Epigenetics 6:7, 875-883; July 2011; © 2011 Landes Bioscience

The emerging role of epigenetic mechanisms in the etiology of neural tube defects

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The molecular requirements for neural tube closure are complex. This is illustrated by the occurrence of neural tube defects (NTDs) in many genetic mouse mutants, which implicate a variety of genes, pathways and cellular functions. NTDs are also prevalent birth defects in humans, affecting around 1 per 1,000 pregnancies worldwide. In humans the causation is thought to involve the interplay of fetal genes and the effect of environmental factors. Recent studies on the etiology of human NTDs, as well as analysis of mouse models, have raised the question of the possible involvement of epigenetic factors in determining susceptibility. A consideration of potential causative factors in human NTDs must now include both alterations in the regulation of gene expression, through mutation of promoter or regulatory elements and the additional analysis of epigenetic regulation. Alterations in the epigenetic status can be directly modified by various environmental insults or maternal dietary factors.

Key words: neural tube defects, diet, folic acid, epigenome, epigenetic regulation, methylation, chromatin, histones, acetylation

Submitted: 05/09/11

Accepted: 05/10/11

DOI: 10.4161/epi.6.7.16400

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Neural Tube Development and Disease

The neural tube is the embryonic precursor of the spinal cord and brain. It develops from a thickened region of the dorsal surface ectoderm, the neural plate, which undergoes a series of shaping and folding events. Briefly, the lateral edges of the neural plate elevate to form paired parallel folds running in an anterior to posterior orientation. Aided by bending along the body axis, these folds then converge and fuse along the dorsal midline to ultimately form the closed neural tube.[1,2] Mammalian neural tube closure is a discontinuous process along the body axis, being initiated at discrete sites and progressing in a "zipper-like" fashion to close the open regions of neural folds, termed neuropores. The initial fusion site occurs at the hindbrain/cervical boundary, at around day 21 of human gestation (day 8 in mice), with closure spreading in both anterior and posterior directions. Another site of initiation occurs at the rostral limit of the neural plate, with progression in a caudal direction to meet the wave of closure from the hindbrain and seal the rostral neuropore, thereby completing closure in the brain.[3,4] In mice, in which the closure process has been most extensively studied, there is a further initiation site at the midbrain-forebrain boundary such that two neuropores (anterior and hindbrain) are observed prior to final closure.[1,5] Closure in the future spinal region proceeds caudally in a unidirectional manner, with final closure of the posterior neuropore at the mid-sacral level at day 26–28. At more caudal levels the neural tube is formed by a process known as secondary neurulation, where canalization of the tail bud region forms the sacro-caudal neural tube which becomes continuous with the primary neural tube.[2] The complete neural tube becomes populated by neurons and surrounded by the protective meninges and surrounding bone of the vertebrae.

Failure in initial fusion or progression of closure results in neural tube defects (NTDs), in which the neural ectoderm remains exposed and subject to degeneration. NTDs are among the most common of human birth defects, with an overall

prevalence of around 0.5–2/1,000 pregnancies and frequently result in infant mortality or major health problems in surviving children.[6] The nature and severity of NTDs is determined by the stage and axial level at which closure fails.[2] Failure to initiate closure at the hindbrain/cervical boundary will result in the most severe defect, known as craniorachischisis, in which the neural folds remain splayed open throughout the midbrain, hindbrain and entire spinal region. Failure to complete closure of the rostral neuropore results in anencephaly, which may involve the midbrain alone, or also involve the hindbrain or more rarely the forebrain. Neither anencephaly nor craniorachischisis are compatible with life after birth, owing to in utero neurodegeneration. Failure of closure of the posterior neuropore leads to open spina bifida (myelomeningocele), a term which refers to the defects in the vertebral arches that obligatorily accompany the presence of the open neural folds. The clinical severity of spina bifida is affected by the axial level at which closure fails but patients frequently suffer paralysis of the legs, as well as bowel and bladder dysfunction.

