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Genomic imprinting and methylation: epigenetic canalization and conflict

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Imprinted genes have patterns of expression that depend on the parent of origin of their alleles. Establishment of imprinting at a locus requires that the two alleles be differentially marked in oogenesis and spermatogenesis, that these marks escape reprogramming after fertilization, and that they are reliably transmitted through development. Recent work on the mammalian DNA methyltransferases involved in these processes suggests mechanisms of epigenetic canalization, which might contribute to the stability of epigenetic inheritance. At the same time, the interactions that determine whether a particular modification will be transmitted or reprogrammed are destabilized by evolutionary conflicts, as the genes and gene products controlling these processes are subject to divergent selective forces. This review summarizes many of the recent advances in our understanding of mammalian systems of epigenetic gene regulation in the context of the long-running evolutionary conflicts that have created them.

Introduction

Advances in DNA sequencing over the past two decades have generated a lot of optimism in the scientific community. During that same time, however, it has become clear that simply collecting DNA sequences will not enable us to address all of the questions that we ultimately want answered. Among the factors that have complicated our understanding of biology is the fact that chromosomes are subject to epigenetic modification: heritable, but reversible, changes that can affect the physical conformation of DNA and its transcriptional activity. Known epigenetic modifications include cytosine methylation, in addition to numerous modifications of chromatin-associated proteins (e.g. methylation and acetylation of histones) [1].

In mammals, DNA methylation occurs predominantly at cytosines in CpG dinucleotides, and the establishment and maintenance of methylation is required for normal development. Differential methylation of the maternally and paternally derived chromosomes correlates with parent-of-origin-dependent differences in gene expression, or genomic imprinting (see Glossary). The mechanisms through which imprinted gene expression is established have drawn interest from developmental, molecular and evolutionary biologists as well as medical researchers. The advances of the past few years point to increasingly complex mechanisms underlying the dynamics of cytosine methylation in imprinting, and suggest a rich evolutionary history.

This review is intended to place certain recent advances in our understanding of mammalian DNA methylation dynamics into an evolutionary context. Specifically, I will focus on the mammalian methylation and demethylation activities in the preimplantation embryo, and the problem of transmitting epigenetic information across cell divisions and generations. Recent molecular findings will be related to the evolutionary theory of imprinting and the intergenomic and intragenomic conflicts involved in establishing imprinted gene expression. The establishment of methylation in the germ line and its interpretation in somatic tissues will be discussed briefly, but are not the focus of this article. Further information on these

Glossary

Bisulfite sequencing: a DNA sequencing technique that distinguishes between methylated and unmethylated cytosine.

***De novo* methyltransferase:** an enzyme that catalyzes the addition of a methyl group in a manner that is not dependent on the presence of methylated cytosine on the opposite strand of the DNA.

Epigenetic canalization: a robustness of epigenetic heritability to small perturbations. For example, the methylation state of CpG islands might be subject to mechanisms that stabilize the region into one of two states (highly methylated and highly unmethylated). Specifically, the methylation state of one cytosine would affect the propensity of nearby cytosines to become methylated, or to maintain their methylation.

Epigenetic modification: a modification to a chromosome that does not involve altering the DNA sequence. The best-known example is cytosine methylation.

Genetic faction: a set of alleles that share a common inclusive fitness, which can differ from that of other genetic factions, even within the same organism. Maternally and paternally derived autosomal genes represent two distinct genetic factions.

Genomic imprinting: the phenomenon in which the expression of an allele depends on whether it was inherited through an egg or a sperm.

Inclusive fitness: a notion of fitness that includes all identical copies of an allele, regardless of which organism they happen to be in. It is often stated as the summed fitness of all individuals, weighted by the probability that they carry an allele identical to one present in the focal individual. This notion is central to explanations of eusociality, sex-ratio distortion and genomic imprinting.

Kinship theory of imprinting: an explanation for the evolutionary origins of genomic imprinting based on the asymmetric effects of changes in gene expression on an individual's matrilineal and patrilineal kin.

Maintenance methyltransferase: a DNA methyltransferase that targets the unmethylated cytosine in a hemimethylated palindromic DNA sequence. In vertebrates, these are typically CpG dinucleotides. These hemimethylated sequences are generated during semiconservative DNA replication.

Maternal-store protein: a protein present in the oocyte or embryo that represents the gene products of both of the mother's alleles at a locus. Natural selection on a maternal-store protein will favor the inclusive fitness interests of the mother, which might not be identical to those of the developing embryo.

Methylation spreading: methylation of DNA stimulated by the presence of other nearby methylation marks.

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and other aspects of genomic imprinting can be found in numerous recent reviews [2–7].

The jobs of the DNA methyltransferases

Methylation associated with imprinted genes is eliminated and re-established every generation during gametogenesis. For example, the maternal and paternal methylation patterns inherited by a male are erased in the male's germ line, and replaced with the paternal methylation pattern, which is carried by each sperm. Similarly, each egg produced by a female carries the maternal methylation pattern. Following fusion of the two gametes, the methylation marks established in gametogenesis are subject to one of three fates: active demethylation, passive demethylation or maintenance (Figure 1). Active demethylation involves the removal of methyl groups from methylated cytosines. Passive demethylation is a gradual dilution of methylation that results from the failure to propagate methylation through cell division. Maintenance requires methylation patterns to be copied onto newly synthesized DNA at each round of replication.

