Early Nutrition, Epigenetic Changes at Transposons and Imprinted Genes, and Enhanced Susceptibility to Adult Chronic Diseases

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INTRODUCTION

Several recent reviews surveying the promising field of nutrigenomics[1,2] have not discussed the important role that epigenetic mechanisms play at the nexus between nutrition and the genome. This is a glaring omission. Certainly, "nutrient–gene interactions" in humans enable various nutrients to transiently influence the expression of specific subsets of genes. In addition to these phenomena, however, it is becoming increasingly evident that by interacting with epigenetic mechanisms, which regulate chromatin conformation across entire genomic regions, transient nutritional stimuli at critical ontogenic stages can wield lasting influences on the expression of various genes.[3] Moreover, such epigenetic changes, if they occur in the gametes, may be heritable. This review focuses on early nutritional influences on cytosine methylation. It proposes that certain genomic regions, including genomically imprinted domains and specific transposon insertion sites, are especially labile to such influences. Considering the critical roles that genomically imprinted genes play in mammalian growth and development[4] and the huge proportion of our genome that is comprised of transposons,[5] early nutritional influences on these genomic components could have a substantial impact on human health. Genomic and epigenetic similarities between these distinct classes of elements are elaborated, and key areas of future research are discussed.

EARLY NUTRITION AND ADULT DISEASE

Extensive human epidemiologic data have indicated that prenatal and early postnatal nutrition influence adult susceptibility to diet-related chronic diseases including cardiovascular disease, type 2 diabetes, obesity, and cancer.[6–10] These epidemiologic data are bolstered by numerous studies in animal models[10,11] clearly showing that subtle nutritional influences during development can influence adult metabolism. Understanding the specific biologic mechanisms underlying such phenomena should enable early life nutritional interventions, or even corrective therapies, aimed at preventing chronic disease in humans. To help focus future mechanistic studies in this area, the term *metabolic imprinting* was proposed to encompass a subset of adaptive responses to early nutrition that is characterized by susceptibility limited to a critical ontogenic period and a persistent effect lasting into adulthood.[10] To avoid confusion, however, the term *imprinting* will be used here to refer to genomic imprinting, an epigenetic phenomenon in which only a single allele of a gene is expressed in a parent-of-origin dependent manner.[4]

We recently demonstrated[12] that mammalian phenotype can be persistently altered via nutritional influences on the establishment and/or maintenance of epigenetic gene regulatory mechanisms. Epigenetics is the study of heritable changes in gene expression that are not mediated by DNA sequence alterations.[13] Because of their inherent malleability, epigenetic mechanisms are susceptible to environmental influences,[14] and as discussed below, this environmental susceptibility is expected to be enhanced during early development. Accordingly, nutritional perturbation of epigenetic gene regulation is a likely link between early nutrition and later metabolism and chronic disease susceptibility.[15–17]

EARLY NUTRITION AND DNA METHYLATION

Epigenetic information is conveyed in mammals via a synergistic interaction between mitotically heritable patterns of DNA methylation and chromatin structure.[18] This review focuses on the epigenetics of cytosine methylation, which occurs on both strands of palindromic CpG dinucleotides in mammals. CpG methylation (the "p" in "CpG" denotes the intervening phosphate group in the dinucleotide), which is critical for mammalian development,[19] affects transcription directly by influencing the binding of methyl-sensitive DNA-binding proteins and indirectly by influencing regional chromatin conformation.[13] Specific patterns of CpG methylation are established in early development and are propagated during DNA replication by DNA-methyltransferase-1 (Dnmt1).[20]