Despite the high prevalence and traumatic consequences for affected individuals and their families, the underlying causes of NTD remain poorly understood in most individuals. In humans, identification of the precise biochemical and cellular factors involved has proved elusive, partly due to the lack of large families with Mendelian inheritance and also because of the high degree of heterogeneity between unrelated sporadic cases. It seems highly likely that the majority of NTD cases result from interaction between both genetic and environmental factors.[7-9] Evidence for a genetic contribution is indicated by the high recurrence risk for siblings of affected individuals and association with specific chromosomal anomalies.[10-12] The complexity of the molecular requirements for neural tube closure is well illustrated by the occurrence of NTDs in more than 200 different mouse genetic models.[13,14] Moreover, the high penetrance observed on the original genetic background, is often seen to diminish if the mice are outcrossed indicating the contribution of modifier loci.[13] Only very few of the NTD-associated genes identified in mice have been confirmed to be involved in humans.[9] The majority of human NTD occur sporadically, with recurrences tending to fit a multifactorial polygenic or oligogenic pattern, rather than either dominant or recessive single gene inheritance with reduced penetrance.[13,14] In addition to mutation of the coding sequence, altered transcriptional regulation of these genes has potential to cause NTDs. Indeed, in some cases, such as *Grhl2*, closure can be prevented by either loss of function or overexpression of a single gene.[15] A consideration of potential causative factors in human NTDs should therefore account for the possibility of deregulation of gene expression through epigenetic mechanisms.

A number of factors have been described to elevate the risk for NTDs. These include medical conditions such as maternal diabetes[16] or maternal obesity,[17] while environmental exposures such as cigarette smoke, mycotoxins or use of anti-epileptic drugs may have teratogenic effects.[18-20] Maternal dietary factors leading to a high dietary glycemic index or a high glycemic load are associated with increased risk of an NTD affected pregnancy,[21] as are sub-optimal levels of folate and vitamin B_{12}.[22,23] Particular attention has been paid to the role of folate one-carbon metabolism, especially owing to the finding that maternal supplementation with folic acid reduces the risk of NTDs.[24-26] Environmental factors may influence neural tube closure through a direct effect on embryonic metabolism/cell biology. Alternatively, some of the established risk factors may act mechanistically via an effect on epigenetic regulation thereby influencing susceptibility through altered gene expression. Evidence for a potential role for epigenetic effects on gene regulation is emerging both from the study of human NTDs and from analysis of mouse models.

Mechanisms of Epigenetic Regulation

The basis of epigenetic regulation of gene expression is complex, involving several interconnecting molecular layers referred to as the "epigenome." These layers include DNA methylation, histone modifications involving post-translational covalent modifications such as acetylation, antisense RNAs, small interfering RNAs and also non-histone proteins that influence chromatin folding. All these processes regulate DNA transcription of specific genes but do not alter the primary sequence. The different mechanisms can act locally or with genome-wide effect, creating an epigenetic landscape reinforcing a transcriptionally favorable or unfavorable chromatin conformation.[27,28] Some specific histone modifications can promote DNA methylation and vice versa.[29,30] For example, methyl-CpG-binding protein MeCP2 attaches to methylated cytosines and attracts other proteins with enzymatic activity that promote nearby histone deacetylation.[31] The establishment and maintenance of epigenetic marks are not fully understood, but it is known that genetic variation in the DNA sequence itself, as well as the sequence of unlinked modifier loci, play additional roles.[32]

DNA methylation in mammals occurs at C5 of the cytosine pyrimidine ring, within CpG dinucleotides, converting them to 5-methylcytosine. This covalent modification inhibits the affinity of methylation-sensitive DNA binding proteins, affects chromatin structure and usually correlates with transcriptional silencing.[33] In normal mammalian cells most of the genomic CpG sites (90–98%) are methylated, including exons, intergenic DNA and the mobile transposable elements called transposons.[34] Conversely, CpG-islands (CGIs), which are found in 50–60% of gene promoter regions, have a high density of CpG, are typically unmethylated and act as a regulation switch.[35] A gene can also be hypermethylated throughout but have a hypomethylated promoter region which controls its expression.[34]

