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# Epigenetic reprogramming of the genome - from the germ line to the embryo and back again

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**ABSTRACT Mammalian parental genomes are not functionally equivalent, and both a maternal and paternal contribution is required for normal development. The differences between the parental genomes are the result of genomic imprinting - a form of gene regulation that results in monoallelic expression of imprinted genes. *Cis*-regulatory elements at imprinted loci are responsible for directing allele-specific epigenetic marks required for correct gene expression. This *cis* information must be interpreted at various points in development, including in the germline where existing imprints are erased and reset. Imprints must also be maintained during preimplantation development, when the genome undergoes dramatic global epigenetic changes.**

KEY WORDS: *Imprinting, epigenetic, reprogramming, germline, preimplantation.*

## Introduction and evolutionary aspects

An apparently unique feature of development in mammals is that both parental genomes are essential for normal development. This is due to genomic imprinting, an unusual mode of gene regulation that is responsible for monoallelic expression of a subset of genes. These imprinted genes have also been implicated in human diseases such as Prader-Willi Syndrome (PWS), Angelman Syndrome (AS) and Beckwith-Wiedemann Syndrome (BWS). It is intriguing that mammals should forfeit the advantage of diploidy for a subset of genes, particularly given the deleterious consequences of either loss or overexpression of these genes resulting from expression from neither or both copies.

It is possible that genomic imprinting originated as part of a progressive trend towards internal development and viviparity during mammalian evolution, which apparently had a critical influence on some aspects of development, reproductive physiology and regulation of gene expression (John and Surani, 2000). An example of a major adaptation towards viviparity was the development of the trophectoderm lineage which in turn had a major impact on the regulation of early postimplantation development. Comparisons amongst the extant mammalian groups illustrate some features of the transition towards viviparity. For example, monotremes, the egg laying mammals, have oocytes that contain large amounts of yolk (telolecithal), the marsupial eggs are less yolky, and this is largely absent in eutherian mammals (Fig. 1). A consequence of telolecithal oocytes is that the early cleavage divisions in monotremes are meroblastic which is typical of yolky zygotes, as opposed to holoblastic cleavage divisions in marsupial and eutherian mammals. Development in monotremes is accompanied by the formation of the blastodisc. In marsupials, blastomeres flatten against the zona pellucida and subsequently a unilaminar blastocyst develops without the formation of a morula. The unilaminar blastocyst contains epiblast and trophectoderm cells. Early eutherian development is accompanied by the formation of a morula in almost all instances, and a multi-layered blastocyst with the inner cell mass which contains epiblast cells plus the outer trophectoderm cells. Placentae of various types develop in marsupials and eutherian mammals followed by fetal development *in utero* and live birth. Monotreme fetal development *in utero* is partly supported by endometrial secretions before they lay their eggs and subsequent development continues. The introduction of the trophectoderm lineage in early development has had a critical effect on early development where the extraembryonic tissues have a major impact on the organisation and differentiation of the pluripotent epiblast cells. Amongst other functions, it is thought that imprinted genes may be particularly important for the development of the placenta.

Currently over thirty imprinted genes have been identified in mice and humans and the list is growing rapidly (http://www.mgu.har.mrc.ac.uk). One group proposes the existence of 100 to 200 imprinted genes (Hayashizaki *et al.*, 1994). Given the potentially low estimate that the human genome contains upwards of 30,000 genes (reviewed in Aparicio, 2000) this may represent a significant proportion of the genome. Some of these genes encode

*Abbreviations used in this paper:* dpc, days post coitum; IC, Imprinting Centre; PcG, Polycomb Group.