Early nutrition can influence DNA methylation because mammalian one-carbon metabolism, which ultimately provides the methyl groups for all biological methylation reactions, is highly dependent on dietary methyl donors and cofactors.[21] For example, dietary methionine and choline are major sources of one-carbon units, and folic acid, vitamin B_{12}, and pyridoxal phosphate are critical cofactors in methyl metabolism. The genome of the preimplantation mammalian embryo undergoes extensive demethylation, and appropriate patterns of cytosine methylation are reestablished after implantation.[4] These DNA methylation patterns must then be maintained over many rounds of rapid cellular proliferation during fetal and early postnatal development. Availability of dietary methyl donors and cofactors during critical ontogenic periods therefore might influence DNA methylation patterns.[10,16] Hence, early methyl donor malnutrition (i.e., overnutrition or undernutrition) could effectively lead to premature "epigenetic aging,"[22] thereby contributing to an enhanced susceptibility to chronic disease in later life.

Given that early nutrition may influence the establishment and maintenance of cytosine methylation, it is logical to wonder whether there are classes of elements in the genome that are

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especially sensitive to such nutritional dysregulation. Transposons[23] and genomically imprinted genes[15,16] may comprise two such classes.

EPIGENETIC LABILITY OF GENOMICALLY IMPRINTED GENES

We carry two copies of all autosomal genes, and the vast majority of these are expressed equally from the paternally inherited and maternally inherited alleles. Some mammalian genes, however, are expressed preferentially from the paternal or maternal allele and are said to be genomically imprinted. The term *imprinted* conveys that these genes must somehow be differentially "marked" in sperm and ova so the developing embryo can distinguish between them. All current data indicate that this marking occurs via allele-specific methylation of specific CpGs.[4] Methylated CpGs serve as primary imprint marks that must resist the global wave of demethylation that occurs in the preimplantation embryo. These primary imprints then serve to "seed" the various layers of epigenetic alterations (including regional cytosine methylation and histone modifications) that maintain monoallelic expression at appropriate stages and in appropriate tissues of the developing mammal.[24,25]

Most imprinted genes are found in clusters, and these imprinted domains are regulated in coordinate fashion via long-range mechanisms such as antisense RNA interference and methylation-sensitive boundary elements.[4] For example, the best-characterized imprinted domain comprises the paternally expressed *insulin-like growth factor 2* (*IGF2*) gene, which shares regulatory elements with the maternally expressed *H19* gene located 100 kb downstream. The regulatory complexity of imprinted domains and the "epigenetic balancing" of imprinted genes between transcriptionally active and inactive states[15] may render them especially susceptible to environmental dysregulation via nutrition.[16,26] Moreover, dysregulation of imprinting can range from loss of imprinting, which results in bi-allelic expression, to silencing of both alleles, which results in complete loss of gene function.

The conjecture that early nutrition might alter imprinting regulation is supported by extensive experimental data in rodents and some alarming observations in humans. Several studies have tested the hypothesis that nutrition, in the form of various culture media used during in vitro manipulations of early mouse embryos, can alter allelic methylation and expression of imprinted genes. Doherty et al.[27] compared the effects of culturing two-cell mouse embryos to the blastocyst stage in Whitten's medium or KSOM medium with amino acids (KSOM + AA). They found that Whitten's medium caused bi-allelic expression of *H19*, which correlated with a loss of methylation in an upstream regulatory region. Conversely, culture in KSOM + AA resulted in blastocysts with appropriate monoallelic expression and methylation similar to that of embryos developed in vivo.

Khosla et al.[28] examined the persistence of such induced epigenetic changes in a slightly different model. They cultured preimplantation mouse embryos in a chemically defined culture medium (M16) with or without fetal calf serum, transferred the resulting blastocysts into recipient females, and assessed expression and methylation of several imprinted genes at 14 d of gestation. Control blastocysts were obtained from females 4 d post coitum and transferred immediately to the uteri of recipient females. At 14 d of gestation, fetuses that were cultured in M16 developed similarly to control fetuses. The addition of fetal calf serum to the M16 culture medium resulted in reduced 14-d viability, and surviving fetuses had lower body weight, decreased expression of *H19* and *IGF2*, and increased DNA methylation at the *H19* imprinting control region compared with controls.[28]

