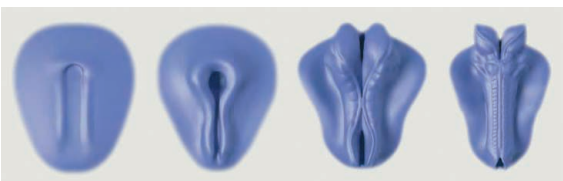
The Continuing Challenge of Understanding, Preventing, and Treating Neural Tube Defects

John B. Wallingford,[1]* Lee A. Niswander,[2] Gary M. Shaw,[3] Richard H. Finnell[4]

Background: Neural tube defects (NTDs) are debilitating birth defects involving the central nervous system (CNS). Despite recent advances, NTDs represent the second most common group of human birth defects. These defects arise when the complex process of early CNS development goes awry. Normally, the brain and the spinal cord begin to form as a flat sheet of cells that rolls up and closes to form a hollow neural tube. Failure in this rolling and sealing process results in an NTD, such as spina bifida. From animal models, we know of over 200 genes that regulate this process, with many more still likely to be discovered. Environmental factors also can have a profound influence on neural tube closure, as evidenced by the impact of folic acid on NTD prevalence. However, the mechanisms by which environmental factors affect the process of neural tube closure and their critical interaction with genetic factors remain largely a mystery.



Successive images showing the progression of neural tube closure in a stylized vertebrate embryo. Initially, the CNS is a flat sheet; paired neural folds elevate along the rostrocaudal axis (rostral = up) and move medially, eventually fusing to enclose the neural tube. Disruption of this process during human embryogenesis results in neural defects, such as spina bifida.

Advances: Three major advances from three different directions—genetics, epidemiology, and surgery—have advanced understanding, prevention, and treatment of NTDs. The rapidly expanding knowledge of the genetic causes of NTDs in animal models is poised to inform high-throughput whole-genome studies of human patients. Epidemiological studies have led to the identification of folic acid as a primary prevention strategy for NTDs. Recent advances in in utero surgical repair of spinal NTDs have improved the clinical outcome by comparison with postnatal surgery.

Outlook: Despite the advances, NTDs remain a very common birth defect and, even with surgical intervention, result in enormous clinical, emotional, financial, and societal costs. The implementation of large-scale genomic studies of human NTD patients is expected to move the field beyond its current focus on individual genetic pathways. Experimental animal systems can complement and extend the information that flows from genomic studies, and animal models can also be exploited to understand the mechanisms by which environmental factors alter the risk for NTDs. The technology exists to create patient-derived stem cells, which may hold a key for understanding this very early developmental process in humans and could provide a platform for screening therapeutic agents. Overall, the key challenge will be to understand the developing neural tube, a complex three-dimensional structure that changes rapidly over time and is dependent on the surrounding tissues for developmental signals and biomechanical forces to drive the dynamic and important process of neural tube closure.

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ARTICLE OUTLINE

BACKGROUND READING

Research with animal models is expanding our understanding of normal and defective neural tube development. These insights can be relayed to intervention strategies to prevent and treat human defects.

H. J. Blom, G. M. Shaw, M. den Heijer, R. H. Finnell, Neural tube defects and folate: Case far from closed. *Nat. Rev. Neurosci.* **7**, 724–731 (2006). doi:10.1038/nrn1986

N. S. Adzick *et al.*, A randomized trial of prenatal versus postnatal repair of myelomeningocele. *N. Engl. J. Med.* **364**, 993–1004 (2011). doi:10.1056/NEJMoa1014379

M. J. Harris, D. M. Juriloff, Mouse mutants with neural tube closure defects and their role in understanding human neural tube defects. *Birth Defects Res. A Clin. Mol. Teratol.* **79**, 187–210 (2007). doi:10.1002/bdra.20333

M. J. Harris, D. M. Juriloff, An update to the list of mouse mutants with neural tube closure defects and advances toward a complete genetic perspective of neural tube closure. *Birth Defects Res. Part A Clin. Mol. Teratol.* **88**, 653–669 (2010). doi:10.1002/bdra.20676

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The Continuing Challenge of Understanding, Preventing, and Treating Neural Tube Defects

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Human birth defects are a major public health burden: The Center for Disease Control estimates that 1 of every 33 United States newborns presents with a birth defect, and worldwide the estimate approaches 6% of all births. Among the most common and debilitating of human birth defects are those affecting the formation of the neural tube, the precursor to the central nervous system. Neural tube defects (NTDs) arise from a complex combination of genetic and environmental interactions. Although substantial advances have been made in the prevention and treatment of these malformations, NTDs remain a substantial public health problem, and we are only now beginning to understand their etiology. Here, we review the process of neural tube development and how defects in this process lead to NTDs, both in humans and in the animal models that serve to inform our understanding of these processes. The insights we are gaining will help generate new intervention strategies to tackle the clinical challenges and to alleviate the personal and societal burdens that accompany these defects.

The vertebrate neural tube serves as the precursor to the central nervous system (CNS): the brain and spinal cord. Structural birth defects of the CNS, called neural tube defects (NTDs), are a multifactorial disorder that arise from a complex interaction of genetic and environmental factors. Although we are only now beginning to understand the etiologies of NTDs, two major advances have been made in their prevention and treatment. First, it is widely known that taking folic acid (FA; vitamin B9) during child-bearing years can greatly reduce a woman's risk of having a baby with a NTD (*1*). Second, studies have demonstrated that in utero repair of spina bifida improves patient outcomes relative to surgery performed in the post-parturition period (*2*). In spite of these advances, we have only just begun to tackle the problem of NTDs. For example, 16 years after mandated folate fortification in the United States, NTD rates remain unacceptably high: 1 in 2000 births in the United States and considerably higher still in developing areas such as China (27.8 per 2000 births) (*3*) and Latin America (4.4 per 2000 births in Argentina; 6.2 per 2000 births in Brazil) (*4*). Furthermore, we understand relatively little about the mechanism by which folate acts on NTDs. Similarly, many NTD patients that have undergone surgery suffer wide-ranging neurological, urological, and orthopedic problems. Thus, NTDs continue to present a major public health burden.

Here, we review the process of neural tube development and how defects in this process lead to NTDs. We discuss both human NTDs and the ways in which animal studies are providing new insights. We focus on recent important findings and highlight unanswered questions about the molecular basis of FA action and genetic susceptibility to NTDs. Lastly, we discuss strategies that could build on our current understanding to lessen the personal and societal burdens that accompany these serious malformations.

NTDs Are a Problem of Embryology

NTDs arise as a defect in embryonic development. During embryogenesis, the central nervous system normally develops as a flat sheet of cells that subsequently rolls up and fuses shut to form the hollow neural tube (Figs. 1 and 2A). NTDs arise when this process of neural tube closure (NTC) is disrupted (Fig. 2B). As discussed below, cellular changes that drive NTC and the molecular mechanisms that control them are largely conserved from humans and mice to birds and amphibians. Indeed, decades of work in animal models have established a comprehensive framework for the developmental processes that underlie NTC (*5*–*8*).

NTDs: Not One Disease, But Many

Although often lumped into a single category, NTDs encompass a wide array of morphologically distinct malformations. In general, failure of NTC is associated with defects in the overlying bony structures (i.e., cranial vault and neural arches) such that neural tissue is exposed. Consequently, most defects of primary NTC are referred to as open NTDs. There are also a number of closed or skin-covered conditions; however, relatively little is known about their etiology. The open NTDs fall into three general categories, but further refinement of these classifications—such as vertebral location and extent of the defect—will help parse some of the differing underlying etiologies.