Establishment of imprinted gene expression therefore involves demethylation in the germ line and the embryo. It also involves two distinct methyltransferase activities: a *de novo* methyltransferase targeting specific unmethylated CpGs, and a maintenance methyltransferase directed at hemimethylated CpGs following DNA replication. Five genes in mammals have been identified as members of the DNA (cytosine-5) methyltransferase (Dnmt) family: *Dnmt1*, *Dnmt2*, *Dnmt3a*, *Dnmt3b* and *Dnmt3L* (reviewed in Refs [8,9]). Exactly how these five genes combine with other factors to perform the two methyltransferase activities is not yet entirely clear, but

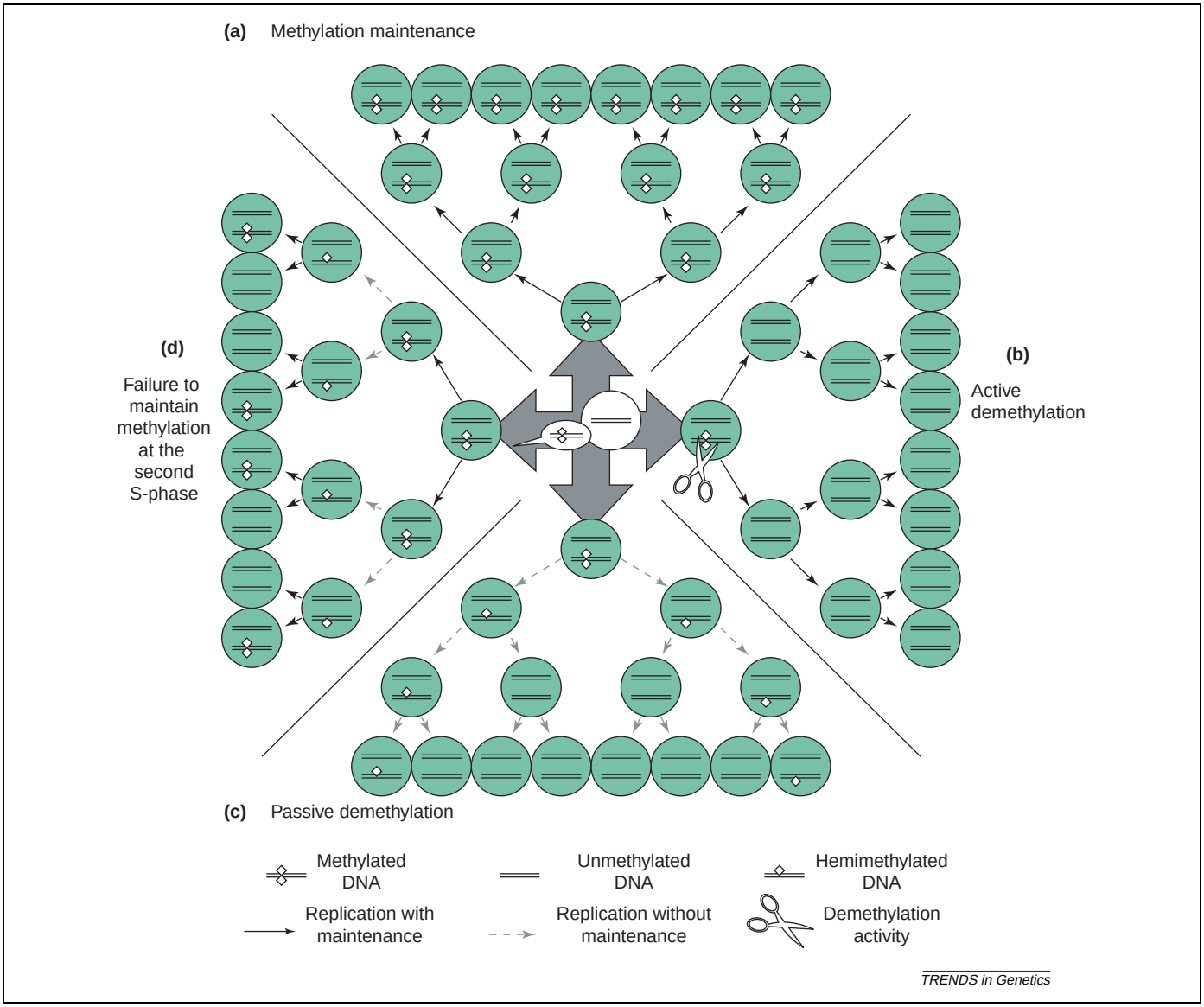

Figure 1. The fate of allele-specific methylation after fertilization. A schematic representation of four different hypothetical outcomes of the interaction of allele-specific methylation marks with the methylation maintenance machinery in the preimplantation embryo. In this example, the paternally derived allele is methylated and the maternally derived allele is unmodified. Three rounds of cell division are pictured, with arrows indicating the patterns of cell parentage. Gray dashed arrows indicate a cell division in which maintenance methylation is not active at this locus. Clockwise, starting from the top, the four quarters of the figure represent **(a)** methylation maintenance, **(b)** active demethylation before the first cell division, **(c)** passive demethylation at all cell divisions and **(d)** failure to propagate methylation at a single cell division, in this case, the second.

active research in the area is beginning to shed light on their possible roles.

Dnmt1: maintenance methyltransferase and more

The *Dnmt1* gene in mice produces three different splicing variants: Dnmt1 (also known as Dnmt1s or Dnmt1a), Dnmt1p and Dnmt1o. Dnmt1p and Dnmt1o are expressed from alternative promoters, and differ from Dnmt1 by the substitution of germ-line-specific versions of exon one [10]. Dnmt1p transcription is restricted to the pachytene spermatocyte, and does not result in detectable levels of Dnmt1 protein, despite high mRNA levels. Dnmt1o is transcribed from an oocyte-specific promoter and encodes a truncated, but enzymatically active, version of Dnmt1 that accumulates to high levels in the oocyte.

Dnmt1 has traditionally been assumed to have the role of the primary maintenance methyltransferase, due to its widespread expression in somatic tissues, its catalytic preference for hemimethylated substrates [11], and the fact that it localizes to the replication fork during DNA replication [12]. Deletion of *Dnmt1* in mice results in genome-wide demethylation and embryonic lethality, consistent with a failure to propagate essential methylation marks [11]. However, the fact that Dnmt1 possesses some *de novo* activity in cell lysates and exhibits little sequence specificity has led to speculation that it might possess a second function (e.g. Ref. [8]).