Together these data demonstrate that epigenetic alterations induced in the early embryo can be maintained to later developmental stages. Although it remains to be determined which specific components of the different media induce these changes, it is clear that subtle differences in "nutrition" of the early embryo can have profound and persistent effects on expression of imprinted genes. Recent data from a genetically engineered murine embryonic stem cell model[29] confirmed that methylation of specific imprinted genes, including *IGF2* and *H19*, is labile to subtle biochemical perturbation. Embryonic stem cells with a two-fold increase in Dnmt1 activity relative to wild-type cells showed increased methylation at the *H19* imprinting control region and an increase in *Igf2* and *H19* expression, suggesting bi-allelic expression. Imprinted *p57Kip2* also showed increased expression, whereas other imprinted genes examined (*IGF2R*, *Peg3*, *Snrpn*, and *Grf1*) were not affected by this excess in Dnmt1 activity.[29]

Recent observations have indicated that in vitro manipulation of human embryos also induces imprinting alterations similar to those characterized in mice. Angelman's syndrome is a human neurogenetic disorder caused by loss of function of the maternal allele of imprinted (normally maternally expressed) *UBE3A*. This loss of function results most often from a genetic mutation or more rarely from a sporadic imprinting error.[30] Recently, there have been several case reports[30,31] of children derived from intracytoplasmic sperm injection who developed Angelman's syndrome associated with a loss of methylation in the *UBE3A* region. Assisted reproduction has similarly been linked to an enhanced incidence of Beckwith-Wiedemann syndrome. Beckwith-Wiedemann syndrome is a congenital disorder caused by loss of imprinting of a group of genes (including *H19* and *IGF2*) on human chromosome 11p15.[32] DeBaun et al. found a six-fold increase in the risk of Beckwith-Wiedemann syndrome in children born after intracytoplasmic sperm injection.[32] Together these studies provide the first evidence that environmental influences encountered during in vitro manipulation of the early embryo can lead to human disease by inducing epigenetic alterations at imprinted loci.

Data from animal models have indicated that the epigenetic lability of imprinted genes is not limited to the early embryonic period. Hu et al. treated mice with 5-azacytidine (an inhibitor of DNA methylation) at postnatal days 11 and 14 and found dramatic alterations in allelic expression of *IGF2* but not of *H19*.[33] Could subtle nutritional influences in the postnatal period also affect genomic imprinting? Waterland and Garza[34] obtained an initial answer to this question by evaluating early and persistent effects on pancreatic islet function and gene expression in rats suckled in divergent litter sizes.

In this model, rats suckled in small litters were overnourished, and those suckled in large litters were undernourished during the suckling period, relative to pups suckled in litters of normal size. Directly after weaning and in adulthood, isolated pancreatic islets from small-litter animals displayed impaired glucose-stimulated insulin secretion that correlated with blunted serum insulin levels during in vivo glucose tolerance tests.[34] Using DNA microarrays, 10 genes were identified that showed differential expression between islets of control and small-litter animals directly after weaning and in adulthood. The quantitative developmental stability of the between-group expression differences of these genes suggested that early postnatal diet had induced epigenetic alterations within the islets cells. Two of the 10 genes identified, *insulin 2* and *neuronatin*, are imprinted genes. Because fewer than 0.5% of rodent genes are currently known to be imprinted,[35] these data are consistent with the hypothesis that imprinted genes have an enhanced epigenetic lability to early nutritional influences.[34]