NTDs restricted to the cranial regions are referred to as anencephaly (Fig. 1C). This invariably lethal condition is characterized by absence of the cranial vault and severe defects in the cerebral hemispheres. The cerebellum is usually absent, and the brain stem may be hypoplastic. NTDs restricted to the caudal portion of the neural tube are referred to generally as spina bifida (meningomyelocele) (Fig. 1C). More prevalent than anencephaly, this condition is associated with defects in the neural arches, through which meninges and spinal cord tissue protrude. The majority of fetuses with meningomyeloceles are live born and, with proper treatment, survive to adulthood. Failure of NTC over the entire body axis, called craniorachischisis (Fig. 1C), is also lethal but relatively rare. Not only do the natural histories of these disorders vary, but even within a single classification, such as meningomyelocele, the degree of handicap is variable. Refinement in the classification of these malformations (e.g., by precise vertebral position of spina bifida) and the assignment of contributing gene pathways should translate into more effective preventative measures and management of affected infants.

Epidemiology of Human NTDs

Prevalence of NTDs has varied over time and geography. Prevalence in the United States is about one in 2000 births and was about 2 in 1000 births in the late 1960s. In neighboring Mexico, the prevalence is significantly higher (3.2 per 2000) (*9*). NTDs are etiologically complex, and both genetic and environmental risk factors have been proposed. In terms of genetic underpinnings, monozygotic twinning and single-gene disorders have long been associated with NTDs (*10*), with concordance rates of monozygotic twins (7.7%) exceeding that of dizygotic twins (4.0%), attesting to some genetic contribution to their etiology (*11*). Numerous studies have explored a variety of candidate gene pathways [reviewed in (*12*)], such as the folate/1-methyl carbon metabolic pathway (*13*, *14*), glucose metabolism and/or transport (*12*), DNA repair (*15*), oxidative stress pathway (*12*), retinoic acid receptors (*16*), and the WNT/planar cell polarity (PCP) signaling network (*17*). In a following section, we discuss animal studies that are shedding new light on genetic factors that govern NTC. However, it is crucial to note that the population burden of

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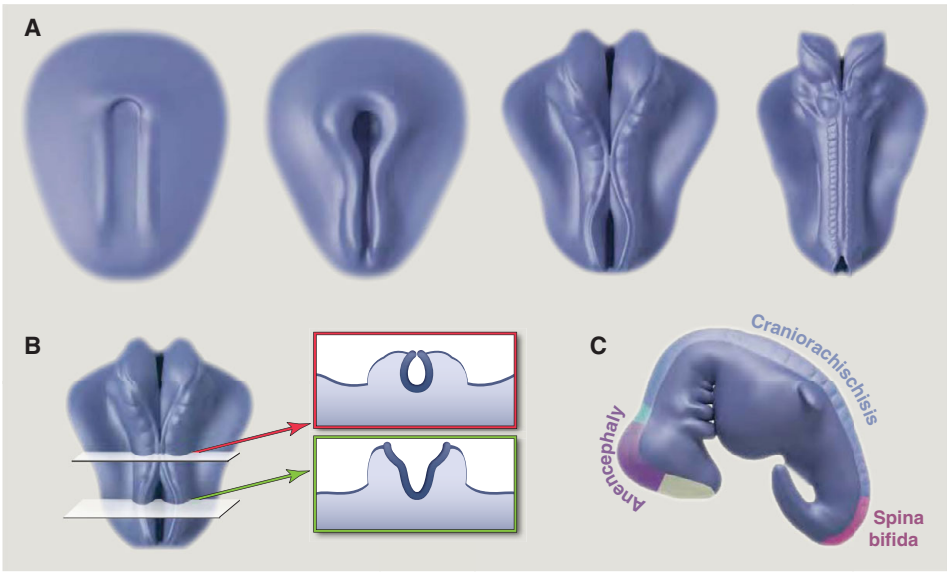

Fig. 1. (**A**) Successive images showing the progression of neural tube closure in a stylized vertebrate embryo (rostral = up). Initially, the CNS is a flat sheet; paired neural folds elevate along the rostrocaudal axis and move medially, eventually fusing to enclose the neural tube. (**B**) Cross sections illustrate closed (red) and open (green) regions of the neural tube. (**C**) Region-specific NTDs.

human NTDs thus far explained by known genetic polymorphisms remains quite small, a fact that almost certainly reflects the relatively modest amount of research effort in this area rather than a relative lack of importance of genetic factors in NTDs. Indeed, no large-scale NTD-focused genomic discovery project (genome-wide association study, exome sequencing, etc.) has yet been published.

The key role that environment plays in NTD etiology is highlighted by the impact of maternal nutrition, specifically folate intake. Both observational studies and randomized trials have provided evidence that FA reduces the risk of NTD-affected pregnancies (*1*, *18*). However, 30 to 50% of NTDs are not folate preventable, and other environmental factors must be considered (*1*). Among the most notable environmental risk factors for NTDs are maternal pregestational insulin-dependent diabetes (*19*) and maternal prepregnant obesity (*20*), as well as maternal use of specific anticonvulsant drugs, including valproic acid (VPA) (*21*).

The teratogenic potential of maternal pregestational diabetes is well established and includes a 2- to 10-fold increase in the risk of CNS malformations (including NTDs) among the offspring of affected women relative to the general population (*22*). Moreover, lack of periconceptional glycemic control and intake of foods of higher glycemic index values have been associated with the risk of NTDs in the offspring of diabetic women (*23*, *24*). Because human embryos lack pancreatic function until after the seventh week of gestation and NTC occurs between weeks 3 and 4, the teratogenic effect of maternal diabetes may be due to embryonic exposure to elevated glucose concentrations. Pre-pregnancy obesity is another consistently observed risk factor for NTDs. The risk increases with increasing maternal body mass index (BMI). Women in the highest BMI categories have a 1.5- to 3.5-fold higher risk of having an NTD-affected child than women with lower BMI (*20*, *25*, *26*). The increased risk of NTDs in the offspring of obese women may be attributable to hyperinsulinemia (*27*). This could provide a common explanation for the associations between NTD risk and maternal obesity and maternal pregestational diabetes, because hyperinsulinemia may coexist with both conditions.

With respect to pharmaceutical NTD teratogens, the anticonvulsant drug VPA (Depakene, Abbott Laboratories) has been associated with a 1 to 2% risk of having a pregnancy affected with spina bifida, whereas the risk of anencephaly does not appear to be increased (*28*). VPA may influence neural tube development via its action as a potent inhibitor of histone deacetylases (HDACs) (*29*, *30*), and studies in model animals are consistent with this idea [see below and (*31*–*33*)]. Class I and II HDACs are involved with histone modification and therefore play a role in the regulation of gene expression. It is also possible that posttranslational gene modification is compromised by VPA exposure, which inhibits HDACs and limits the availability of folate molecules (*34*), thereby increasing the risk of NTDs.

Diagnosis, Treatment, and Outcome

Screening to identify pregnant women at risk for carrying NTD-affected fetuses is achieved by evaluation of maternal serum alpha-fetoprotein levels, amniocentesis, and ultrasound imaging (*35*). Individuals with spina bifida can generally survive if they undergo surgical repair in utero or soon after birth (*36*, *37*). A recent randomized trial showed that in utero repair of spina bifida improved patient outcomes in terms of improved motor function and ambulation and mental development compared with those receiving surgery postpartum (*2*). Moreover, 40% of infants receiving in utero surgery required a ventriculoperitoneal shunt at 12 months of age, compared with 82% of infants undergoing postnatal surgery (*2*).

However, even after surgery (in utero or postnatally), individuals with spina bifida remain at elevated risk for nervous system malformations, including hydrocephalus and skull malformations that press the brain downward into the spinal canal. Lower extremity weakness and paralysis, sensory loss, and bowel and bladder dysfunction are also common. Individuals with spina bifida are also at risk for a range of orthopedic abnormalities including clubfoot, contractures, hip dislocation, scoliosis, and kyphosis. Moreover, although most individuals with spina bifida have normal intelligence, specific cognitive and language difficulties are common (*38*).

Even successful surgical repairs represent only a partial solution. Because NTC occurs early in development (~3 weeks postconception), a NTD will leave the delicate neural tissue, normally encased within bone, exposed and subjected to trauma in utero until the much later time point for surgical intervention. As such, substantial effort should be focused on understanding the mechanisms of NTC and the biological basis of NTDs, with an eye toward developing preventive strategies.