Epigenetic regulation has been found to operate very actively in normal mammal cell functioning, from conception to aging and death.[36] Reprogramming of DNA methylation of the totipotent zygote takes place in germ cells and during pre-implantation[35] (**Fig. 1**). Mammal sperm and egg genomes are highly methylated when compared with somatic cells. However, a few hours after fertilization, rapid demethylation of the paternal

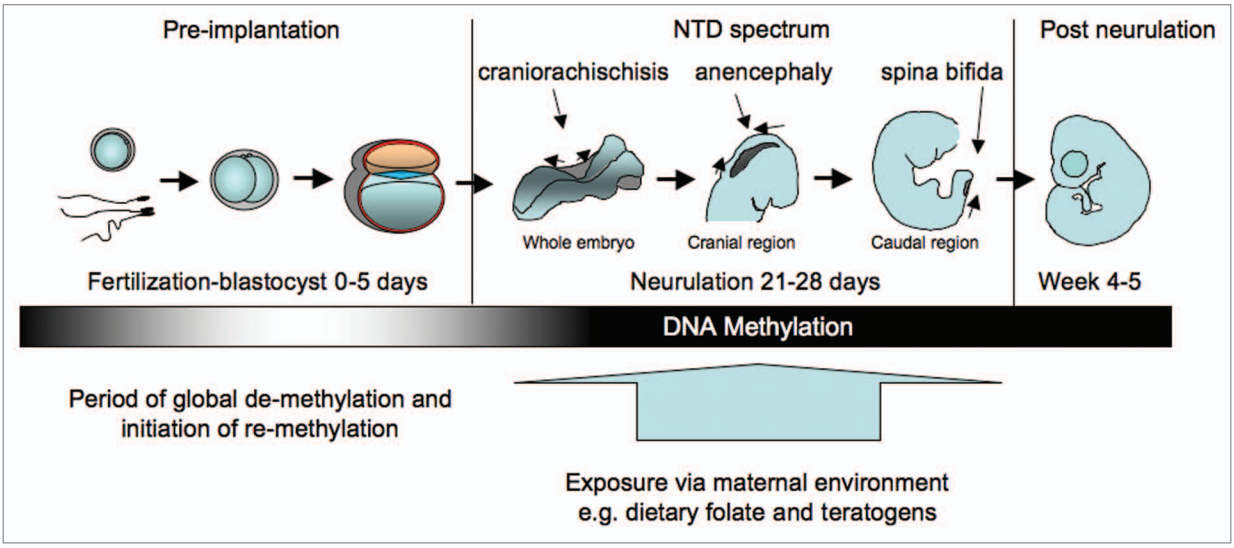

Figure 1. Methylation changes during early development. The epigenome is a dynamic process where rapid de-methylation occurs immediately post fertilization but is then followed by re-methylation in the blastocyst and early embryo. Developmental stages are shown diagrammatically. During neurulation stages, the direction of neural tube fusion is shown by arrows, resulting in closure of the rostral neuropore (dark shaded area) in the cranial region and the posterior neuropore in the caudal region. Craniorachischisis results from failure to initiate closure of the neural folds, anencephaly and spina bifida from incomplete closure in cranial and caudal regions respectively.

genome takes place by active but yet undefined mechanisms, in addition to histone modification acquisition.[37] The maternal genome follows a slower and more passive process, with demethylation by simple dilution of DNA methylation during replication, preventing DNMT activity at the replication fork.[38] Up to the morula stage, DNA methylation remains reduced and cells are pluripotent, with all genes potentially active. Simultaneously, primordial germ cells (PGCs) undergo changes in histones and reorganization of chromatin. After implantation, genome-wide resetting occurs for most of the genome in a lineage-specific manner and continues, to a lesser extent over the rest of the fetal development (**Fig. 1**). Re-methylation at this stage varies upon the part of the embryo concerned and, whereas ectoderm and mesoderm become hypermethylated, primary endoderm and trophoblast remain hypomethylated. There seems to be a sequence of re-methylation dictating the structure and function of each formatting somatic tissue.[39]