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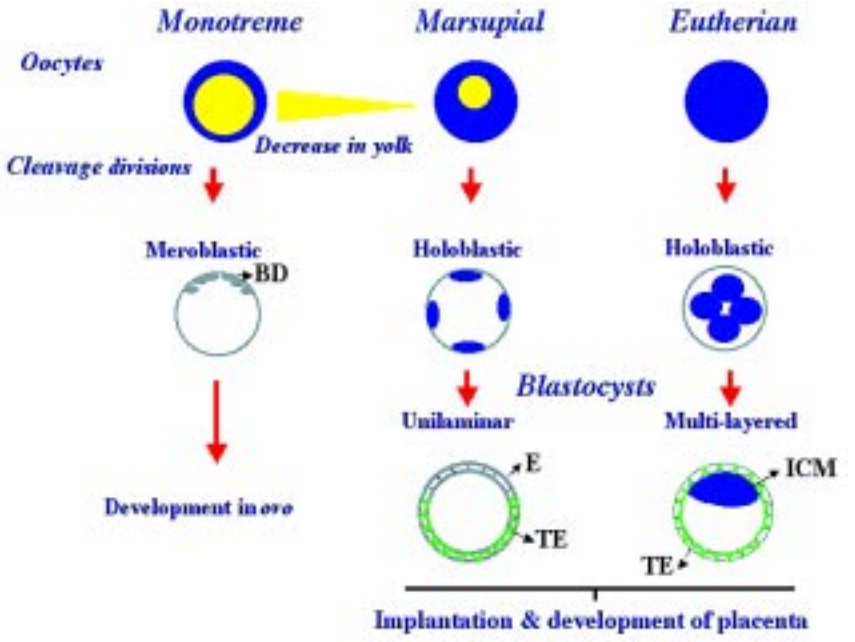


**Fig. 1. Mammalian oocytes and early development.** *Monotreme oocytes contain large amounts of yolk (telocithal), the marsupial eggs are less yolky, and yolk is largely absent in eutherian mammals. Development in monotremes is accompanied by the formation of the blastodisc (BD). In marsupials and eutherians, early development results in the formation of a unilaminar and a multi-layered blastocyst, respectively. These blastocysts contain epiblast (EP) and trophectoderm (TE) cells. Early eutherian development is accompanied by the formation of a morula in almost all instances, with the inner cell mass (ICM) which contains epiblast cells, and the outer trophectoderm (TE) cells. Placentae of various types develop in marsupials and eutherians, followed by fetal development in utero and live birth.*

products with roles in embryonic growth, for example the insulin-like growth factor-2 (*Igf2*), its receptor *Igf2r* and the cyclin-dependent kinase inhibitor *p57*$^{kip2}$. Others have more oblique functions, such as the epoxide hydrolase *Mest* and the ubiquitin ligase UBE3A. *Peg3* is a transcription factor which has an effect on growth and behaviour, while *Mash2* has a critical role in development of the trophectoderm.

Genomic imprinting requires reversible epigenetic modifications which occur in the germ line. This review will address key aspects of epigenetic regulation- the role of *cis*-acting elements in directing imprinting and how this *cis*-information is interpreted at various points in development. Much attention has been focused on the germ line, as it is only here that both alleles of imprinted genes first become indistinguishable, and the imprints are subsequently reset according to the sex of the animal. Following fertilisation these imprints must be maintained through the epigenetic changes that precede implantation, before finally establishing correct expression patterns at the appropriate point in development.

## *Cis*-acting elements: regulators of imprinted gene expression

To achieve monoallelic expression of imprinted genes requires the presence of *cis* control elements. Silencing of a parental allele involves epigenetic modifications at imprinted loci, such as chromatin modifications and DNA methylation. A great number of imprinted genes are clustered in chromosomal regions (http://www.mgu.har.mrc.ac.uk, Beechey and Cattanach, 1996), This genomic organisation raises the possibility that the imprinting of these genes is regulated by *cis*-acting control elements capable of directing gene expression across entire domains. Transgenic experiments in mice and the characterisation of mutations in human patients with diseases have led to the identification of a number of such *cis*-acting control elements at different imprinted loci. The organisation and function of these control centres appears to be quite disparate, suggesting that the regulation of gene expression in individual clusters of imprinted genes is subject to variation.

The imprinted locus on chromosome 15q11-13 in humans is syntenic to an imprinted region in the centre of mouse chromosome 7. It is associated with the human diseases Prader-Willi syndrome (PWS) and Angelman syndrome (AS) which have an imprinted inheritance pattern. Analysis of patients with familial PWS or AS revealed that relatively small deletions in a region upstream of the *SNRPN* gene were capable of disrupting gene expression over 2/3Mb of surrounding sequence (Buiting *et al.*, 1995). This indicated that the 5' *SNRPN* region functions as an imprinting centre (IC), regulating imprinted gene expression in *cis* throughout the 15q11-13 domain. The different deletions are transmitted silently through the germline of one sex and cause a disease phenotype only after inheritance from the opposite sex, suggesting that the mutations are blocking the resetting of the imprint in gametogenesis (Buiting *et al.*, 1995). The recent characterisation of a human patient with a deletion which overlaps the IC and results in a post-zygotic paternal to maternal epigenetic switch indicates that the IC is not only required for the establishment of the paternal imprint, but also its maintenance (Bielinska *et al.*, 2000).