Primary Prevention of NTDs by Folic Acid

NTDs stand out as one of few birth defects for which primary prevention strategies are available. Research spanning decades, including randomized and community-based trials, demonstrate that maternal, periconceptional supplementation with FA alone or multivitamins containing FA can reduce the risk of NTDs in offspring (*1*, *39*, *40*). Fortification of FA in the U.S. food supply has been associated with upward of a 20% decline of anencephaly and a 34% decline in spina bifida (*41*–*43*). Reported prevalence declines have been higher in Canada and Chile (*44*, *45*). How FA acts to prevent NTDs is a major outstanding question, and the answer will be complex because folate is central to numerous cellular reactions. These include production of purines and thymidylate, the building blocks for DNA and RNA biosynthesis, and production of the universal methyl donor *S*-adenosyl-methionine (SAM), used in methylation of DNA, histones, proteins, and lipids. Therefore, deficits in FA metabolism could affect cell proliferation, cell survival, transcriptional regulation, or a host of other cellular reactions; defects in any of these processes could disrupt NTC. To bring insight into the mechanisms by which FA acts during NTC, studies have turned to animal models, in particular mouse NTD models, which are thought to be representative

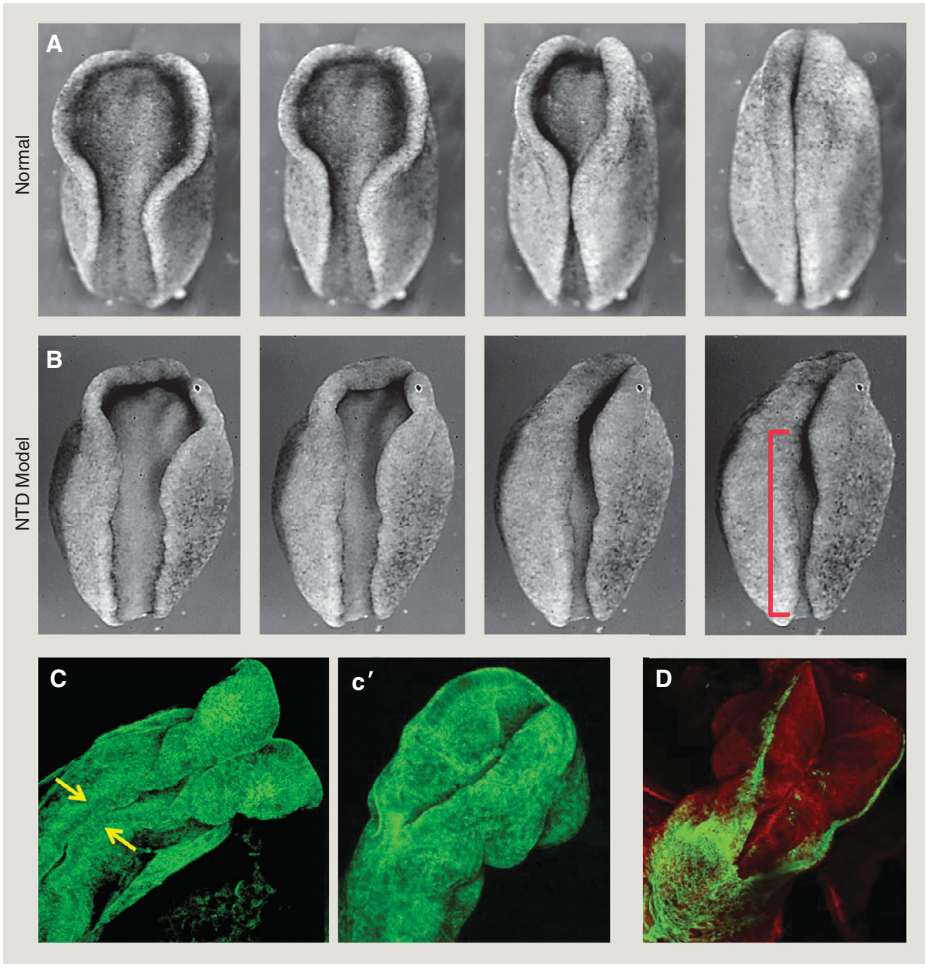

Fig. 2. (**A**) Still frames from a time-lapse movie showing the progress of NTC in an amphibian embryo (rostral = up). (**B**) Disruption of PCP signaling results in disrupted NTC (red bracket). (**C**) Still frame from a movie of mouse neural tube closure; arrows indicate initial meeting of neural folds (*66*). (**c′**) NTC has progressed in a later time point. (**D**) Genetically inducible fluorescent reporters allow visualization of specific tissues, in this case the ectoderm (green) that borders the neural tissue (red).

of human neurulation anatomically and molecularly and in which folate levels can be altered before or during pregnancy.

Mutations have been made in numerous mouse genes required for FA metabolism or use; however, only three mutated genes [*Folr1*, *Slc19a1* (previously *Rfc*), and *Mthfd1l*] have overt NTD phenotypes (*46*–*48*). Even under conditions of folate deficiency, there is relatively little evidence of altered NTD incidence for mouse FA pathway mutations (*49*). Moreover, there is as yet no compelling association between *Folr1* or *Slc19a1* and human NTDs, although a few *Mthfr* polymorphisms have been identified as possible risk factors for human NTDs (*14*). Currently, in the folate-replete population in the United States, the majority of human pregnancies are within the normal range of FA levels, and recent studies found little association between NTD risk and maternal FA intake, perhaps suggesting that FA-sensitive NTDs have largely been prevented (*50*). Therefore, data to date suggest that deficits in the FA pathway likely represent only a modest fraction of NTD risk.

Looking beyond the folate pathway to elucidate gene-environment interactions. The large number of mouse NTD models with no apparent link to the FA pathway provides enormous potential to explore how genetics impact responsiveness to FA and to define mechanisms by which FA influences NTC. This potential has been only minimally realized, because only 23 of the >200 mouse NTD mutants have been tested for FA responsiveness (*49*, *51*, *52*). FA treatment has a preventive effect in 11 mouse NTD models; some show a correlation with compromised FA use, but others do not, indicating that disrupted FA metabolism is unlikely to be the full explanation for FA-mediated effects. Currently there is little that ties together NTD models that are FA-responsive or FA-resistant at a mechanistic level. Testing a far greater number of mouse mutants will expand this data set, may reveal common pathways or targets, and should lead to better predictions as to whether FA or perhaps another treatment would be most effective in preventing NTDs.

Contrary to expectations, FA treatment in a few mouse NTD models resulted in detrimental effects, including an increased risk for NTDs and embryo loss before the time of NTC (*51*, *52*). These findings of early embryo loss are consistent with the possibility raised in 1997 based on miscarriage risk that embryo loss may explain some of the decrease in human NTD occurrence upon FA supplementation (*53*). If these findings are relevant to human NTDs, it could suggest that, for certain mutations, FA may not be protective or even neutral in its action. Additional studies in animal models will be required to determine the basis for the observed detrimental effect and whether there are particular gene or pathway mutations that are more susceptible, either positively or negatively, to FA effects.

Possible epigenetic changes induced by FA. FA is known to cause epigenetic changes. Alterations in SAM levels could affect DNA methylation and histone modification, both of which can influence gene transcription. Indeed, there is evidence that methyl donor–enriched diets can induce alterations in gene expression, and long-term generational exposure can result in increasing variation in DNA methylation even in wild-type mice (*54*). Moreover, questions have been raised as to whether the increase in FA intake acting through the methylation cycle may predispose to allergic airway disease, although the current evidence is conflicting (*55*–*57*). With respect to NTD risk, some mutant mice showed a beneficial response to increased FA over a single gestation period but a detrimental response over multiple generations (*52*). This contradictory response depending on the length of FA exposure highlights the difficulties in considering how best to model human exposure to FA as currently implemented. Moreover, it raises the question of whether there may be unexpected effects of long-term FA fortification and supplementation in humans or potential effects because of increased levels of metabolized and unmetabolized FA.