The methyl groups used for DNA methylation are supplied by S-adenosylmethionine (SAM), and are catalyzed by various types of DNA methyltransferases (DNMTs) (**Fig. 2**). De novo methylation patterns during gametogenesis, embryogenesis and tissue differentiation are established mainly by the enzymes DNMT3A and DNMT3B, whose activity is downregulated upon differentiation of embryonic stem cells and remain low during adult life. Maintenance of these patterns in somatic cells is dependent on different DNMT1 variants, which also have some de novo activity.[40] The accuracy of copying DNA methylation patterns at cell division is estimated at ~96%. DNMT enzymes are believed to have a dual role, both in methylation and demethylation.[41,42] Recent studies have also suggested that demethylation of DNA is indirect with modification of the methyl-cytosine by deamination or oxidation, sometimes referred to as 'hydroxymethlyation', followed by DNA repair.[43]

Several lines of evidence from epidemiologic studies are suggestive of a link between impaired methylation cycle and human NTDs. These include the association with elevated homocysteine and sub-optimal levels of folate and/or vitamin B_{12} in maternal blood.[23,44-46] Probably the most extensively studied genetic risk factor for NTDs is the 677C>T SNP in *5, 10-methylenetetrahydrofolate reductase* (*MTHFR*) (reviewed in ref. 47), which is associated with elevated homocysteine,[48] presumably due to diminished production of 5-methyl THF (see below). An alternative folate-independent mechanism for re-methylation of homocysteine involves transfer of a one-carbon unit from betaine, catalyzed by betaine homocysteinemethyltransferase (BHMT) (**Fig. 2**). It is intriguing that higher maternal dietary intake of betaine and choline, the precursor of betaine, shows a striking association with reduced risk of NTDs.[49] Furthermore, choline levels were found to be correlated with NTD risk in analysis of second trimester maternal serum, where low levels increases risk and high levels are protective.[50] Interestingly, the study population was North American and therefore the diet was folate-fortified, such that other one-carbon metabolites including homocysteine, methionine, folate, vitamin B_{12} and betaine were not found to be altered in NTD pregnancies. Inhibition of choline uptake or metabolism causes NTDs in cultured mouse embryos.[51] Whether these defects relate to a methylation deficit or lack of phosphatidylcholine synthesis is not entirely clear. The lack of association between betaine level and risk of human NTDs perhaps argues against a methylation-related mechanism, but further analysis of the relationship between maternal choline/betaine and embryonic levels would be merited. Later in development,