Recent studies suggest that Dnmt1 functions as a maintenance methyltransferase in two senses. In addition to its role as the primary maintenance methyltransferase, it is involved in methylation spreading, in which *de novo* methyltransferase activity is specifically targeted to regions that are already partially methylated. Both *de novo* and maintenance methylation activities increase with methylation density, even in the absence of the canonical *de novo* methyltransferases, Dnmt3a and Dnmt3b [13]. Dnmt1 is allosterically activated by binding of its N-terminal regulatory domain to methylated DNA [14–16], and functionally cooperates with Dnmt3a and Dnmt3b [17–19]. Whereas the Dnmt3 enzymes are required to initiate methylation, Dnmt1 could be important for converting this initial mark into a local region of dense methylation [15].

The evidence for this methylation spreading activity is limited to *in vitro* studies, and its relevance to patterns of methylation *in vivo* remains unclear. However, these studies do suggest a possible mechanism of epigenetic canalization, by which chromosomal regions would be stabilized into one of two states: highly methylated or largely (or completely) unmethylated. This mechanism can be distinguished from epigenetic heritability, in which epigenetic marks are reconstituted following DNA replication. Canalization implies a robustness of epigenetic heritability to small perturbations, such as the failure to propagate methylation at a particular cytosine. If Dnmt1 possesses methylation spreading activity *in vivo*, the presence of nearby methylated cytosines could stimulate the replacement of a methyl group that is lost by chance. In this scenario, the *de novo* activity of Dnmt1 would compensate for occasional failures of its methylation maintenance activity. Whereas heritability can be thought of as being associated with individual methylation marks, canalization would be a regional property, involving positive feedback among the methylation states of groups of nearby cytosines, limited perhaps by insulator elements or CpG-poor regions.

The hypothesis that epigenetic states are regionally canalized could be tested by eliminating specific methylation sites. The prediction would be that this would reduce the fidelity of methylation maintenance at nearby sites. The specific role of Dnmt1 in the canalization process could be tested through manipulation of the N-terminal regulatory region of the enzyme. For example, inactivation of the allosteric-binding site would be predicted to destabilize epigenetic inheritance, possibly producing heterogeneous methylation patterns in differentiated cell lineages. However, such experiments might have to await a more detailed biophysical characterization of Dnmt1.

Dnmt1o: there and back again

The oocyte-specific Dnmt1 splicing variant Dnmt1o is of particular interest owing to its unusual trafficking behavior and its specific role in maintaining methylation at imprinted loci in mice [20]. Dnmt1o is sequestered in the cytoplasm of mature oocytes, and throughout the first three rounds of cell division in the embryo. At the eight-cell stage, Dnmt1o moves transiently into the nucleus, and then returns to the cytoplasm at the 16-cell stage. Heterozygous embryos of mothers homozygous for a knockout of the Dnmt1o promoter die in the last third of gestation. Bisulfite sequencing of DNA from these embryos showed a loss of methylation at certain imprinted loci on approximately half of the methylated alleles, consistent with a failure to propagate methylation at a single cell division (Figure 1).

Dnmt1o is apparently required in mice for methylation maintenance at the fourth S-phase, but specifically excluded from participating in the same reaction during any other cell division. This interpretation suggests the involvement of at least one other maintenance methyltransferase that would be active during the other cell divisions, but held inactive by some mechanism during the fourth S-phase. The full-length Dnmt1 protein is not present at detectable levels until embryonic day seven [10,21], despite the presence of low levels of Dnmt1 mRNA. When full-length Dnmt1 was expressed in oocytes from the Dnmt1o promoter, the protein trafficked into the nucleus at the eight-cell stage like Dnmt1o [21], suggesting that if low levels of Dnmt1 protein were present in the preimplantation embryo, they would be subject to the same temporal regulation as Dnmt1o. This seems to make Dnmt1 an unlikely candidate for the maintenance methyltransferase active during the first three replication cycles, although it is difficult to rule out the possibility that very low levels of the protein might be present.

The fact that Dnmt1 and Dnmt1o share the same activity and trafficking behavior raises the question of why an oocyte-specific form should exist. One possible answer is suggested by the fact that Dnmt1o is resistant to a developmentally regulated mechanism that degrades Dnmt1 [22]. The need for this additional stability could be

related to the fact that Dnmt1o is a maternal-store protein. That is, Dnmt1o mRNA is produced in the developing oocyte before the completion of meiosis, significantly in advance of the time when the Dnmt1o protein performs its enzymatic function.

The identity of Dnmt1o as a maternal-store protein is crucial to our understanding of its function in an evolutionary context (Box 1). Specifically, the Dnmt1o present in the preimplantation embryo represents the products of both of the mother's alleles at the *Dnmt1* locus. The embryonic phenotype depends on the maternal genotype, and evolution of the function of Dnmt1o should be interpreted in terms of how the embryonic phenotype affects the mother's inclusive fitness [2,23]. Because the proteins involved in methylation maintenance at the other preimplantation cell divisions have not yet been identified, it is impossible to know what the genetic origin of those proteins might be. However, the scarcity of proteins in sperm and the early transcriptional silencing in the zygote make these unlikely sources of additional maintenance methyltransferase activity, suggesting that other important maternal-store proteins are likely to exist.