A recent study in mice[36] showed that, even in the postweaning period, transient nutritional exposures can persistently alter allelic expression of imprinted genes. C57/Castaneus F1 hybrid mice were weaned onto a natural-ingredient rodent diet, an amino acid–defined diet, or the amino acid–defined diet deficient in methionine, choline, vitamin B_{12}, and folic acid. The intent was to determine whether the deficient diet would induce relaxation of *Igf2* imprinting. Surprisingly, by 60 d postweaning, the amino acid

diet caused an even greater increase in renal expression of the normally silent maternal allele of *Igf2* than did the deficient diet. Furthermore, this differential relaxation was maintained after all animals were provided the natural-ingredient diet for an additional 100 d.[36] Because the amino acid diet was considered nutritionally complete, these data showed that even subtle differences in diet during the postweaning period can induce persistent changes in the regulation of genomically imprinted genes. Notably, if early nutrition induces similar epigenetic alterations at imprinted genes in the developing germline, it is possible that these could be transmitted to the next generation.

EPIGENETIC LABILITY OF TRANSPOSONS

Transposons are parasitic, repetitive mobile elements that are dispersed throughout the genome and can be classified as DNA transposons (which transpose by a direct DNA "cut-and-paste" mechanism) or retrotransposons (which transpose via an RNA intermediate).[37] Retrotransposons are by far the predominant class of transposons in the mammalian genome. They are grouped into two broad classes: long-terminal repeat (LTR)–containing retrotransposons, such as human endogenous retroviruses, and non-LTR retrotransposons, such as the L1 and Alu elements in humans.[38] All of these elements are remnants of ancestral infections that became fixed in the germline DNA and subsequently increased in copy number. The sequencing of the human genome provided the rather humbling finding that transposons comprise roughly 45% of our genome.[5] This mass of genomic baggage seems particularly excessive when compared with the 2% of our genome that is exonic.

Most transposons in the human genome have accumulated mutations that render them incapable of transposition, but their promoters often remain transcriptionally competent.[23] Except for a brief period of global demethylation in the early mammalian embryo, transposons are normally silenced by promoter CpG methylation.[39] Transposons that escape this epigenetic silencing, however, can interfere with the expression of neighboring genes in several ways.[37] Transcription originating at a transposon promoter can cause inappropriate expression of neighboring genes.[40] For example, the LTR promoters of human endogenous retroviruses are used as alternative promoters for the endothelin-B receptor and the apolipoprotein-C-I genes.[41] In contrast, epigenetic interference of expression can occur when CpG hypermethylation "spreads" from transposon DNA into neighboring genes, leading to their inadvertent silencing.[42]

Whitelaw and Martin recently proposed that stochastic epigenetic variability, which occurs at specific retrotransposon insertion sites, may underlie a substantial amount of phenotypic variability in mammals.[23] One model for such epigenetically based phenotypic variability is the viable yellow agouti (A^{vy}) mouse. The A^{vy} mutation resulted from the insertion of an intracisternal-A particle (IAP, a murine LTR retrotransposon) into an exon of the *agouti* gene.[12] Normally expressed only in hair follicles during a specific stage of hair growth, *agouti* encodes a signaling molecule that causes yellow hair pigmentation. In A^{vy} animals, ectopic *agouti* transcription originates from a cryptic promoter in the proximal LTR of the inserted IAP, causing pleiotropic effects including yellow coat color. CpG methylation in the IAP LTR varies stochastically among individuals, such that even among isogenic A^{vy}/a littermates, a broad range of coat-color phenotypes is observed (Figure 1A).[12,43] Another unique characteristic of the A^{vy} mouse is its capacity for epigenetic inheritance. When A^{vy}/a animals inherit the A^{vy} allele maternally, *agouti* expression and coat-color phenotype are correlated with maternal phenotype.[44]

Rakyan et al.[45] proposed the term *metastable epiallele* to describe alleles such as A^{vy}. *Metastable* conveys the labile nature of the epigenetic state of such alleles, and *epiallele* alludes to their capacity to maintain their epigenetic state transgenerationally. In addition to various IAP insertions at the *agouti* locus, examples of metastable epialleles include the mouse *axin fused* ($Axin^{Fu}$) locus (which causes a variably expressed tail kink) and the fox *star* gene (which causes piebald spotting).[45] All metastable epialleles that have been characterized at the molecular level are associated with a transposon insertion, thus underscoring the unique ability of these viral elements to cause epigenetic instability. It was shown recently that the IAP insertion responsible for the $Axin^{Fu}$ mutation demonstrates individual variations in CpG methylation that correlates with severity of phenotype,[46] reminiscent of the A^{vy} IAP.