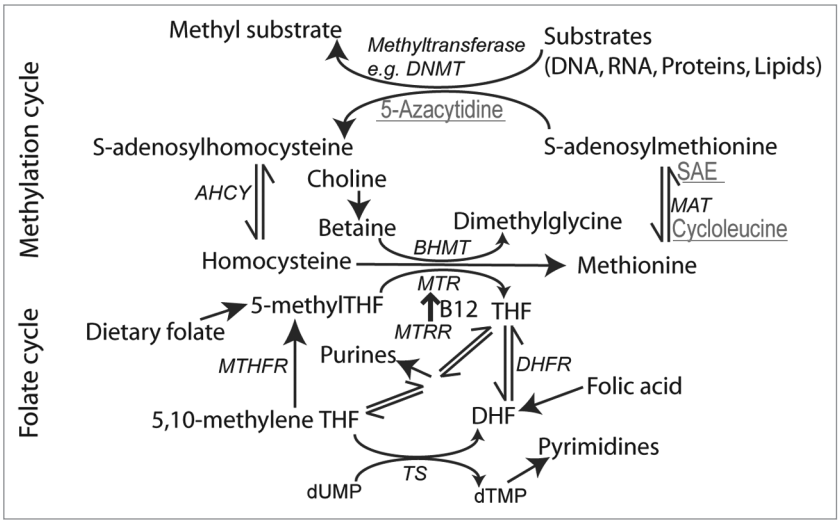

Figure 2. Summary of folate one-carbon metabolism. A simplified diagram showing the key functions of the folate cycle (lower half of diagram), involving transfer of 1C groups between folate molecules, required for pyrimidine and purine biosynthesis. The action of MTHFR (5,10-methylene tetrahydrofolate reductase) produces 5-methylTHF for re-methylation of homocysteine by MTR (methionine synthase). Alternatively, homocysteine is re-methylated by the action of BHMT (betaine-homocysteine methyltransferase). In the methylation cycle (upper half), S-adenosylmethionine (SAM) acts as the methyl group donor in a variety of methylation reactions. Reagents that inhibit steps of the methylation cycle and produce NTDs in cultured mouse embryos are shown in grey and underlined. S-adenosylethionine (SAE) is a non-metabolized analogue of SAM. Enzymes are shown in italics. AHCY, S-adenosylhomocysteine hydrolase; DHFR, dihydrofolate reductase; MAT, methionine adenosyltransferase; MTRR, methionine synthase reductase; TS, thymidylate synthase.

dietary imposition of choline deficiency has been found to alter DNA and histone methylation patterns in fetal mouse hippocampus.[52,53]

Evidence that Folate One-Carbon Metabolism Influences Risk of NTDs via its Role in Methylation?

Several lines of evidence suggest that folate one-carbon metabolism is a key determinant of NTD susceptibility (reviewed by ref. 47 and 54–56). However, NTDs do not appear to simply result from a maternal folate deficiency (which is corrected by folic acid supplementation), since maternal levels in most affected pregnancies fall within the "normal" range.[57] Moreover, in experimental models of folate deficiency, the developing mouse embryo appears relatively resistant to maternal folate depletion. Whereas profound dietary folate deficiency affects embryonic folate levels at neurulation stages, a moderate deficiency produces significant effects on maternal circulating folate and homocysteine but has no apparent effect on embryonic folate content.[58] Therefore, it appears likely that supplemental folic acid acts either to overcome a defect in embryonic folate one-carbon metabolism or to compensate for a predisposing factor that itself may be unrelated to folate status.

Circulating folate, principally in the form of 5-methyl tetrahydrofolate (5-MeTHF), is taken up into cells by folate receptors (FOLR1 and FOLR2) or by the reduced folate carrier (RFC1). Mice that are deficient for *Folr1* develop NTDs but can be rescued from early lethality by folic acid treatment, thereby demonstrating that folate uptake is essential for embryonic development.[59,60] Within the cell, folates act as cofactors in a network of reactions for the transfer of one-carbon units, termed "folate one-carbon metabolism," that is essential for the production of purines and pyrimidines (**Fig. 2**).[55] Defective thymidylate biosynthesis has been implicated in NTDs in humans and mouse models,[61-63] suggesting that adequate supply of nucleotides for cellular proliferation is essential for closure of the neural tube.

In addition to nucleotide biosynthesis, folate one-carbon metabolism also generates SAM, which as mentioned above is the methyl donor for methylation of DNA, proteins, RNA and lipids and hence a key requirement for epigenetic regulatory mechanisms. It is therefore not surprising that research on NTDs has also focused on this aspect as a key function of the folate cycle. It is thought that suboptimal folate one-carbon metabolism could be associated with an increased risk of NTDs, directly as a result of diminished or decreased essential methylation.[64,65] The folate and methylation cycles are interlinked by the transfer of a one-carbon unit from 5-methyl tetrahydrofolate to homocysteine catalyzed by methionine synthase, for which vitamin B_{12} (cobalamin) acts as cofactor. This reaction serves to generate methionine and tetrahydrofolate (THF) and is important for methylation but also for folate cycle flux, as the reaction that generates 5-methyl THF is essentially irreversible (**Fig. 2**). Inhibition of methionine synthase would result in accumulation of homocysteine, but also 5-methyl THF and therefore effective depletion of other folates, the so-called methyl trap.[47,55]

Are the Predicted Correlations Between Biomarkers of One-Carbon Metabolism and Methylation Born Out by Analysis of DNA in Humans?