The trafficking behavior of Dnmt1o has not yet been examined in any species other than the mouse. The presence of the Dnmt1o transcript and absence of the full-length Dnmt1 have, however, been reproduced in the oocytes of the marsupial *Monodelphis domestica* [24], and in human oocytes and very early embryos [25]. The oocytes of *Xenopus* and zebrafish, by contrast, contain only the full-length somatic form of Dnmt1 [26,27]. This phylogenetic distribution is consistent with the Dnmt1o transcript arising in the common ancestor of marsupials and eutherians, coincident with the most likely origin of genomic imprinting [28,29] (Figure 2), although no evidence is yet available on the presence or absence of Dnmt1o in monotremes. A recent study found no evidence of Dnmt1o expression in bovine embryos [30], suggesting the possibility of a subsequent loss of this specific Dnmt1o function in at least one mammalian lineage.

Box 1. Maternal and maternally derived genetic factions

Natural selection favors alleles that are successful at passing copies of themselves on to future generations. An allele can accomplish this by facilitating the survival and reproduction of the organism in which it is physically present, in addition to other organisms possessing copies of the same allele. Selection will therefore act on allelic variants on the basis of their inclusive fitness effects: the total effect on all identical copies of an allele. The likelihood that another organism shares an allele depends on the relatedness of the two organisms. For example, a maternally derived autosomal allele in an outbred population is present in full sibs and maternal half-sibs with probability 1/2, but is in paternal half-sibs with probability zero. The inclusive fitness of this allele would include the fitness of the focal individual carrying the allele, as well as half of the fitness of each of that individual's full sibs and maternal half-sibs.

A genetic faction is a set of alleles that share a common pattern of relatedness, and therefore a common inclusive fitness. Maternally derived autosomal genes are one genetic faction, and paternally derived autosomal genes are another. Important to the understanding of imprinting is the fact that maternal and maternally derived genes represent distinct genetic factions. The maternal faction, representing both of the mother's alleles at a locus, is equally related to each of the mother's offspring. The maternally derived faction differs because it is more closely related to one of the mother's offspring (the offspring carrying that allele) than it is to the others.

In a sense, the maternal faction has one degree of uncertainty that the maternally derived faction does not. For example, an allele encoding a DNA methyltransferase in the female germ line might or might not end up in the same egg with a particular cytosine that it methylates. However, the nucleotides immediately surrounding that methylation site almost certainly will. The fitness consequences of methylation will therefore be different for the methyltransferase and its target. More generally, *trans*-acting factors in oogenesis are 'maternal', whereas *cis*-acting factors are 'maternally derived', so long as they act on a scale that is unlikely to be disrupted by recombination in any generation. Similarly, premeiotic transcripts, including maternal-store proteins, are maternal, whereas postmeiotic transcripts are maternally derived. A similar distinction applies to the paternal and paternally derived genetic factions.

Dnmt2: mystery methyltransferase

Dnmt2 continues to be the least understood member of the Dnmt family. Until recently, methyltransferase activity had not been observed in Dnmt2, leading to speculation that it might be catalytically inactive, despite the presence of sequence motifs shared with the catalytic sites of other Dnmt proteins. Recent experiments provide some evidence that Dnmt2 homologs from mouse and *Drosophila* can methylate cytosine *in vivo* [31–33], and evidence of human Dnmt2 activity has been observed *in vitro* [34]. In all cases, however, activity is low, and primarily targets non-CpG cytosines, suggesting that it could be involved in an unrelated set of phenomena. The conservation of enzymatically active homologs in *Drosophila* and mammals suggests some selectable function for Dnmt2, although the gene is not essential for mouse development [9]. Interestingly, the same study that failed to find Dnmt1o transcripts found Dnmt2 to be the most highly expressed methyltransferase in bovine embryos [30]. As yet there are no data to suggest whether there are any functional differences between bovine Dnmt2 and the other mammalian forms.

The Dnmt3 family: two-and-a-half more methyltransferases

Dnmt3a and *Dnmt3b* encode essential *de novo* methyltransferases in mice [35] that are actively expressed in male and female germ lines [36,37]. Both enzymes have been studied extensively *in vitro* and in various cellular contexts [9], but the lethal phenotype of both knockouts has made it difficult to assess their functions *in vivo*. Recently Kaneda *et al.* [38] constructed conditional knockouts of both genes in mice, and surveyed the effects of disrupting gene expression specifically in germ cells. In oogenesis, the *Dnmt3a* conditional knockout disrupted the establishment of imprinted gene expression at all loci examined, whereas in spermatogenesis, imprinting was disrupted at two out of three loci (the exception being *Rasgrf1*). The *Dnmt3b* conditional knockouts produced no disruption of imprinting, and resulted in phenotypically normal pups. Thus although Dnmt3b is likely to have a role in methylation of centromeric satellite repeats [35], Dnmt3a appears to be the primary *de novo* methyltransferase involved in germ-line establishment of genomic imprinting.

In addition to their roles in initiating methylation, Dnmt3a and Dnmt3b are important for epigenetic