There is also evidence that an IC is located on human chromosome 11p15.5, a region syntenic with the distal region of mouse chromosome 7, which contains at least 12 imprinted genes which are predominantly expressed from the maternal chromosome. This locus is associated with Beckwith-Wiedemann syndrome (BWS) in humans. Extensive studies at the *H19*/*Igf2* locus within this domain in the mouse have revealed that the imprinted expression of these two genes is indeed regulated by an IC, which harbours complex *cis*-acting elements.

*H19* and *Igf2* are reciprocally imprinted, with *H19* expressed only from the maternal chromosome and *Igf2* expressed only from the paternal chromosome. The *H19* gene is subject to a number of different epigenetic modifications of the active and silent alleles which may be involved in regulating imprinting, the most comprehensively studied of which is DNA methylation. Methylated CpG sites are thought to exert a repressive effect on gene activity by recruiting DNA binding proteins such as MeCP2 (Boyes and Bird, 1991; Meehan *et al.*, 1992) and histone deacetylases (Jones *et al.*, 1998; Nan *et al.*, 1997). *H19* is hypermethylated on the inactive paternal allele and hypomethylated on the maternal allele (Bartolomei *et al.*, 1993; Brandeis *et al.*, 1993; Ferguson-Smith *et al.*, 1993; Tremblay *et al.*, 1995). A definitive regulatory role for

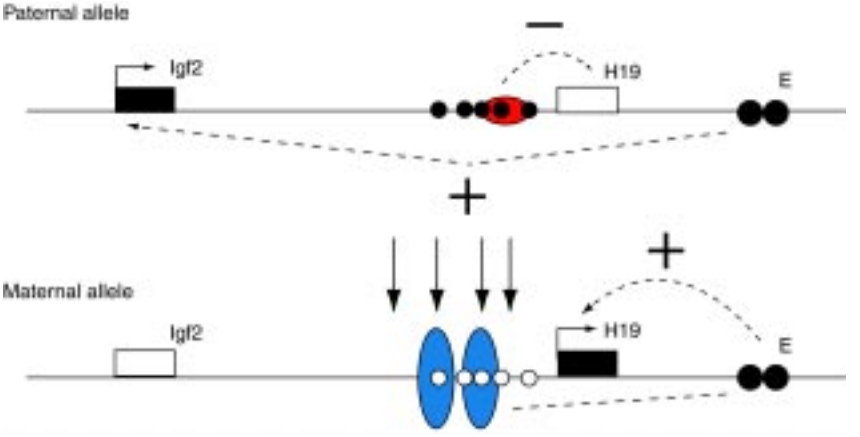


**Fig. 2. Model for the role of the *H19/Igf2* imprinting centre.** *The silencer element (red ellipse) functions on the paternal chromosome to prevent transcriptional activation of H19. Hypermethylation at the DMD (small black circles) and other epigenetic modifications preclude the formation of a functional insulator element, enabling downstream enhancers (E) to interact with the Igf2 gene. The hypomethylated-associated epigenetic state (small white circles) and accessible chromatin structure (vertical arrows) of the maternal chromosome allows the insulator to form (blue ellipses) and in the absence of a functional silencer directs the enhancers to the H19 gene. Deletion of the silencer element sequence from the maternal chromosome does not disrupt the insulator element function. The function of the additional hypersensitivity sites (vertical arrows) is not known, but they may be indicative of a further cis-element responsible for initiation of the epigenetic imprint for the locus.*

methylation in *H19* imprinting was demonstrated in mice homozygous for a deletion in the DNA methyltransferase (*Dnmt1*) gene, which showed a complete loss of methylation at the locus and consequent biallelic *H19* expression (Li *et al*., 1992).