The variation in NTD risk depending on the length of FA exposure (*52*) points toward the possibility of epigenetic changes. Consistent with this idea, mutations in genes that affect DNA methylation, histone modification (in particular acetylation), or chromatin remodeling result in NTDs in mice (*6*, *31*, *58*). As previously described, the antiepileptic drug VPA is a HDAC inhibitor, and it is a well-known risk factor for NTDs in humans (*21*). Interestingly, NTDs in mice bearing mutations in the HDACs GCN5 or CITED2 can be prevented with FA supplementation (*32*, *33*). Epigenetic influence has also been suggested to help explain the predominance of cranial NTDs in females versus males. X chromosome inactivation is maintained by DNA methylation, and hence there is more demand on the methylation cycle in female cells after every

division relative to male cells (*59*). Epigenomic studies should bring new insights into how FA may affect transcriptional programs during NTC.

The Cell Biology of NTDs

One complication that has hindered our understanding of NTDs generally, and of FA action specifically, is the generally underappreciated complexity of NTC. Although it can be described simply and succinctly as a sheet rolling up to form a tube, NTC is actually the sum of several autonomous and region-specific changes in cell behavior (*5*–*8*). For example, NTC begins not with rolling but instead with a thickening of the neural ectoderm, only after which do the neural folds elevate and begin moving toward the midline. This medial movement of the folds is facilitated by bending of the epithelial sheet and by narrowing and lengthening of the neural tissue. In addition, some studies suggest that the epidermis generates a pushing force that aids the movement of neural folds toward the midline (*8*). Lastly, a poorly defined process of epithelial fusion links the two neural folds into a sheet of epidermal cells covering the hollow neural tube [e.g., (*60*)].

Adding to this complexity is the discontinuity of the NTC process along the rostrocaudal axis. Rather than progressing continuously from one end or the other, NTC initiates at multiple sites along the axis and progresses rostrally and/or caudally from those sites. The cellular machines that drive the rolling up of the neural tube differ regionally along the length of the neural tube. This point is important in light of the heterogeneity observed in human NTDs (e.g., spina bifida versus anencephalyl; Fig. 1C), because studies in animals now make clear that these spatially restricted subtypes of NTD stem from regional differences in the underlying cell behaviors.

One example is the process of convergent extension, whereby cells interdigitate mediolaterally in order to elongate the tissue perpendicularly along the rostrocaudal axis (Fig. 3, pink/green cells). This process acts specifically in the hindbrain and spinal cord, with disruption of genes governing convergent extension resulting in craniorachischisis (*7*, *17*). In contrast, another cellular process called apical constriction converts columnar cells into wedge-shaped cells, leading to localized bending of the neural epithelium and facilitating NTC (Fig. 3, blue cells). Apical constriction is most important in the future brain and the caudal-most spinal cord, because disruption of genes controlling apical constriction is associated most strongly with anencephaly and caudally restricted spina bifida (*7*).

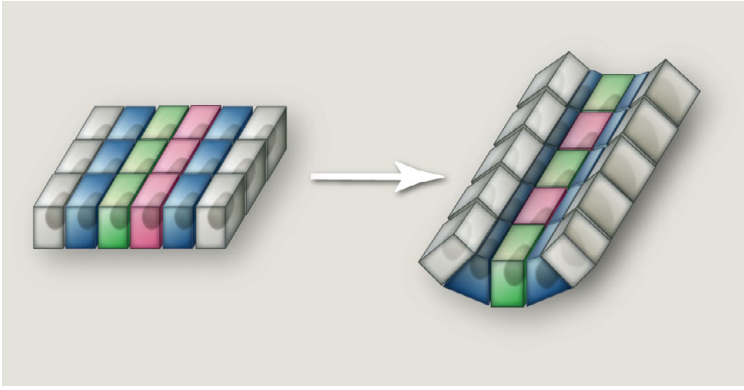

Fig. 3. Multiple cell behaviors contribute to neural tube morphogenesis. In this schematic, pink and green cells illustrate convergent extension. By exchanging neighbors specifically in the mediolateral (horizontal) axis, the sheet of cells is elongated in the anteroposterior (vertical) axis. Blue cells illustrate apical constriction. These cells do not move but rather change their shape, leading to a bend in the tissue sheet. [Schematic adapted from (*7*)]

Lastly, much of what is known about the dynamic events of vertebrate NTC comes from live imaging studies of amphibian and chick embryos (Fig. 2A and Movie 1) (*61*–*64*), but important advances have recently been made such that dynamic imaging of NTC is now possible in the living mouse embryo (Fig. 2, C and D, and Movie 2) (*60*, *65*, *66*). The emerging ability to combine prolonged live imaging with the wealth of mouse genetic mutants will drive a deeper understanding of how gene function regulates the cell and tissue behaviors necessary for this critical and sensitive stage of embryonic development.

The Genetics of NTDs in Animal Models

Animal studies have identified hundreds of genes as candidates for human genetic studies. In the mouse, over 200 genes are causative for NTDs (*58*, *67*), and many others have been provided by studies in amphibians (*7*). These genes fall into diverse functional classes and include regulators of actin dynamics, cell adhesion, electron transport, DNA damage repair, and other processes (*58*, *67*). Because most studies reporting NTDs in mutant animals were focused primarily on other biological questions, the majority of these models are only cursorily characterized. Nonetheless, some themes are emerging.

1) Genetic modules govern closure in discrete regions of the CNS. NTDs in mutant mice fall into heterogeneous subtypes similar to those seen in humans, and genes with common cell-biological functions tend to associate with the same subtype. For example, disruption of genes encoding Shroom3, Abl, or Mena results in highly penetrant exencephaly and caudally restricted spina bifida (*68*–*70*), and each of these genes has been implicated in the control of apical constriction (*71*–*73*). As described below, exencephaly also consistently results from disruption of any of a large number of genes associated with the assembly or function of cilia, whereas mutations in genes associated with the PCP signaling network associate with craniorachischisis (*17*, *74*, *75*). These linkages of genetic modules to particular NTD subtypes is important, because they suggest that more accurate and more specific diagnosis of human NTD subtypes will facilitate studies of how specific genetic polymorphisms confer NTD risk in humans.

2) Cilia and NTDs. One genetic module that is well defined and has emerged as a critical regulator of NTC is that governing ciliogenesis (*75*). Cilia are small microtubule-based cellular protrusions that are essential for cell-cell signaling (*76*), a discovery first made in the course of mouse genetic screens focused on neural tube morphogenesis (*77*, *78*). Roughly two dozen genes associated with ciliogenesis have now been implicated in NTC in mice (*75*). The cell biological mechanism linking cilia to NTDs remains unclear, but there is evidence to suggest defects in apical constriction. First, like mutation of genes associated with apical constriction, mutation of cilia-related genes elicits exencephaly specifically (*75*). Second, cilia are crucial organelles for Hedgehog signal transduction (*76*), and Hedgehog signals have been linked directly to neural patterning and to neural epithelium bending, a process facilitated by apical constriction (*79*).

This work in animals has paralleled work in humans revealing a link between cilia and an array of human diseases, and this "ciliopathy" disease spectrum includes both severe and milder forms of NTDs (*80*–*82*). For example, the *fuz* gene is crucial for ciliogenesis and NTC in animal models (*83*, *84*), and mutations in *fuz* have also been associated with human NTDs (*85*). Moreover, a number of genes associated with Meckel-Gruber syndrome, in which occipital encephaloceles are frequently observed, are also known to compromise cilia formation (*81*, *82*).

3) PCP and NTDs. Another genetic module for which a link to NTDs is emerging is the PCP network, which was discovered in *Drosophila* and has now been shown to govern a wide array of polarized cell behaviors (*74*). Beginning with studies of the mouse mutant *loop-tail*, researchers have found that mutations in many PCP genes (*Fz*, *Dvl*, *Vangl*, and *Celsr*) lead to NTDs (*17*, *74*, *86*). In mice, most manipulations of PCP function result in craniorachischisis, and studies in frogs revealed that craniorachischisis was in fact a secondary phenotype stemming from an essential role for PCP genes in neural convergent extension (*61*, *87*). In the absence of PCP function, cells fail to interdigitate mediolaterally, and the resulting overly wide neural tissue cannot fuse (*61*, *87*). Subsequent studies demonstrated the same mechanism in mice with

craniorachischisis (*88*, *89*). Importantly, human fetuses with craniorachischisis frequently display shortened and widened axes, consistent with a failure of convergent extension (*90*, *91*). Taking cues from these animal studies, concerted efforts have now identified mutations in several PCP genes in patients with NTDs (*17*, *86*).