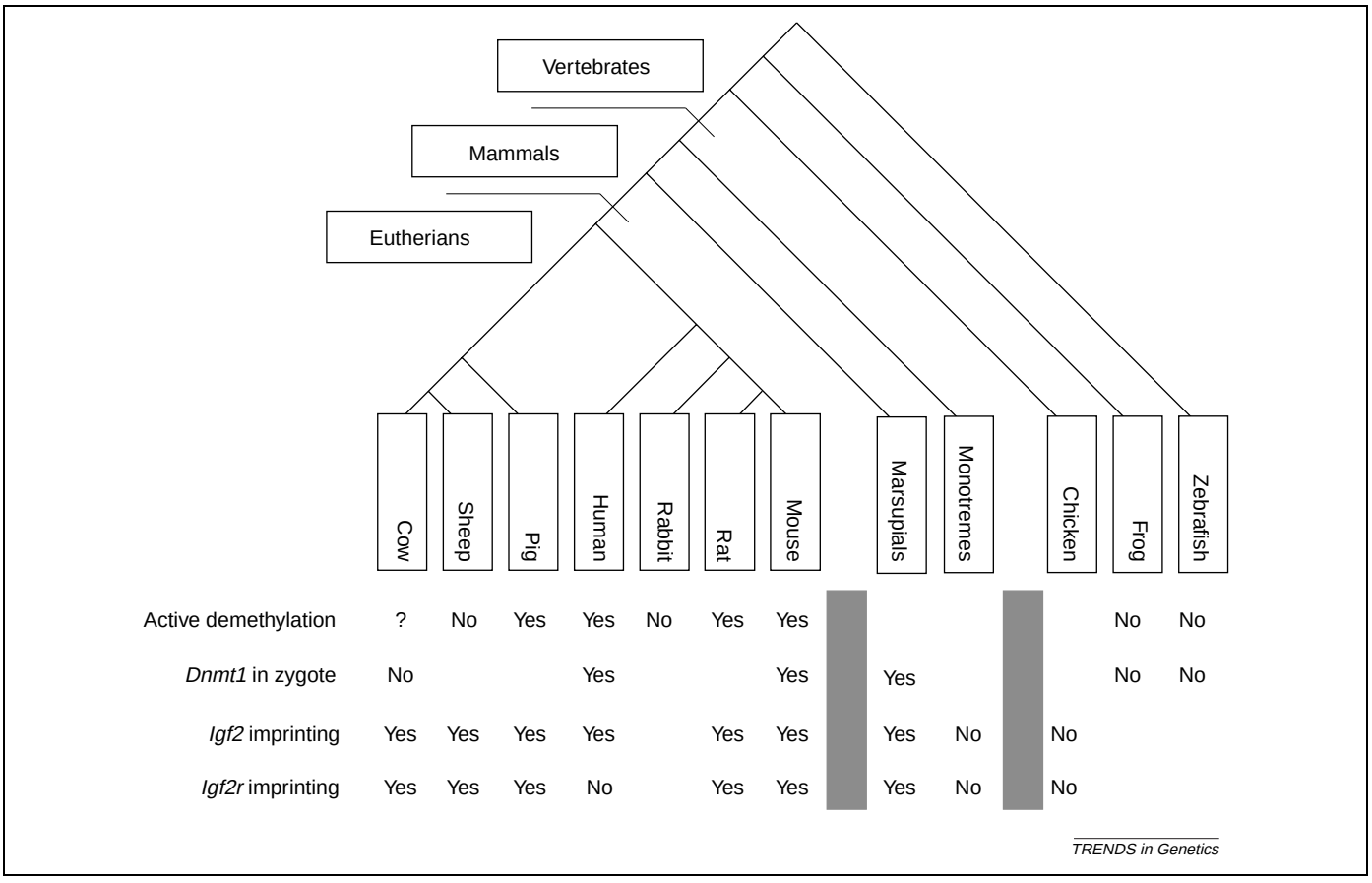

Figure 2. A phylogenetic view of imprinting and methylation. Some of the results referred to in the text are placed into a phylogenetic context. 'Active demethylation' refers to a genome-wide demethylation of the paternal genome following fertilization, as inferred from immunofluorescence studies. 'Dnmt1o in zygote' refers to the presence of a splicing variant of Dnmt1 in oocytes and early embryos that is distinct from the somatic form. This implies nothing about trafficking behavior, which has been studied only in mice. Presence of a trait is indicated by the word 'yes', and absence by the word 'no'. The question mark refers to the evidence for partial demethylation in bovines. Empty cells indicate a lack of direct evidence about the presence or absence of a trait in a particular taxon. The distribution of genomic imprinting is included for comparison. Only two genes, the insulin-like growth factor type 2 (*Igf2*) and the insulin-like growth factor type 2 receptor (*Igf2r*), have been characterized in more than a few species, so these loci serve as a crude proxy for the presence or absence of imprinting in a particular species.

stability [19,39]. At least in some circumstances, Dnmt1 alone is not sufficient to maintain methylation through multiple cell divisions. Maintenance in these cases might require a periodic 'topping off' by *de novo* methylation. For imprinted genes, this would require a mechanism to direct *de novo* methyltransferases specifically to the previously methylated allele. There is, in fact, some evidence suggesting that it is easier to re-establish recently erased methylation than it is to methylate previously unmodified DNA. During gametogenesis, the imprints of the previous generation are erased before establishment of germ-line-specific methylation. At the paternally derived allele in males (or the maternally derived one in females), the methylation pattern that is established is similar or identical to the one that has just been removed. At some loci, methylation is established on this allele earlier in development than on the allele whose methylation pattern is being altered [37,40]. This implies that other aspects of chromatin structure associated with DNA methylation persist for some time following the loss of that methylation.

The changes in chromatin structure induced by DNA methylation include histone methylation and deacetylation [1,41]. This altered chromatin structure could then favor recruitment of *de novo* methyltransferases [42,43]. If the mechanisms that give rise to asynchronous methylation in gametogenesis are active elsewhere, this positive feedback loop between DNA methylation and histone modification would produce a second possible mechanism of epigenetic canalization. The hypothesis that *Dnmt3a* and/or *Dnmt3b* might contribute to the robustness of epigenetic heritability could be tested through variations on the conditional knockout experiments of Kaneda *et al.* [38]. Disruption of these genes in specific cell lineages would be predicted to destabilize the propagation of previously established methylation marks, in addition to the expected effect of inhibiting *de novo* methylation.