Evidence for a potential link between one-carbon metabolites and the fetal epigenome has been suggested by small scale studies in which DNA methylation and one-carbon metabolites were assayed in cord blood, collected at term following normal pregnancy.[66,67] Methylation of long interspersed nucleotide element-1 (LINE-1) sequences, assayed as an indicator of genome wide DNA methylation, was not associated with either use of folic acid supplements or serum folate. However, there was a significant inverse correlation with fetal plasma homocysteine level and this correlation was association was also apparent in more detailed analysis of CpG methylation patterns.[66,67] While few neurulation-stage human embryos are available for study, initial

data on DNA methylation in NTDs is now emerging from analysis of fetal DNA of nervous tissue (or its remnants) following termination of pregnancy.[68] Methylation of genomic DNA (5-methyl cytosine content) and LINE-1 sequences was found to be lower among NTD cases involving the cranial region compared to controls, but not among spina bifida cases.[68] The reduction in methylation was associated with reduced maternal vitamin B_{12} level, but not folate status, although a subsequent study shows an inverse correlation between methylation of genomic DNA in abortus brain tissue and maternal serum folate.[69]

Does the Experimental Evidence from Model Systems Support the Hypothesis that Diminished Methylation Could Contribute to the Development of NTDs?

Cranial NTDs arise in homozygous null embryos for *Dnmt3b*[70] and in embryos cultured in the presence of 5-azacytidine,[71] suggesting that there is a requirement for DNA methylation in neural tube closure. Similarly, exposure to methylation cycle inhibitors or excess methionine causes cranial NTDs without other major defects, even in non-mutant strains.[72,73] Both these treatments result in a reduced ratio of S-adenosylmethionine (SAM) to S-adenosylhomocysteine (SAH), indicative of reduced methylation potential. A corresponding reduction in global DNA methylation was observed in embryos treated with the methylation cycle inhibitor, ethionine.[58] On the other hand, NTDs are not observed in null embryos for *Mthfr*, despite a significant reduction in SAM/SAH ratio and global DNA methylation.[74] Therefore, evidence that the apparent association of *MTHFR* polymorphism with human NTDs is mediated through an epigenetic mechanism is currently unsupported. Similarly, NTDs are not observed in mice carrying a hypomorphic allele of *Mtrr*, encoding methionine synthase reductase, which is required for methionine synthase activity,[75] although there is some evidence of an association with NTDs in humans.[9,76] These models emphasize the point that elevated homocysteine is likely to be a marker of impaired one carbon metabolism in NTDs rather than directly causative. This is further supported by the fact that homocysteine treatment of mouse embryos developing in culture or in vivo does not cause NTDs.[77,78]

Folic acid treatment can alter global DNA methylation in adult rats.[79] It is also apparent that genomic DNA methylation can be altered in the offspring of mice fed a methyl donor-rich diet, at least at transposable elements.[80] However, the converse situation of folate deficiency is less clear. NTDs do not arise in wild-type mouse embryos under conditions of profound maternal folate-deficiency, which is imposed by both folate-deficient diet and treatment with antibiotics (that removes gut flora that synthesize folates), despite a significant reduction in SAM/SAH ratio.[58,81] Folate-deficiency can, however, exacerbate susceptibility in embryos that carry a genetic predisposition to NTDs, such as *splotch (Pax3*) mutants. In this model there was no apparent effect of reduced SAM/SAH ratio on global DNA methylation, although an effect on specific loci cannot be ruled out.[58] Similarly, overall reduction in methylation does not increase the frequency of NTDs in splotch embryos made doubly mutant for *Mthfr* null alleles.[82] Maternal folate deficiency also induces cranial NTDs in mouse embryos with a curly tail genetic background.[83] Again, a mechanism in which overall impairment of DNA methylation causes NTDs appears unlikely, since breeding the *Mthfr* null allele into the curly tail strain does not increase the frequency of defects despite major reduction in SAM/SAH ratio.[84]

Histone Methylation and NTDs

To date few studies have addressed the possible role of altered histone methylation in development of NTDs. However, a recent study demonstrated increased methylation of lysine 27 of histone H3 (H3K27) in cultured neural crest cells and neural tube explants from *splotch* (*Pax3*) mutant embryos, which develop NTDs.[85] Interestingly, this abnormality was not observed following treatment with folic acid, that is known to rescue *splotch* NTDs,[62] raising the possibility that *splotch* NTDs could be associated with altered epigenetic regulation. The role of folic acid in this context appears complex since increased folic acid has previously been associated with elevated DNA methylation potential. The finding of diminished H3K27 methylation following folic acid treatment therefore appears likely to reflect prevention of the underlying defect rather than a direct effect via stimulation of methylation.