Although the association of PCP gene variants with human NTDs is exciting, these data also highlight the difficulty in predicting genotype-phenotype relationships in human or in animal models: The NTDs in patients with PCP gene mutations range from the expected craniorachischisis to open spina bifida and even to closed NTDs (*17*). This outcome likely reflects the hypomorphic nature of the particular PCP gene mutations in these patients, because strong homozygous mutation of PCP genes in mice generally leads to craniorachischisis, whereas trans-heterozygosity for many of these mouse mutations results in other types of NTDs (*17*).

The growing abundance of genotype-phenotype data for genes with a shared biological function demonstrates the importance of cilia-related and PCP genes in determining susceptibility to NTDs, and studies are now needed to determine the proportion of human NTDs accounted for by variation in these genetic modules and how these variants place embryos at risk of failed NTC. As the first of what is likely to be many such genetic modules to be discerned, these data provide a conceptual framework for future NTD gene identification.

Future Directions

Identification of NTD-associated mutations in humans. A major advance in the past 10 years has been the discovery in animal models of over 200 genes whose function is required for NTC, and many new genes continue to be discovered [e.g., (*58*)]. These insights should now be translated into medical sequencing efforts in human NTD patients and the existing large cohorts of NTD patient DNA. Going forward, it will be important to move beyond the analysis of single candidate genes to genome-wide sequencing efforts of large sets of patient samples in order to have the power to reveal significant associations and to begin to understand the multifactorial nature of NTDs in humans. Polymorphisms and possible genetic interactions identified in human NTD cases can then be validated in animal models, where both specific mutations as well as multiple genetic changes can be tested.

Epigenomic studies. Epigenetic mechanisms can underlie human disease, as is becoming evident for neurological diseases and cancer (*92*–*94*). On the basis of findings that epigenetic regulators play key roles in mouse NTC and that such factors may be affected by FA (*31*–*33*), it is likely that genome-wide analyses will reveal epigenomic changes associated with NTDs. In animal models, advanced technologies can evaluate the transcriptional profile of specific cell types and correlate this with changes in DNA methylation, histone marks, and higher-order chromatin states. These technologies can also be used in human tissues, with the caveat that transcriptional and epigenetic signatures may be vastly different between the available tissue (generally collected at the time of delivery or in infancy) and the early embryonic tissues that mediate NTC. Nonetheless, substantial efforts have begun to define the DNA methylation and histone states in numerous control and diseased tissues from human patients, including some preliminary studies of DNA methylation from human NTD tissue (*95*). Future studies incorporating information on FA status should help to define potential FA-mediated changes, with a particular focus on the genes known to be necessary for NTC.

Developing new therapies for FA-resistant NTD based on knowledge of molecular pathways. Not all NTDs are preventable with FA treatment in human or in animal models (*49*, *50*, *58*), underscoring the need to consider alternative therapies. Mouse NTD models provide a substantial resource to develop alternative strategies based on biochemical and mechanistic information. For example, there is evidence for some preventive effect of inositol in *curly tail* (*Grhl3*) mutants; inositol deficiency increases NTD incidence in mice and rats, and mutations in genes associated with inositol metabolism and use can lead to NTDs in mice (*6*, *96*). This evidence has led to a small PONTI Study (Prevention of NTDs by Inositol, www.ucl.ac.uk/ich/research-ich/neural-development/ponti_study), in conjunction with FA for women with a previous NTD-affected pregnancy. Expansion of studies to additional micronutrients—for instance, vitamin B12 or zinc, low levels of which appear to be associated with NTD risk in humans (*97*, *98*)—carry the possibility of defining which genetic risk factors may be best targeted by therapies beyond FA.

Stem cell alternatives to animal models. Although animal models clearly serve as important tools, work with induced pluripotent stem cells now allows direct study of basic biological processes in cells derived from human patients. With the recent demonstration that complex three-dimensional morphogenetic events can be recapitulated in vitro using stem cells (*99*, *100*), it is now conceivable that NTC could also be modeled in this way. The promise of such an approach would be threefold. First, it would allow for direct, dynamic studies of neural morphogenesis in human cells. Second, by using cells derived from human NTD patients, this approach would allow direct comparison of normal and affected tissues as they engage in NTC; this should increase the efficacy of transcriptomic and epigenomic studies proposed above. Lastly, a stem cell approach could provide more abundant material and a relatively fast time frame to analysis; such increased efficiency could provide a tractable platform with which to screen small molecules for therapeutic or preventive potential.

In conclusion, although the risk of NTD remains high and the occurrence of NTDs translates to a great cost in terms of physical, emotional, and financial burden on the affected child and family, much remains undefined for NTD etiology. New approaches should overcome the technical barriers to ascertaining the causes, whether genetic or environmental. Important and rich human data sources have now become available such as the National Birth Defects Prevention Study (*101*), which has collected environmental and lifestyle data and DNA samples on thousands of women and their infants. Such data will be excellent resources to which new technologies for risk factor discovery can be applied. Furthermore, the rapid advances being made in animal models are contributing substantially to understanding the genetic basis of human NTD and the intersection with environmental factors. Increased attention to this problem is essential for the development of alternative therapies to help to prevent NTDs.

Movie 1. Live imaging of NTC in a frog (*Xenopus*) embryo. These large embryos have large cells, and by labeling cell membranes with a fluorescent marker every cell in the early CNS can be monitored during NTC. A few still frames in this movie were published previously (*102*). [Credit: C. Lee]

Movie 2. Live imaging of NTC in the hindbrain and midbrain of a mouse embryo visualized by mosaic fluorescent labeling (membrane tomato in red, membrane GFP in green). Zipping proceeds rostrally from closure point I, and caudally from closure point II/III, to continue to close the cranial neural tube. [Credit: C. Fees and L.A.N.]

References and Notes

1. H. J. Blom, G. M. Shaw, M. den Heijer, R. H. Finnell, Neural tube defects and folate: Case far from closed. *Nat. Rev. Neurosci.* **7**, 724 (2006). doi: 10.1038/nrn1986; pmid: 16924261
2. N. S. Adzick *et al.*, A randomized trial of prenatal versus postnatal repair of myelomeningocele. *N. Engl. J. Med.* **364**, 993 (2011). doi: 10.1056/NEJMoa1014379; pmid: 21306277
3. Z. Li *et al.*, Extremely high prevalence of neural tube defects in a 4-county area in Shanxi Province, China. *Birth Defects Res. A Clin. Mol. Teratol.* **76**, 237 (2006). doi: 10.1002/bdra.20248; pmid: 16575897
4. E. E. Castilla, I. M. Orioli, J. S. Lopez-Camelo, M. G. Dutra, J. Nazer-Herrera, Preliminary data on changes in neural tube defect prevalence rates after folic acid fortification in South America. *Am. J. Med. Genet. A* **123A**, 123 (2003). doi: 10.1002/ajmg.a.20230; pmid: 14598335
5. Y. Yamaguchi, M. Miura, How to form and close the brain: Insight into the mechanism of cranial neural tube closure in mammals. *Cell. Mol. Life Sci.* (2012). doi: 10.1007/s00018-012-1227-7; pmid: 23242429
6. A. J. Copp, N. D. E. Greene, Genetics and development of neural tube defects. *J. Pathol.* **220**, 217 (2010). pmid: 19918803
7. J. B. Wallingford, Neural tube closure and neural tube defects: Studies in animal models reveal known knowns and known unknowns. *Am. J. Med. Genet.* **135C**, 59 (2005). doi: 10.1002/ajmg.c.30054; pmid: 15806594
8. J. F. Colas, G. C. Schoenwolf, Towards a cellular and molecular understanding of neurulation. *Dev. Dyn.* **221**, 117 (2001). doi: 10.1002/dvdy.1144; pmid: 11376482