Dnmt3L encodes a protein with regions of homology to *Dnmt3a* and *Dnmt3b*, but lacking enzymatic activity itself due to the absence of conserved catalytic motifs. Nevertheless, *Dnmt3L* has an important role in the establishment of methylation marks on imprinted genes in both the male and female germ lines. Targeted disruption of *Dnmt3L* in mice causes sterility in males, and maternal-effect lethality in females, associated with a failure to establish methylation at imprinted loci during oogenesis [44,45]. The *Dnmt3L* knockouts are otherwise phenotypically normal, and disruption of *Dnmt3L* does not result in a genome-wide loss of methylation [44]. *Dnmt3L*, *Dnmt3a* and *Dnmt3b* show similar expression

profiles in oogenesis [36,37], and Dnmt3L can interact directly with Dnmt3a and Dnmt3b to stimulate their methyltransferase activities [45–47]. The *Dnmt3L* knockouts are phenotypically similar to the *Dnmt3a* conditional knockouts [38], pointing to regulatory interactions with Dnmt3a as the essential function of Dnmt3L *in vivo*. In spermatogenesis, loss of Dnmt3L results in demethylation and subsequent activation of retrotransposons, but has no effect on the satellite methylation associated with Dnmt3b [48].

Active demethylation of the paternal genome

The paternally derived genome of mice undergoes active demethylation following fertilization, in which most of the epigenetic marks established during spermatogenesis are eliminated [49–52]. This demethylation occurs before the first cell division, independent of DNA replication. The maternally derived genome retains its methylation during this process, but subsequently undergoes passive demethylation as the genome is replicated. The differentially methylated regions associated with imprinted genes are presumed to be resistant to both demethylation processes, as are some, but not all, retrotransposable elements [53–56]. Paternally derived genome demethylation also occurs in humans [57], rats [51] and pigs [51], but not in zebrafish [58], *Xenopus* [59], sheep [57,60] or rabbits [57,61]. The case of bovines remains somewhat controversial, but it currently appears that the paternally derived genome might undergo an intermediate degree of demethylation [51,57,62].

The details of active demethylation are, as yet, poorly understood. In particular, most evidence on the phenomenon is based on labeling cells with fluorescent antibodies to 5-methylcytosine. This technique provides a gross quantitative measure of overall methylation, and, before pronuclear fusion, is capable of distinguishing between the maternally derived and paternally derived genomes. However, these results provide no information regarding the fate of specific methylation marks; direct observation of active paternal demethylation from DNA bisulfite sequencing is currently limited to *Igf2*, *α-actin* and a transgene, *TKZ751*, in mice [50]. Furthermore, many of these results are based on somatic cell nuclear transfer experiments, and there is no guarantee that patterns of epigenetic reprogramming observed in this context will be identical to those present in normal development.

The apparent absence of active demethylation in fish and frogs suggests that this phenomenon could have a phylogenetic distribution similar to that of genomic imprinting (Figure 2). The kinship theory of imprinting [2,63] predicts that paternal epigenetic modifications will often result in increased demands on maternal resources (Box 2); Reik and Walter [64] have suggested that demethylation of the paternally derived chromosomes is an attempt by the maternal genes to eliminate this epigenetic information. In particular, maternal genes could benefit from reactivation of paternally silenced imprinted genes [23].

Our understanding of how methylation dynamics vary across mammalian taxa is likely to change as more detailed information becomes available. However, current evidence suggests a phylogenetic distribution requiring loss of active demethylation from some lineages, or multiple independent origins. This variation could present opportunities to test specific hypotheses about the factors favoring active demethylation. Specifically, a conflict based explanation of demethylation would predict that active demethylation would be correlated with the intensity of the conflicts over maternal resource distribution between the mother and the fetus, and between the maternally and paternally derived alleles within the fetus.

Retrotransposons, genetic conflict and the origins of epigenetic complexity

The recent advances covered here hint at a remarkable degree of complexity in the epigenetic machinery of mammals. It might be tempting to believe that this complexity simply reflects the enhanced role of methylation in vertebrate gene regulation [65]. However, these systems represent the products of a history of antagonistic coevolution among their constituent parts. The kinship theory attributes imprinted gene expression to the fact that natural selection acts differently on maternally and paternally derived alleles. Analogous reasoning applies to

Box 2. The kinship theory of imprinting and phenotypic plasticity

The kinship theory of imprinting identifies factors favoring alleles whose expression pattern is dependent on their parent of origin [2,64]. Central to the theory is the fact that the inclusive fitness effect of a change in gene expression can be different for maternally and paternally derived alleles. Although the logic of the theory applies to any gene, its consequences have been best characterized in the context of prenatal and postnatal growth effects. In mammals, offspring have an opportunity to influence the availability of maternal resources. The consequence to an offspring of acquiring additional resources is to increase that offspring's fitness, but at a cost to the mother's other offspring. Because these other offspring might not share the same father, the fitness consequences of this cost will fall more heavily on the offspring's maternally derived alleles. An allele will therefore favor placing a greater demand on the maternal resources when it is paternally derived than when it is maternally derived. The magnitude of the difference between the two preferred demand levels will depend on the details of the system (e.g. biochemical properties of the gene product, degree of polyandry and reproductive life history).

In one sense, genomic imprinting is a special form of phenotypic plasticity, similar to other cases of conditional gene regulation. Sex-regulated gene expression, for instance, is a conditional strategy that permits an allele to adapt simultaneously to two separate environments: a male body and a female body. Similarly, an imprinted allele is one that evolves two separate strategies that it employs in the 'maternally derived' and 'paternally derived' environments. There is, however, an important difference between imprinting and other forms of plasticity. In many cases it is reasonable to assume that all of the alleles in a single organism are under selection to adapt to the same environment: it is presumably in the interests of all of the alleles in my body to be adapted to the 'male body' environment. However, an individual organism consists of nearly equal numbers of maternally and paternally derived alleles, which are under selection to produce different phenotypes (e.g. less and more resource demand, respectively). The realized phenotype depends on interactions among those alleles, so that the 'maternally derived' environment consists partly of paternally derived alleles and vice versa. This leads to an antagonistic coevolution of two conditional expression strategies at a single locus.

other elements of the epigenetic machinery representing different genetic factions [23,64,66,67].