Histone Acetylation and NTDs

In addition to methylation, other histone modifications can also affect chromatin function and thereby contribute to modulation of gene expression.[86] In the context of neural tube closure, regulation of acetylation appears to play a critical role. In addition to N-terminal acetylation, which occurs cotranslationally on most proteins, some proteins are subject to post-translational acetylation of specific lysine residues. Acetylation neutralizes the positive charge on the lysine amino group and can therefore influence electrostatic properties and function of the protein. The acetylation status of specific proteins depends on an equilibrium between the activity of histone acetylases (HATs) and deacetylases (HDACs), which respectively add and remove acetyl groups.[86,87] Whereas HATs (such as p300) frequently act as transcriptional co-activators, HDAC activity results in chromatin compaction and transcriptional repression.[88]

The observation of cranial NTDs among knock-out embryos for p300 suggested that histone acetylase activity is essential for neural tube closure.[87] Further evidence of a requirement for acetylation in neural tube closure was provided by generation of a knock-in allele of the acetyltransferase encoding *Kat2a (Gcn5)* gene. The $Gcn5^{hat}$ allele carries point mutations in the catalytic domain that abolish HAT activity.[89] In contrast to embryos that are completely null for *Gcn5*, which die early in gestation, $Gcn5^{hat/hat}$ embryos survive beyond neurulation stages but exhibit cranial NTDs,[89] as do mice carrying a hypomorphic allele of *Gcn5* with reduced expression.[90] Cranial NTDs also occur in knockout embryos for *Cited2*, encoding a member of the CITED (CBP/p300 interacting transactivator with ED-rich

tail) protein family, which, as its name suggests, can bind to p300 and its paralogue CBP (cAMP-responsive element-binding protein).[91,92] These interactions may interfere with, and thereby modulate the acetylation of, p300 target proteins such as HIF-1α. Interestingly, HIF-1α-responsive genes are upregulated in *Cited2* null embryos.[92,93]

The cellular and developmental mechanisms underlying NTDs in acetylation-related mouse models are not completely clear. A proportion of *Gcn5*$^{hat/hat}$ embryos are growth retarded and exhibit increased rates of apoptosis. However, NTDs still arise even in those embryos in which overall growth, neuroepithelial proliferation and apoptosis are comparable to wild-types.[89] NTDs in *Cited2* mutants are associated with a dramatic increase in apoptosis in the neural folds prior to failure of closure and could therefore potentially play a causative role.[94] Nevertheless, prevention of NTDs by folic acid in *Cited2* mutants is not associated with an obvious reduction in apoptosis, suggesting either that apoptosis is not causative or that folic acid does not act by amelioration of the underlying defect.[94]

Changes in regulation of acetylation has also been implicated in failure of neural tube closure owing to the teratogenic effect of pharmacological inhibitors of HDACs, such as valproic acid (VPA) and trichostatin A, which cause NTDs as well as defects of the axial skeleton.[95,96] In accordance with a downstream effect mediated through HDAC inhibition, expression analyses of valproic acid-treated embryos showed misregulation of the HDAC ontology group and elevated acetylation of histone H4 in the neural tube.[96-98] Among VPA derivatives, HDAC activity correlates with teratogenic potential.[99] Knock-out of *Hdac1* results in early embryonic lethality with reduced proliferation, probably due to upregulation of *p21*.[100] Altered proliferation would have potential to have an impact on neural tube closure. However, while conditional mutants of both *Hdac1* and *Hdac2* have been generated and show a requirement in neuronal development,[101] we are not aware that experiments to selectively ablate their activity in the neural folds have yet been performed. However, a proportion of *Hdac4* mutants are reported to develop cranial NTDs,[102] as do some knock-out embryos for *Sirt1*, another histone deacetylase.[103]