But are metastable epialleles epigenetically labile to early nutrition? Wolff et al. conducted an initial test of this question by comparing the coat-color distribution of A^{vy}/a offspring born to a/a dams that were fed an NIH-31 diet or NIH-31 supplemented with the methyl donors and cofactors betaine, choline, folic acid, and vitamin B_{12}.[47] Compared with pups born to non-supplemented dams, the coat-color distribution of those born to supplemented dams was shifted toward the brown (pseudo-agouti) phenotype.

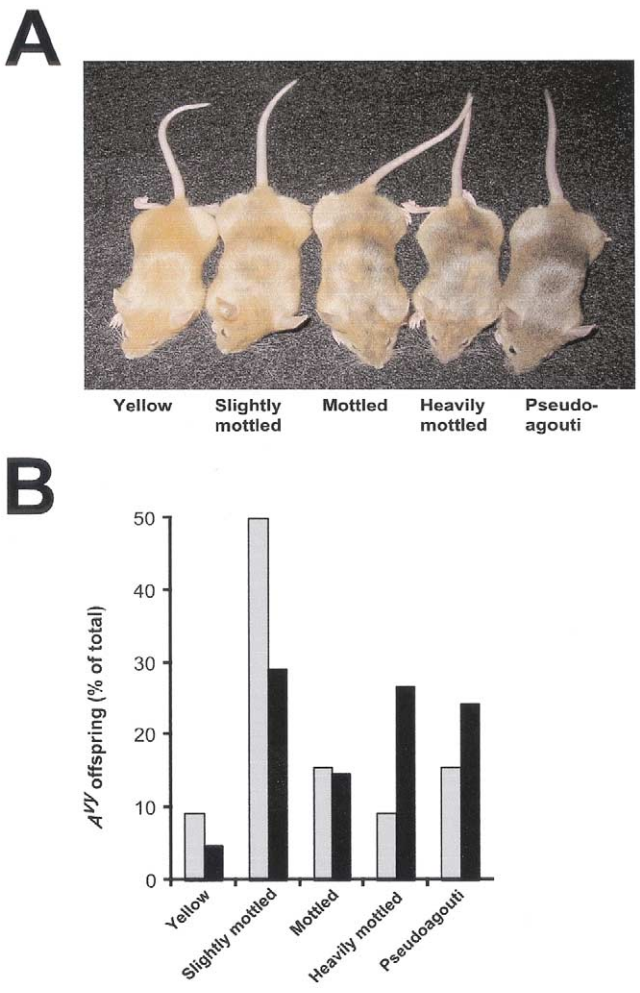

FIG. 1. Maternal dietary methyl supplementation and coat-color phenotype of A^{vy}/a offspring. (A) Isogenic A^{vy}/a animals representing the five coat-color classes used to classify phenotype. The A^{vy} alleles of yellow mice are hypomethylated, allowing maximal ectopic *agouti* expression. A^{vy} hypermethylation silences ectopic *agouti* expression in pseudo-agouti animals, recapitulating the agouti phenotype. (B) Coat-color distribution of all A^{vy}/a offspring born to 9 non-supplemented (30 offspring, gray bars) and 10 supplemented dams (39 offspring, black bars). The coat-color distribution of supplemented offspring is shifted toward the pseudo-agouti phenotype relative to that of non-supplemented offspring ($P = 0.008$). Reprinted with permission from Waterland and Jirtle.[12]