The complexity of the interactions in mammalian epigenetics creates numerous opportunities for genetic conflict and antagonistic coevolution. Methylation, for example, requires the interaction of *cis*-acting elements (e.g. a DNA sequence) and *trans*-acting elements (e.g. a methyltransferase), which will often be subject to divergent selective pressures (Box 1). Natural selection acting on the *cis*-acting elements associated with imprinted gene expression will favor preservation of the differential marks established in oogenesis and spermatogenesis [23]. The *trans*-acting elements involved in active demethylation and maintenance methylation immediately following fertilization are likely to be maternal-store proteins, such as Dnmt1o [20]. These proteins will be selected to maintain maternal methylation patterns at imprinted loci, but will favor a loss of the paternal methylation patterns associated with imprinting [23,64].

Bestor [53] has recently highlighted the role of cytosine methylation in silencing retrotransposable elements. The similarities between the mechanisms of imprinting and retrotransposon silencing [48,54] might serve to further complicate epigenetic interactions (Figure 3). If retrotransposable elements can benefit from transposition after fertilization, their associated *cis*-acting elements will favor elimination of methylation marks involved in silencing transposition. Maternal-store proteins and proteins transcribed from the zygote will favor maintenance of methylation involved in retrotransposon silencing. Immediately following fertilization, the interaction between *cis*- and *trans*-acting factors involved in methylation maintenance will be fully cooperative only for the case of imprinting on the maternally derived chromosomes. In each of the other cases, the sequence specificity of the *trans*-acting factors and their DNA target sequences could be evolving antagonistically.

The involvement of methylation in retrotransposon silencing could create an opportunity for paternally methylated imprinted genes to evade the active demethylation process. Wilkins and Haig [23] argue that antisense RNA production provides long-term evolutionary stability to paternal gene silencing in the face of maternally directed demethylation. A second possible mechanism would be retrotransposon mimickry; paternal silencing could be stabilized if it were impossible for maternal-store proteins to reactivate a paternally derived imprinted gene without simultaneously activating multiple retrotransposable elements. The retrotransposon-mimickry hypothesis would apply specifically to paternally methylated alleles, and would therefore predict that paternally methylated imprinted loci would resemble retrotransposons more than maternally methylated loci. This hypothesis also implies a potential trade-off faced by the maternal-store proteins between the dangers posed by paternal epigenetic information and the danger of inadvertent retrotransposon activation. The phylogenetic distribution of active demethylation might be comprehensible in terms of the relative magnitudes of these two dangers in different taxa.

The discussion here has focused on genetic conflicts immediately following fertilization. However, the potential for genetic conflict over epigenetic gene regulation exists throughout the life cycle. The details of the conflict will vary, however, as the genetic origins of the *cis*- and