9. Centers for Disease Control and Prevention (CDC), Neural tube defect surveillance and folic acid intervention—Texas-Mexico border, 1993-1998. *MMWR Morb. Mortal. Wkly. Rep.* **49**, 1 (2000). pmid: 10993565
10. K. L. Deak *et al.*, Further evidence for a maternal genetic effect and a sex-influenced effect contributing to risk for human neural tube defects. *Birth Defects Res. A Clin. Mol. Teratol.* **82**, 662 (2008). doi: 10.1002/bdra.20511; pmid: 18937341
11. J. L. Hall, M. J. Harris, D. M. Juriloff, Effect of multifactorial genetic liability to exencephaly on the teratogenic effect of valproic acid in mice. *Teratology* **55**, 306 (1997). doi: 10.1002/(SICI)1096-9926(199705)55:5<306::AID-TERA2>3.0.CO;2-X; pmid: 9261924
12. K. S. Au, A. Ashley-Koch, H. Northrup, Epidemiologic and genetic aspects of spina bifida and other neural tube defects. *Dev. Disabil. Res. Rev.* **16**, 6 (2010). doi: 10.1002/ddrr.93; pmid: 20419766
13. G. M. Shaw *et al.*, 118 SNPs of folate-related genes and risks of spina bifida and conotruncal heart defects. *BMC Med. Genet.* **10**, 49 (2009). doi: 10.1186/1471-2350-10-49; pmid: 19493349
14. A. M. Molloy, L. C. Brody, J. L. Mills, J. M. Scott, P. N. Kirke, The search for genetic polymorphisms in the homocysteine/folate pathway that contribute to the etiology of human neural tube defects. *Birth Defects Res. A Clin. Mol. Teratol.* **85**, 285 (2009). doi: 10.1002/bdra.20566; pmid: 19235830
15. A. F. Olshan, G. M. Shaw, R. C. Millikan, C. Laurent, R. H. Finnell, Polymorphisms in DNA repair genes as risk factors for spina bifida and orofacial clefts. *Am. J. Med. Genet. A* **135A**, 268 (2005). doi: 10.1002/ajmg.a.30713; pmid: 15887293
16. P. X. Tran *et al.*, Association of retinoic acid receptor genes with meningomyelocele. *Birth Defects Res. A Clin. Mol. Teratol.* **91**, 39 (2011). doi: 10.1002/bdra.20744; pmid: 21254357
17. D. M. Juriloff, M. J. Harris, A consideration of the evidence that genetic defects in planar cell polarity contribute to the etiology of human neural tube defects. *Birth Defects Res. A Clin. Mol. Teratol.* **94**, 824 (2012). doi: 10.1002/bdra.23079; pmid: 23024041
18. S. G. Obican, R. H. Finnell, J. L. Mills, G. M. Shaw, A. R. Scialli, Folic acid in early pregnancy: A public health success story. *FASEB J.* **24**, 4167 (2010). doi: 10.1096/fj.10-165084; pmid: 20631328
19. J. S. Sheffield, E. L. Butler-Koster, B. M. Casey, D. D. McIntire, K. J. Leveno, Maternal diabetes mellitus and infant malformations. *Obstet. Gynecol.* **100**, 925 (2002). doi: 10.1016/S0029-7844(02)02242-1; pmid: 12423854
20. G. M. Shaw, E. M. Velie, D. Schaffer, Risk of neural tube defect-affected pregnancies among obese women. *JAMA* **275**, 1093 (1996). doi: 10.1001/jama.1996.03530380035028; pmid: 8601928
21. E. J. Lammer, L. E. Sever, G. P. Oakley Jr., Valproic acid. *Teratology* **35**, 465 (1987). doi: 10.1002/tera.1420350319; pmid: 3114906
22. L. McLeod, J. G. Ray, Prevention and detection of diabetic embryopathy. *Community Genet.* **5**, 33 (2002). doi: 10.1159/000064629; pmid: 14960898
23. J. G. Ray, T. E. O'Brien, W. S. Chan, Preconception care and the risk of congenital anomalies in the offspring of women with diabetes mellitus: A meta-analysis. *QJM* **94**, 435 (2001). doi: 10.1093/qjmed/94.8.435; pmid: 11493721
24. G. M. Shaw *et al.*, Neural tube defects associated with maternal periconceptional dietary intake of simple sugars and glycemic index. *Am. J. Clin. Nutr.* **78**, 972 (2003). pmid: 14594784
25. D. K. Waller *et al.*, Are obese women at higher risk for producing malformed offspring? *Am. J. Obstet. Gynecol.* **170**, 541 (1994). pmid: 8116710
26. M. M. Werler, C. Louik, S. Shapiro, A. A. Mitchell, Prepregnant weight in relation to risk of neural tube defects. *JAMA* **275**, 1089 (1996). doi: 10.1001/jama.1996.03530380031027; pmid: 8601927
27. K. A. Hendricks, O. M. Nuno, L. Suarez, R. Larsen, Effects of hyperinsulinemia and obesity on risk of neural tube defects among Mexican Americans. *Epidemiology* **12**, 630 (2001). doi: 10.1097/00001648-200111000-00009; pmid: 11679789
28. K. J. Meador *et al.*, In utero antiepileptic drug exposure: Fetal death and malformations. *Neurology* **67**, 407 (2006). doi: 10.1212/01.wnl.0000227919.81208.b2; pmid: 16894099
29. H. Kanai, A. Sawa, R.-W. Chen, P. Leeds, D.-M. Chuang, Valproic acid inhibits histone deacetylase activity and suppresses excitotoxicity-induced GAPDH nuclear accumulation and apoptotic death in neurons. *Pharmacogenomics J.* **4**, 336 (2004). doi: 10.1038/sj.tpj.6500269; pmid: 15289798
30. E. Yildirim *et al.*, Valproate administration to mice increases histone acetylation and 5-lipoxygenase content in the hippocampus. *Neurosci. Lett.* **345**, 141 (2003). doi: 10.1016/S0304-3940(03)00490-7; pmid: 12821190
31. N. D. Greene, P. Stanier, G. E. Moore, The emerging role of epigenetic mechanisms in the etiology of neural tube defects. *Epigenetics* **6**, 875 (2011). doi: 10.4161/epi.6.7.16400; pmid: 21613818
32. W. Lin *et al.*, Proper expression of the Gcn5 histone acetyltransferase is required for neural tube closure in mouse embryos. *Dev. Dyn.* **237**, 928 (2008). doi: 10.1002/dvdy.21479; pmid: 18330926
33. J. P. M. Barbera *et al.*, Folic acid prevents exencephaly in Cited2 deficient mice. *Hum. Mol. Genet.* **11**, 283 (2002). doi: 10.1093/hmg/11.3.283; pmid: 11823447
34. C. J. Phiel *et al.*, Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. *J. Biol. Chem.* **276**, 36734 (2001). doi: 10.1074/jbc.M101287200; pmid: 11473107
35. A. Drugan, A. Weissman, M. I. Evans, Screening for neural tube defects. *Clin. Perinatol.* **28**, 279, vii (2001). doi: 10.1016/S0095-5108(05)70083-X; pmid: 11499052
36. R. M. Bowman, D. G. McLone, J. A. Grant, T. Tomita, J. A. Ito, Spina bifida outcome: A 25-year prospective. *Pediatr. Neurosurg.* **34**, 114 (2001). doi: 10.1159/000056005; pmid: 11359098
37. N. S. Adzick, Fetal surgery for myelomeningocele: trials and tribulations. *J. Pediatr. Surg.* **47**, 273 (2012). doi: 10.1016/j.jpedsurg.2011.11.021; pmid: 22325376