Overall, it appears that both decreased acetylation (HAT mutants) and increased acetylation (HDAC mutant or inhibitor-treated) are associated with development of NTDs. When interpreting the mechanisms by which acetylation influences neural tube closure it should be considered that, while many of the functions of HATs are mediated through histones, it is also apparent that other proteins are also targets for acetylation/deacetylation.[104,105] In the context of neural tube closure, proteins such as p53 and Rb are of interest owing to their role in regulation of cell cycle progression and apoptosis.[103,104] Acetylation may also mediate interplay between different mechanisms of epigenetic regulation. For example, studies in yeast indicate that the SWI/SNF chromatin remodeling complex is regulated by acetylation of its constituent proteins in addition to histone acetylation.[106]

Chromatin Remodeling and NTDs

Although not directly participating in chromatin modifications, three types of proteins are critical for epigenetic regulation: chromatin remodeling complexes, effector binding-proteins and insulator proteins.[35] Specific CpG-binding proteins such as MeCP2 interpret DNA methylation by recruiting, transcribing or repressing complexes that decipher the histone/DNA methylation marks. Polycomb-group proteins can remodel chromatin and target human gene promoters, throughout development.[107] Other factors which are also involved in chromatin structure and gene regulation include nucleosome positioning, especially in the vicinity of the transcription start site results in inactivation or activation respectively, maybe due to affecting access for binding of transcription factors.[108]

The occurrence of NTDs in several mouse mutants for chromatin remodeling enzymes[13,14] further emphasizes the multiplicity of mechanisms by which transcriptional regulation may be altered with potential detrimental effects on closure. Chromatin binding/remodeling proteins associated with mouse NTDs include SMARCA4 (BRG1);[109] CERCR2;[110] SMARCC1 (SRG3).[111] Cranial NTDs also occur at high frequency, in association with profound growth retardation, in embryos lacking *BRD2* (bromodomain-containing protein 2),[112] which binds acetylated histones and chromatin remodeling proteins. Composition of the chromatin remodeling complex provides differential specificity of binding to target loci.[113,114] Interrogation of data-sets generated from recent genome-wide mapping screens for targets and interactors of chromatin remodeling complexes may therefore inform further analysis of the mechanism by which loss of function of some components (e.g., BRG1) results in NTDs.

Summary

Studies in model organisms have provided valuable insight into the developmental and cellular basis of neural tube closure. Similarities in the anatomy and pathogenesis of mouse and human NTDs imply that there is considerable overlap in the underlying causative mechanisms. However, despite NTDs being common birth defects in humans, in most cases the underlying molecular pathology continues to remain obscure. This is perhaps surprising given the large number of mouse single gene mutants that develop NTDs with high penetrance. The most likely explanation lies in the apparent multigenic inheritance of human NTDs, under the influence of modifier genes and environmental factors. In addition to the large number of potential candidate genes, further complexity comes from the fact that in addition to coding mutations there may be causative involvement of gene expression, perhaps resulting from regulatory sequence variants or altered epigenetic architecture. Among the many different epigenetic mechanisms known to be involved in early development, the best understood is currently that of DNA methylation. Animal studies suggest that impaired DNA methylation can interfere with normal neural tube closure and preliminary data indicate that it may potentially contribute to human NTDs. Other key mechanisms such as histone modifications and chromatin remodeling have

also been associated with NTDs in mice and, at least in the case of histone acetylation, in humans as well. It is well known that phenotypic expression of the developing embryo is strongly influenced by the maternal environment, especially diet. This raises the possibility that the relationship between folic acid supplementation and/or folate one-carbon metabolism with the risk of NTDs may be mediated in part through their effects on methylation. In this respect, there is certainly experimental evidence to show that DNA methylation can be increased following folic acid supplementation, but it is less clear whether sub-optimal folate status results in diminished methylation. The next challenge will be to determine whether maternal dietary factors that affect NTD risk do so by altering epigenetic regulation, and then to identify the key genes and pathways that are differentially regulated in this way.

Acknowledgments

The authors would like to thank the Wellcome Trust, Medical Research Council, SPARKS, WellBeing of Woman and the UCL Biomedical Research Centre for funding research in their laboratories. P.S. is supported by Great Ormond Street Hospital Children's Charity.

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