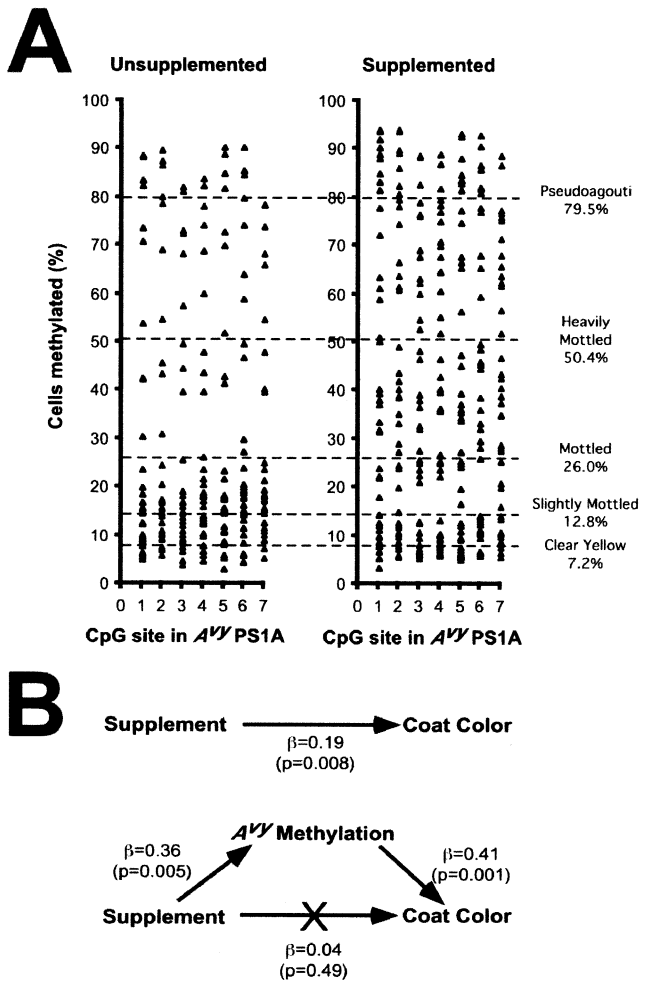

FIG. 2. CG dinucleotide (CpG) methylation within the A^{vy} region of A^{vy}/a offspring from non-supplemented and methyl-supplemented dams. (A) Percentage of cells methylated at each of seven CpG sites in the A^{vy} region in all A^{vy}/a offspring of 9 non-supplemented and 10 supplemented dams. DNA was isolated from tail tips at weaning. The seven CpG sites studied are located approximately 600 bp downstream from the A^{vy} intracisternal-A particle (IAP) insertion site. The percentage of methylation is distributed bimodally in non-supplemented offspring, with fewer than 20% of the cells being methylated at each site in most animals. Maternal methyl supplementation increases mean methylation at each site, generating a more uniform distribution. Dotted lines show the average percentage of methylation across the seven sites in all A^{vy}/a offspring according to coat-color phenotype. (B) Mediational regression analysis of supplementation, A^{vy} methylation, and coat color. Supplementation significantly affects offspring coat color (top), but this relation is nullified when A^{vy} methylation is included in the regression model (bottom). This indicates that A^{vy} CpG methylation completely mediates the effect of supplementation on coat color. Reprinted with permission from Waterland and Jirtle.[12]

Because methylation of A^{vy} correlates with coat color,[43] these data suggested that maternal methyl donor supplementation leads to increased A^{vy} methylation in the offspring. Recently, we conducted a direct test of this hypothesis[12] by measuring site-specific A^{vy} CpG methylation in all offspring born to several litters of supplemented and non-supplemented dams. We confirmed that maternal diet influences offspring coat-color phenotype (Figure 1) and, for the first time, showed that this effect is mediated by increased A^{vy} methylation in the offspring of supplemented dams (Figure 2). Future studies will determine whether other metastable epialleles in the mouse are epigenetically labile to early nutrition.

