cells reorganize around a lumen forming the caudal-most regions of the neural tube that then becomes continuous with the remainder of the tube formed by primary neurulation (note that the junction between the two processes is well below the site of virtually all occurrences of spina bifida and so cannot be considered a potential factor in the origin of the vast majority of these NTDs).

NEURAL TUBE DEFECTS

As might be expected with such a complex process, NTDs are common, occurring in the United States with an overall frequency of approximately 1/1,000 births. However, rates vary in different regions of the country with higher rates in the East, lowest in the West, and the highest in the Southeast, particularly in North and South Carolina. Rates also vary in different populations with people of Mexican and Irish descent having higher rates than Caucasians. Even among peoples of similar ethnicity, rates can vary greatly. For example, in Northern China rates of 1/200 were observed compared to 1/1,000 among those living in the Southern part of the country [Berry et al., 1999].

The term neural tube defect is applied to a variety of abnormalities, most of which result from a lack of closure of the neural tube. The most severe are "open" defects in which neural tissue is exposed. These defects may occur cranially, causing anencephaly, which is fatal, or caudally, usually in the lumbosacral area, causing spina bifida cystica. Regardless of where they occur, the vast majority of these types of closure defects result from failure of the neural folds to elevate and fuse. Other NTDs may involve only the coverings of the brain and cord, such as meningoceles or myeloceles, or may include neural tissue, as in the cases of meningoencephalocele and meningomyeloceles. In these types of defects the folds may have come together, but the normal fusion process was disrupted. Bony defects overlying these abnormalities may be caused by a lack of signaling between underlying neural tissue and overlying mesoderm and ectoderm.

Most NTDs are multifactorial in origin, having both genetic and environmental components. With respect to genetics, there is not just one neural tube defect gene. Instead, misregulation of any of a number of different genes may result in an NTD. As mentioned, *Dishevelled* appears to be important for convergent extension and its disruption may account for some NTDs [Wallingford and Harland, 2001, 2002]. *Sonic Hedgehog* is responsible for floor plate induction and MHP formation [Jessell and Sanes, 2000], but NTDs do not occur when this gene is misregulated, even though an MHP fails to form [Chiang et al., 1996]. Other gene candidates include those in the folic acid pathway. One of these, the

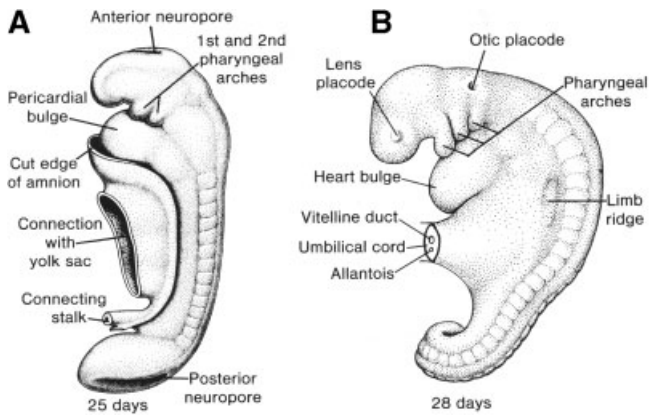

Figure 6. Lateral views of 25 and 28 day (postfertilization) embryos. **A**: Closure of the anterior neuropore is completed on day 25. **B**: Closure of the posterior neuropore is completed on day 28.

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MTHFR gene for methylene tetrahydrofolate reductase that catalyzes the reduction of folate as part of the methylation pathway, appears to play a role in some NTDs [Shields et al., 1999]. However, it is a relatively minor one accounting for at most 15% of these defects [Botto and Yang, 2000]. The search for other genes in the folic acid pathway that might contribute to NTDs has failed to demonstrate any other associations. Emphasis on folic acid metabolism as a major contributor to NTDs has occurred because unequivocal evidence shows that folic acid supplementation (400 μg/day), initiated 2–3 months prior to pregnancy reduces the risk of NTDs by up to 70% [MRC Vitamin Study Research Group, 1991; Ceizel and Dudas, 1992].

While genetic mutations in key genes in the folic acid metabolic pathway may yet be discovered to explain the origin of additional NTDs, it is important to note that folic acid itself is also essential for maintaining basic cell processes, including de novo synthesis of nucleotides for DNA synthesis. This pathway is especially important for embryos during neurulation because it probably represents their only source of these nucleotides [Rowe and McEwen, 1983]. Nucleotides are essential for sustaining mitosis, especially in rapidly proliferating cell populations, such as those found in the neurectoderm, and for DNA repair. Cells normally have a 5 min supply of these DNA precursors [Skoog and Nordenskjold, 1971; Skoog et al., 1974] and if the proper ratios are not maintained, mutations increase, DNA repair mechanisms fail, and DNA synthesis decreases [Meuth, 1984; Yoshioka et al., 1987; Hirota et al., 1989; James et al., 1994]. Since mitosis plays a key role in neurulation [Smith and Schoenwolf, 1987, 1988], it is easy to consider a link between NTDs and this aspect of folate metabolism.

It is also possible that compromised DNA synthesis produced by an inadequate folate supply may increase the embryo's sensitivity to teratogens. Some evidence for this hypothesis is derived from studies showing that embryos from mice who were almost completely folic acid deficient had developmental delays, but no overt birth defects, although here was an increase in spontaneous abortions [Burgoon et al., 2002]. Thus, even while maternal folate levels were extremely low, surviving embryos were spared, but were they also more vulnerable to a second insult? The fact that they had developmental delays suggests they were, but this potential remains to be investigated. In support of this hypothesis, that folic acid provides essential components for a general process, such as DNA synthesis and mitosis, are these observations: (1) in increased amounts, the vitamin protects against heart, craniofacial, and other birth defects [For review, see Antony and Hansen, 2000], e.g., a variety of defects that might have their origins in the disruption of a general metabolic pathway as a common denominator; (2) the vitamin also affords protection against some genetic [Zhao et al., 1996; Flemming and Copp, 1998] and environmental causes of birth defects, including methanol [Sakarashi et al., 1998], valproic acid [Wegner and Nau, 1991], hyperthermia [Shin and Shiota, 1999; Botto et al., 2002; Shaw et al., 2002], and some mycotoxins [Sadler et al., 2002], again suggesting that a general effect of the vitamin protects the embryo. Such an effect on DNA synthesis might allow embryos to recover more quickly from an insult by producing more cells or by avoiding genetic mutations by providing large nucleotide pools for DNA repair.

Certainly, other genetic causes of human NTDs will be discovered. In fact, in mice a number of key "neurulation" genes have been identified [Harris and Jurriloff, 1999; Copp et al., 2000]. Colas and Schoenwolf [2001] provide an excellent discussion of the potential genes involved and have listed a number of candidates. However, not all NTDs will be shown to have a single gene mutation; most will remain multifactorial in origin with a strong environmental component.

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