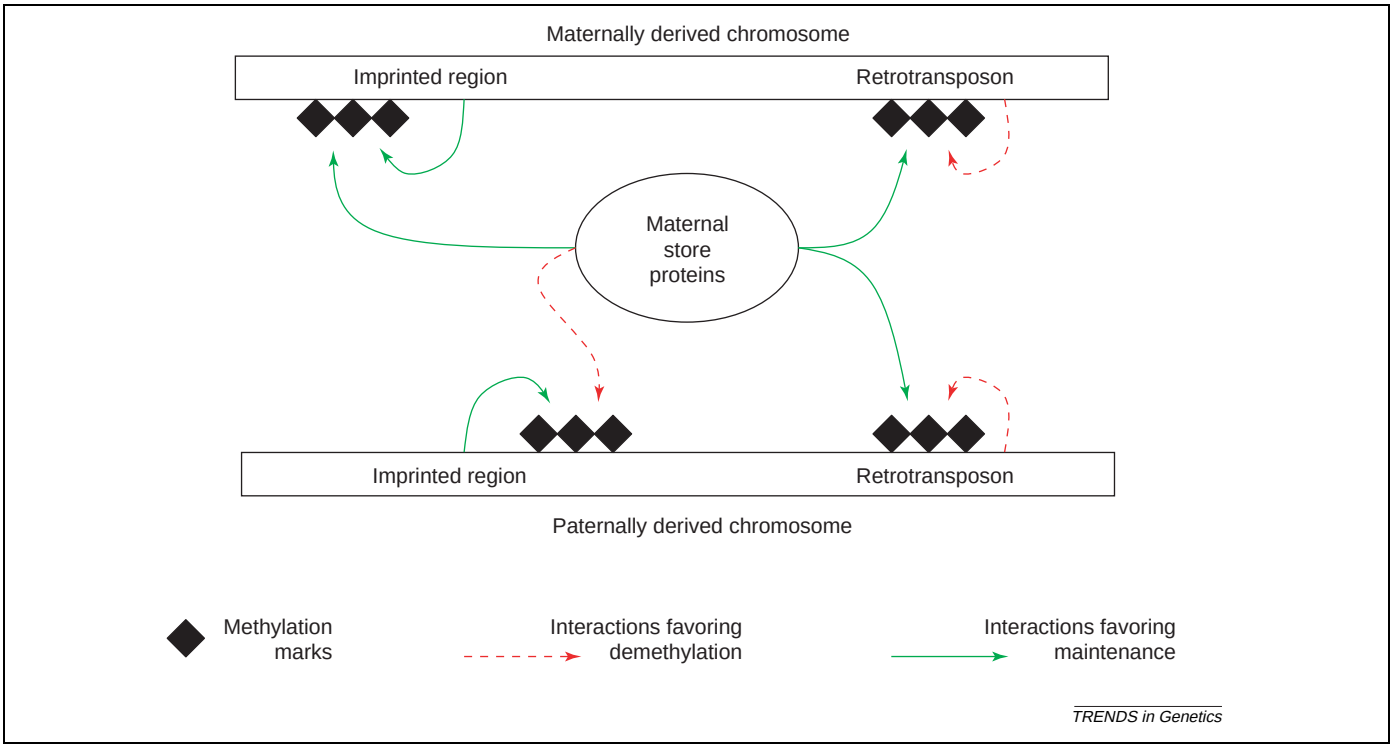

Figure 3. Genetic conflicts over methylation maintenance. Immediately following fertilization, the *cis*- and *trans*-acting elements involved in regulating the methylation patterns established in gametogenesis are subject to evolutionary conflicts. *Cis*-acting elements associated with imprinted genes will favor maintenance of their methylation marks, whereas those associated with retrotransposons will favor demethylation. The *trans*-acting, maternal-store proteins present in the preimplantation embryo will favor methylation maintenance at retrotransposons and maternally methylated imprinted genes, but will favor a loss of paternal methylation at imprinted loci.

Table 1. A list of some of the genetic factions that participate in, and can potentially influence, particular epigenetic processes

Process	Maternal genes	Paternal genes	Maternally derived genes	Paternally derived genes	Fetal (biallelic) genes	Transposons
De novo methylation in oogenesis	Yes		Yes			Yes
De novo methylation in spermatogenesis		Yes		Yes		Yes
Active demethylation after fertilization	Yes		Yes	Yes		Yes
Maintenance methylation (before zygote activation)	Yes		Yes	Yes		
Maintenance methylation (after zygote activation)			Yes	Yes	Yes	
Regulation of transcription			Yes	Yes	Yes	

trans-acting factors change. For instance, the *cis*- and *trans*-acting elements involved in the establishment of methylation during spermatogenesis represent the paternally derived and paternal genetic factions, respectively. The *cis*-acting elements involved in methylation maintenance following fertilization represent the maternally derived, paternally derived and retrotransposon factions. The *trans*-acting elements will represent the maternal genetic faction (maternal-store proteins) before transcriptional activation of the zygote, but will represent the fetal genetic faction following zygote activation. The genetic factions that participate in (and can therefore potentially affect) various epigenetic processes are indicated in Table 1.

Box 3. Outstanding questions

- What factors participate in the active demethylation of the paternal genome after fertilization?
- Which enzyme(s) in addition to Dnmt1o perform methylation maintenance in the preimplantation embryo?
- Why does Dnmt1o act specifically at the fourth cell division in mouse embryogenesis?
- What is the role of Dnmt2?
- How does the architecture of the epigenetic machinery vary across mammalian species, including marsupials and monotremes?
- How does the active demethylation process affect specific loci, including imprinted genes, unimprinted genes and transposable elements?
- What other regulatory factors contribute to the stage-, tissue- and locus-specificity of epigenetic modification?

Concluding remarks

The members of the mammalian DNA methyltransferase family are part of a complex system for establishing and propagating epigenetic signals across cell divisions and generations. Recent research suggests that in addition to its canonical role as the primary maintenance methyltransferase, Dnmt1 could possess *de novo* methyltransferase activity. Conversely, the canonical *de novo* methyltransferases, Dnmt3a and Dnmt3b, could be involved in methylation maintenance. Each of these noncanonical activities suggests possible mechanisms of epigenetic canalization. However, testing the hypothesis that these mechanisms are relevant to the robustness of epigenetic inheritance *in vivo* will have to await future empirical work.

In addition to the material presented here, there is already evidence for complications beyond the scope of this review. Both Dnmt3a and Dnmt3b produce multiple isoforms that have different developmentally regulated patterns of expression and subcellular localization (reviewed in Ref. [9]). Some of these forms lack some of the conserved catalytic motifs, suggesting that they could have altered activity [68], or function in a regulatory role, such as inhibition of other isoforms [39,69]. Many imprinted loci are associated with noncoding RNA transcripts, some of which are required for appropriate epigenetic regulation. This suggests that RNA-directed DNA modification could be yet another mechanism linking transcriptional activity, DNA methylation and chromatin structure. It remains to be seen what relationship, if any, exists between the mechanisms involved in RNA interference and the *cis*-acting regulatory effects of imprinted antisense RNAs.

Most of what we know about methylation dynamics in early embryogenesis comes from the mouse and, to a lesser extent, humans. It is becoming clear that many of the features of epigenetic inheritance are not going to be shared by all mammals. The bad news is that understanding mammalian epigenetics is going to require extensive work in non-model organisms. The good news is that this variation should provide a window onto a rich evolutionary history. Unraveling that history will require not only a deeper knowledge of the systems of epigenetic regulation but also a broader knowledge of how those systems vary across species (Box 3). Our epigenetics is closely linked to our mode of reproduction, which is a battleground for conflicting selective pressures; we should not be surprised if it is impossible to understand these systems as a set of adaptive responses to an external environment. Mammalian epigenetics presents many technical and intellectual challenges, but also an excellent opportunity to study the complex evolutionary dynamics at the interface of imperfectly allied genomes.

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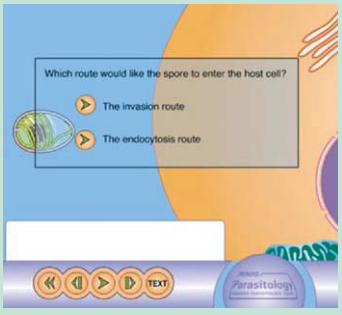

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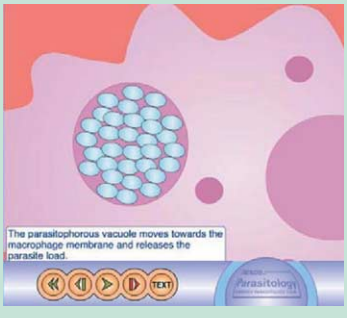

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