Epigenetic metastability does not appear to be specific to LTR-containing retrotransposons such as the A^{vy} IAP because Sutherland et al.[48] also demonstrated epigenetic metastability at a specific metallothionein-I transgene insert adjacent to an L1 retrovirus in the mouse. We should note that endogenous retroviruses, which are structurally similar to murine IAPs, comprise fully 8% of the human genome.[49] Therefore, even if epigenetic metastability is associated mainly with LTR-containing retrotransposons, these elements could render a host of human genes epigenetically labile to early environmental influences.

Epigenetic metastability appears to depend on the genomic region into which a transposon is inserted. Contrary to the individually variable methylation at the A^{vy} IAP,[12] most murine IAPs are hypermethylated during early embryonic development and remain so through adulthood.[50] The characteristics of the surrounding genomic DNA that confer epigenetic metastability upon a specific transposon insertion are unknown. The A^{vy} IAP is inserted into an *agouti* promoter region,[12] whereas the IAP associated with the $Axin^{Fu}$ metastable epiallele is positioned in *Axin* intron 6.[51] It is important to compare these two regions to look for genomic similarities that may contribute to their epigenetic metastability. The determination of common characteristics of metastable epialleles in animal models ultimately may lead to the development of genomic approaches for their identification in humans.

GENOMIC AND EPIGENETIC SIMILARITIES OF IMPRINTED GENES AND TRANSPOSONS

Genomically imprinted genes and specific transposon insertion sites at first may seem to not have much in common. A close inspection, however, reveals that these apparently disparate genomic elements share several genomic and epigenetic characteristics that might help us understand their apparent enhanced epigenetic susceptibility to the influence of early nutrition.

The most obvious such shared characteristic is that imprinted genes and transposons are transcriptionally regulated by CpG methylation. Differential methylation of maternal and paternal alleles plays a primary role in regulating monoallelic expression of imprinted genes,[4] and CpG methylation is necessary to transcriptionally silence most transposons.[39,50] Accordingly, imprinted genes and transposons are associated with regions of high CpG density.

Another characteristic shared by imprinted genes and transposons is their tendency to be clustered within the genome. The clustering of imprinted genes is in some cases necessary to facilitate long-range coordinate regulation between pairs of genes within an imprinted domain.[52] Clustering of imprinted genes also might occur to minimize deleterious epigenetic influences on non-imprinted genes from neighboring imprinted genes.[4] Similarly, transposons tend to cluster within the genome, being enriched in areas where they cause the least genetic and epigenetic harm to the host organism.[53] Analogous to the epigenetic influences that imprinted genes can exert on neighboring genes, CpG hypermethylation tends to spread from transposons into adjacent genomic DNA.[42] A recent bioinformatics analysis[54] demonstrated that a specific class of transposons, short interspersed nuclear elements, has been preferentially excluded from imprinted domains over the course of evolution. This result is consistent with the conjecture that their shared tendency to influence epigenetic regulation of neighboring genomic regions prevents imprinted genes and specific transposons from coexisting in close proximity.[54] Such regional conflicts in epigenetic regulation could contribute to epigenetic instability, conferring lability to environmental influences at specific imprinted loci and transposon insertion sites.

The parent-of-origin effects common to imprinted genes and specific transposons may be mechanistically linked to their shared

susceptibility to environmental influences. L1 elements and IAPs are preferentially methylated in sperm and unmethylated in the oocyte, whereas Alu elements display the opposite behavior. Hence, certain transposons behave somewhat like imprinted genes.[39] Parental effects at imprinted genes are possible because 1) differential epigenetic marks are established on sperm and egg DNA during gametogenesis and 2) these marks are capable of withstanding the genome-wide demethylation that occurs in the preimplantation embryo.[55]