38. M. Dennis, M. A. Barnes, The cognitive phenotype of spina bifida meningomyelocele. *Dev Disabil Res Rev* **16**, 31 (2010). doi: 10.1002/ddrr.89; pmid: 20419769
39. MRC Vitamin Study Research Group, Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study. *Lancet* **338**, 131 (1991). doi: 10.1016/0140-6736(91)90133-A; pmid: 1677062
40. R. J. Berry *et al.*, Prevention of neural-tube defects with folic acid in China. China-U.S. *N. Engl. J. Med.* **341**, 1485 (1999). doi: 10.1056/NEJM199911113412001; pmid: 10559448
41. M. A. Canfield *et al.*, Changes in the birth prevalence of selected birth defects after grain fortification with folic acid in the United States: Findings from a multi-state population-based study. *Birth Defects Res. A Clin. Mol. Teratol.* **73**, 679 (2005). doi: 10.1002/bdra.20210; pmid: 16240378
42. M. A. Honein, L. J. Paulozzi, T. J. Mathews, J. D. Erickson, L. Y. Wong, Impact of folic acid fortification of the US food supply on the occurrence of neural tube defects. *JAMA* **285**, 2981 (2001). doi: 10.1001/jama.285.23.2981; pmid: 11410096
43. L. J. Williams *et al.*, Prevalence of spina bifida and anencephaly during the transition to mandatory folic acid fortification in the United States. *Teratology* **66**, 33 (2002). doi: 10.1002/tera.10060; pmid: 12115778
44. P. De Wals *et al.*, Reduction in neural-tube defects after folic acid fortification in Canada. *N. Engl. J. Med.* **357**, 135 (2007). doi: 10.1056/NEJMoa067103; pmid: 17625125
45. J. S. López-Camelo *et al.*, Reduction of birth prevalence rates of neural tube defects after folic acid fortification in Chile. *Am. J. Med. Genet. A* **135A**, 120 (2005). doi: 10.1002/ajmg.a.30651; pmid: 15846825
46. J. A. Piedrahita *et al.*, Mice lacking the folic acid-binding protein Folbp1 are defective in early embryonic development. *Nat. Genet.* **23**, 228 (1999). doi: 10.1038/13861; pmid: 10508523
47. J. Gelineau-van Waes *et al.*, Embryonic development in the reduced folate carrier knockout mouse is modulated by maternal folate supplementation. *Birth Defects Res. A Clin. Mol. Teratol.* **82**, 494 (2008). doi: 10.1002/bdra.20453; pmid: 18383508
48. J. Momb *et al.*, Deletion of Mthfd1l causes embryonic lethality and neural tube and craniofacial defects in mice. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 549 (2013). doi: 10.1073/pnas.1211199110; pmid: 23267094
49. M. J. Harris, Insights into prevention of human neural tube defects by folic acid arising from consideration of mouse mutants. *Birth Defects Res. A Clin. Mol. Teratol.* **85**, 331 (2009). doi: 10.1002/bdra.20552; pmid: 19117321
50. B. S. Mosley *et al.*, Neural tube defects and maternal folate intake among pregnancies conceived after folic acid fortification in the United States. *Am. J. Epidemiol.* **169**, 9 (2009). doi: 10.1093/aje/kwn331; pmid: 18953063
51. J. D. Gray *et al.*, Functional interactions between the LRP6 WNT co-receptor and folate supplementation. *Hum. Mol. Genet.* **19**, 4560 (2010). doi: 10.1093/hmg/ddq384; pmid: 20843827
52. A. Marean, A. Graf, Y. Zhang, L. Niswander, Folic acid supplementation can adversely affect murine neural tube closure and embryonic survival. *Hum. Mol. Genet.* **20**, 3678 (2011). doi: 10.1093/hmg/ddr289; pmid: 21693562
53. E. B. Hook, A. E. Czeizel, Can terathanasia explain the protective effect of folic-acid supplementation on birth defects? *Lancet* **350**, 513 (1997). doi: 10.1016/S0140-6736(97)01342-1; pmid: 9274597
54. C. C. Y. Li *et al.*, A sustained dietary change increases epigenetic variation in isogenic mice. *PLoS Genet.* **7**, e1001380 (2011). doi: 10.1371/journal.pgen.1001380; pmid: 21541011
55. J. W. Hollingsworth *et al.*, In utero supplementation with methyl donors enhances allergic airway disease in mice. *J. Clin. Invest.* **118**, 3462 (2008). pmid: 18802477
56. M. J. Whitrow, V. M. Moore, A. R. Rumbold, M. J. Davies, Effect of supplemental folic acid in pregnancy on childhood asthma: A prospective birth cohort study. *Am. J. Epidemiol.* **170**, 1486 (2009). doi: 10.1093/aje/kwp315; pmid: 19880541
57. F. J. H. Magdelijns, M. Mommers, J. Penders, L. Smits, C. Thijs, Folic acid use in pregnancy and the development of atopy, asthma, and lung function in childhood. *Pediatrics* **128**, e135 (2011). doi: 10.1542/peds.2010-1690; pmid: 21690114
58. M. J. Harris, D. M. Juriloff, An update to the list of mouse mutants with neural tube closure defects and advances toward a complete genetic perspective of neural tube closure. *Birth Defects Res. A Clin. Mol. Teratol.* **88**, 653 (2010). doi: 10.1002/bdra.20676; pmid: 20740593
59. D. M. Juriloff, M. J. Harris, Hypothesis: The female excess in cranial neural tube defects reflects an epigenetic drag of the inactivating X chromosome on the molecular mechanisms of neural fold elevation. *Birth Defects Res. A Clin. Mol. Teratol.* **94**, 849 (2012). doi: 10.1002/bdra.23036; pmid: 22753363
60. C. Pyrgaki, P. Trainor, A.-K. Hadjantonakis, L. Niswander, Dynamic imaging of mammalian neural tube closure. *Dev. Biol.* **344**, 941 (2010). doi: 10.1016/j.ydbio.2010.06.010; pmid: 20558153
61. J. B. Wallingford, R. M. Harland, Neural tube closure requires Dishevelled-dependent convergent extension of the midline. *Development* **129**, 5815 (2002). doi: 10.1242/dev.00123; pmid: 12421719
62. H. Morita *et al.*, Cell movements of the deep layer of non-neural ectoderm underlie complete neural tube closure in *Xenopus*. *Development* **139**, 1417 (2012). doi: 10.1242/dev.073239; pmid: 22378637
63. H. W. Van Straaten, H. C. Janssen, M. C. Peeters, A. J. Copp, J. W. Hekking, Neural tube closure in the chick embryo is multiphasic. *Dev. Dyn.* **207**, 309 (1996). doi: 10.1002/(SICI)1097-0177(199611)207:3<309::AID-AJA8>3.0.CO;2-L; pmid: 8922530
64. H. W. Van Straaten, I. Sieben, J. W. Hekking, Multistep role for actin in initial closure of the mesencephalic neural groove in the chick embryo. *Dev. Dyn.* **224**, 103 (2002). doi: 10.1002/dvdy.10078; pmid: 11984878
65. E. A. Jones *et al.*, Dynamic in vivo imaging of postimplantation mammalian embryos using whole