It was previously thought that parent-of-origin effects at metastable epialleles (such as A^{vy}) resulted from incomplete erasure of epigenetic marks in the sperm or egg. For example, Morgan et al.[43] concluded that maternal effects at the A^{vy} locus occur because A^{vy} methylation is incompletely erased in the oocyte, whereas that in the sperm is completely "wiped clean." More recent data,[46] however, have shown that A^{vy} methylation in sperm correlates with that in somatic tissue. If A^{vy} methylation is not completely reset in the egg or sperm, the maternal effects observed at this locus must result from differential erasure of those residual marks in the early embryo. Hence, imprinted genes and, to some extent, metastable epialleles associated with transposons are able to resist the global demethylation that sweeps through the genome of the preimplantation embryo. It may be important to consider that this shared developmental resistance to demethylation might be associated mechanistically with epigenetic susceptibility to environmental influences.

CpG methylation at each imprinted gene functions as an "epigenetic switch" enabling the gene's primary imprint status to assume one of two distinct states. The "switch" is flipped in one direction or the other based on the different "environments" encountered during spermatogenesis and oogenesis, respectively. Metastable epialleles associated with specific transposon insertions also appear to behave as epigenetic switches. Contrary to the majority of mammalian alleles, which fairly predictably become methylated or not in specific cell lineages,[55,56] in each cell of the early embryo CpG sites within metastable epialleles stochastically assume a methylated or unmethylated state. Once set, this state is propagated to all the founder cell's progeny.[45] The prevailing understanding is that these two types of epigenetic switches are quite distinct; methylation at imprinted loci is determined by parental inheritance, whereas that at metastable epialleles is determined probabilistically.[45]

This distinction, however, is not so absolute. For example, it is customary to refer to imprinted genes as being expressed from the maternal or the paternal allele. Nevertheless, when highly sensitive methods are used to determine allelic expression, it is evident that a small subpopulation of cells within any tissue may express both alleles of any given imprinted gene,[57] and the degree of biallelic expression of specific imprinted genes varies widely among different individuals.[57–59] Beyond this normal low level of "leakiness" of imprinting, more substantial relaxation of imprinting occurs in several developmental diseases[35,60] and cancers.[61,62] This demonstrates that there is also a probabilistic component to imprinting.

In some imprinted domains, the probabilistic nature of allelic expression is further reflected at the level of allele-specific differential methylation, which contributes to their regulation. Contrary to the near-absolute allele-specific methylation found at specific CpG sites within the differentially methylated regions of many imprinted genes, some imprinted differentially methylated regions maintain only quantitative differences in allelic methylation. For example, on the paternal allele of murine *IGF2*, specific CpG sites that comprise differentially methylated region-1 are methylated in approximately 80% of cells, versus approximately 40% of cells on the maternal allele.[63] Hence, even though methylation in such regions is certainly influenced by parental inheritance, there is also a probabilistic component to their epigenetic setting, similar to that of metastable epialleles associated with transposons. This probabilistic nature of epigenetic setting may directly confer epigenetic lability to early environmental influences.

FUTURE DIRECTIONS: NUTRITIONAL EPIGENOMICS

New technologies now make it possible to test the hypothesis that specific genomic regions have an enhanced epigenetic lability to nutritional (and other environmental) influences during development. Rather than using genomic approaches to identify genes whose expression is persistently altered by early nutrition, epigenomic approaches now promise the potential to measure gene-specific changes in DNA methylation of many genes simultaneously. Genome-wide methylation profiling offers important advantages over genome-wide (mRNA) expression analyses,[64] and several different approaches have already been developed.[65–67] Only by applying such epigenomic approaches will we eventually be able to determine, in animal models, the specificity and breadth of genomically imprinted genes and transposon insertion sites that act as targets for early nutritional effects on epigenetic gene regulation. By using these models to identify the genomic and epigenetic characteristics that confer epigenetic lability to early nutrition, we will be poised to identify mechanistically similar regions that serve as epigenetic templates for metabolic imprinting in humans.

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