embryo culture. *Genesis* **34**, 228 (2002). doi: 10.1002/gene.10162; pmid: 12434332
66. R. Massarwa, L. Niswander, In toto live imaging of mouse morphogenesis and new insights into neural tube closure. *Development* **140**, 226 (2013). doi: 10.1242/dev.085001; pmid: 23175632
67. M. J. Harris, D. M. Juriloff, Mouse mutants with neural tube closure defects and their role in understanding human neural tube defects. *Birth Defects Res. A Clin. Mol. Teratol.* **79**, 187 (2007). doi: 10.1002/bdra.20333; pmid: 17177317
68. J. D. Hildebrand, P. Soriano, Shroom, a PDZ domain-containing actin-binding protein, is required for neural tube morphogenesis in mice. *Cell* **99**, 485 (1999). doi: 10.1016/S0092-8674(00)81537-8; pmid: 10589677
69. L. M. Lanier *et al*., Mena is required for neurulation and commissure formation. *Neuron* **22**, 313 (1999). doi: 10.1016/S0896-6273(00)81092-2; pmid: 10069337
70. A. J. Koleske *et al*., Essential roles for the Abl and Arg tyrosine kinases in neurulation. *Neuron* **21**, 1259 (1998). doi: 10.1016/S0896-6273(00)80646-7; pmid: 9883720
71. S. L. Haigo, J. D. Hildebrand, R. M. Harland, J. B. Wallingford, Shroom induces apical constriction and is required for hingepoint formation during neural tube closure. *Curr. Biol.* **13**, 2125 (2003). doi: 10.1016/j.cub.2003.11.054; pmid: 14680628
72. J. Roffers-Agarwal, J. B. Xanthos, K. A. Kragtorp, J. R. Miller, Enabled (Xena) regulates neural plate morphogenesis, apical constriction, and cellular adhesion required for neural tube closure in *Xenopus*. *Dev. Biol.* **314**, 393 (2008). doi: 10.1016/j.ydbio.2007.12.010; pmid: 18201691
73. D. T. Fox, M. Peifer, Abelson kinase (Abl) and RhoGEF2 regulate actin organization during cell constriction in *Drosophila*. *Development* **134**, 567 (2007). doi: 10.1242/dev.02748; pmid: 17202187
74. J. B. Wallingford, Planar cell polarity and the developmental control of cell behavior in vertebrate embryos. *Annu. Rev. Cell Dev. Biol.* **28**, 627 (2012). doi: 10.1146/annurev-cellbio-092910-154208; pmid: 22905955
75. J. N. Murdoch, A. J. Copp, The relationship between sonic Hedgehog signaling, cilia, and neural tube defects. *Birth Defects Res. A Clin. Mol. Teratol.* **88**, 633 (2010). doi: 10.1002/bdra.20686; pmid: 20544799
76. S. C. Goetz, K. V. Anderson, The primary cilium: A signalling centre during vertebrate development. *Nat. Rev. Genet.* **11**, 331 (2010). doi: 10.1038/nrg2774; pmid: 20395968
77. D. Huangfu *et al*., Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature* **426**, 83 (2003). doi: 10.1038/nature02061; pmid: 14603322
78. I. E. Zohn, K. V. Anderson, L. Niswander, Using genomewide mutagenesis screens to identify the genes required for neural tube closure in the mouse. *Birth Defects Res. A Clin. Mol. Teratol.* **73**, 583 (2005). doi: 10.1002/bdra.20164; pmid: 15971254
79. P. Ybot-Gonzalez, P. Cogram, D. Gerrelli, A. J. Copp, Sonic hedgehog and the molecular regulation of mouse neural tube closure. *Development* **129**, 2507 (2002). pmid: 11973281
80. E. C. Oh, N. Katsanis, Cilia in vertebrate development and disease. *Development* **139**, 443 (2012). doi: 10.1242/dev.050054; pmid: 22223675
81. C. V. Logan, Z. Abdel-Hamed, C. A. Johnson, Molecular genetics and pathogenic mechanisms for the severe ciliopathies: Insights into neurodevelopment and pathogenesis of neural tube defects. *Mol. Neurobiol.* **43**, 12 (2011). doi: 10.1007/s12035-010-8154-0; pmid: 21110233
82. T. W. Vogel, C. S. Carter, K. Abode-Iyamah, Q. Zhang, S. Robinson, The role of primary cilia in the pathophysiology of neural tube defects. *Neurosurg. Focus* **33**, E2 (2012). doi: 10.3171/2012.6.FOCUS12222; pmid: 23025443
83. T. J. Park, S. L. Haigo, J. B. Wallingford, Ciliogenesis defects in embryos lacking inturned or fuzzy function are associated with failure of planar cell polarity and Hedgehog signaling. *Nat. Genet.* **38**, 303 (2006). doi: 10.1038/ng1753; pmid: 16493421
84. R. S. Gray *et al*., The planar cell polarity effector Fuz is essential for targeted membrane trafficking, ciliogenesis and mouse embryonic development. *Nat. Cell Biol.* **11**, 1225 (2009). doi: 10.1038/ncb1966; pmid: 19767740
85. J. H. Seo *et al*., Mutations in the planar cell polarity gene, Fuzzy, are associated with neural tube defects in humans. *Hum. Mol. Genet.* **20**, 4324 (2011). doi: 10.1093/hmg/ddr359; pmid: 21840926
86. Z. Kibar *et al*., Mutations in VANGL1 associated with neural-tube defects. *N. Engl. J. Med.* **356**, 1432 (2007). doi: 10.1056/NEJMoa060651; pmid: 17409324
87. J. B. Wallingford, R. M. Harland, *Xenopus* Dishevelled signaling regulates both neural and mesodermal convergent extension: Parallel forces elongating the body axis. *Development* **128**, 2581 (2001). pmid: 11493574
88. J. Wang *et al*., Dishevelled genes mediate a conserved mammalian PCP pathway to regulate convergent extension during neurulation. *Development* **133**, 1767 (2006). doi: 10.1242/dev.02347; pmid: 16571627
89. P. Ybot-Gonzalez *et al*., Convergent extension, planar-cell-polarity signalling and initiation of mouse neural tube closure. *Development* **134**, 789 (2007). doi: 10.1242/dev.000380; pmid: 17229766
90. M. Marin-Padilla, Study of the vertebral column in human craniorhachischisis. The significance of the notochordal alterations. *Acta Anat. (Basel)* **63**, 32 (1966). doi: 10.1159/000142778; pmid: 5911674
91. I. Kirillova *et al*., Expression of the sonic hedgehog gene in human embryos with neural tube defects. *Teratology* **61**, 347 (2000). doi: 10.1002/(SICI)1096-9926(200005)61:5<347::AID-TERA6>3.0.CO;2-#;pmid: 10777830
92. G. W. E. Santen *et al*., Mutations in SWI/SNF chromatin remodeling complex gene ARID1B cause Coffin-Siris syndrome. *Nat. Genet.* **44**, 379 (2012). doi: 10.1038/ng.2217; pmid: 22426309
93. Y. Tsurusaki *et al*., Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome. *Nat. Genet.* **44**, 376 (2012). doi: 10.1038/ng.2219; pmid: 22426308
94. D. C. Hargreaves, G. R. Crabtree, ATP-dependent chromatin remodeling: Genetics, genomics and mechanisms. *Cell Res.* **21**, 396 (2011). doi: 10.1038/cr.2011.32; pmid: 21358755
95. L. Wang *et al*., Relation between hypomethylation of long interspersed nucleotide elements and risk of neural tube defects. *Am. J. Clin. Nutr.* **91**, 1359 (2010). doi: 10.3945/ajcn.2009.28858; pmid: 20164316
96. N. D. Greene, A. J. Copp, Inositol prevents folate-resistant neural tube defects in the mouse. *Nat. Med.* **3**, 60 (1997). doi: 10.1038/nm0197-60; pmid: 8986742
97. J. G. Ray *et al*., Vitamin B12 and the risk of neural tube defects in a folic-acid-fortified population. *Epidemiology* **18**, 362 (2007). doi: 10.1097/01.ede.0000257063.77411.e9; pmid: 17474166
98. A. C. Dey *et al*., Maternal and neonatal serum zinc level and its relationship with neural tube defects. *J. Health Popul. Nutr.* **28**, 343 (2010). doi: 10.3329/jhpn.v28i4.6040; pmid: 20824977
99. M. Eiraku *et al*., Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature* **472**, 51 (2011). doi: 10.1038/nature09941; pmid: 21475194
100. H. Suga *et al*., Self-formation of functional adenohypophysis in three-dimensional culture. *Nature* **480**, 57 (2011). doi: 10.1038/nature10637; pmid: 22080957
101. P. W. Yoon *et al*., The National Birth Defects Prevention Study. *Public Health Rep.* **116** (suppl. 1), 32 (2001). doi: 10.1093/phr/116.S1.32; pmid: 11889273
102. C. Lee, H. M. Scherr, J. B. Wallingford, Shroom family proteins regulate gamma-tubulin distribution and microtubule architecture during epithelial cell shape change. *Development* **134**, 1431 (2007). doi: 10.1242/dev.02828; pmid: 17329357

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