In addition the silent paternal allele of *H19* is found in a compacted chromatin configuration (see Fig. 2), when compared to the nuclease accessibility of the maternal allele (Hark and Tilghman, 1998; Khosla *et al*., 1999). Studies on DNA replication timing at *H19* have also revealed that the two alleles replicate asynchronously, with the paternal chromosome replicating earlier than the maternal chromosome (Greally *et al*., 1998; Kitsberg *et al*., 1993). Recent experimental evidence has suggested that histones associated with the paternally inherited *H19* allele are less acetylated than those associated with the maternal allele. Inhibition of histone deacetylation and DNA methylation in cells growing in culture, but not either modification on its own, was capable of derepressing the silencing of the paternal allele, suggesting these two modifications in combination are essential for the maintenance of *H19* imprinting (Pedone *et al*., 1999).

Data from targeted deletion studies and transgenic experiments have indicated that an imprinting control element which is capable of regulating both *H19* and *Igf2* imprinted expression lies in the 4kb region immediately upstream of *H19*, shown in Fig. 2 (Elson and Bartolomei, 1997; Leighton *et al*., 1995; Ripoche *et al*., 1997).This region contains a 2kb differentially methylated domain (DMD) located between -2 and -4 kb from the start of transcription which is required for parental origin specific silencing in the mouse (Thorvaldsen *et al*., 1998; Tremblay *et al*., 1997) (see Fig. 2). A 1.2kb element from the DMD can function as a *cis*-acting silencer of transgenic reporter genes in *Drosophila* (Lyko *et al*., 1997) and mice (Brenton *et al*., 1999). Targeted deletion of this silencer element at the endogenous locus (see Fig. 2) results in reactivation of the paternal *H19* allele, but does not disrupt the DNA methylation or asynchronous replication pattern of the locus (Drewell *et al*., 2000). This suggests that the imprinted epigenotype of *H19* may be initiated by a different *cis*-acting element to that responsible for transcriptional silencing of the gene and that different epigenetic modifications, other than DNA methylation or asynchronous replication patterns, may be the primary targets for regulation by the *cis*-control elements at *H19*. Furthermore, this deletion does not affect *Igf2* expression, indicating that an insulator capable of protecting *Igf2* from transcriptional disruption is still intact at the IC after deletion of the silencer. There are nuclease hypersensitivity sites within the DMD on the maternal chromosome of unknown function (Hark and Tilghman, 1998; Khosla *et al*., 1999) (Fig. 2). The 1.2kb deletion leaves 1.1kb of the DMD intact (Fig. 2), including some hypersensitive sites which may constitute an insulator element. Indeed, recent studies have identified a number of CTCF protein binding sites which map close to some of these hypersensitivity sites, suggesting that at the endogenous locus these sequences are important for an insulator function (Bell and Felsenfeld, 2000; Hark *et al*., 2000; Szabo *et al*., 2000).

There is evidence suggesting that regulatory regions which contain transcriptional silencers are often complex and can display both functional redundancy as well as divergent functions within the control region (Frisch and Morisaki, 1990; Hagstrom *et al*., 1996; Perkins *et al*., 1995; Weissman and Singer, 1991; Zhou and Levine, 1999). A multi-functional role for the imprinting centre at *H19*, including a silencer, imprint initiator and insulator, is reminiscent of regulatory systems at non-imprinted loci in other organisms, such as the mating type locus silencing in yeast (Donze *et al*., 1999; Fourel *et al*., 1999) and *Hox* gene regulation in *Drosophila* (Hagstrom *et al*., 1997; Mihaly *et al*., 1997).

## Epigenetic modifications in the germ line

The key events of imprinting occur in the germ line. The initial event in primordial germ cells (PGCs) results in the erasure of all the epigenetic modifications inherited from the previous generation, followed by the re-initiation of the new imprints in male and female gametogenesis. The discovery of differential methylation patterns at imprinted loci, described above, suggested that this may function as the principle epigenetic mark (Brandeis *et al*., 1993; Li *et al*., 1993). Mutation of the putative maintenance methyltransferase *Dnmt1* results in genome-wide demethylation and loss of imprints in ES and somatic cells. Reintroduction of *Dnmt1* cDNA allows restoration of genome-wide methylation, but not of methylation patterns associated with imprinted loci. The appropriate 'imprints' were only established in these cells after transmission through the germ line. This indicates that events that only take place during gametogenesis are required for imprinting (Tucker *et al*., 1996), or that *Dnmt1* does not play a role in establishing imprints.

The recently discovered *de novo* methyltransferases *Dnmt3a* and *Dnmt3b* are highly expressed in undifferentiated ES cells. After

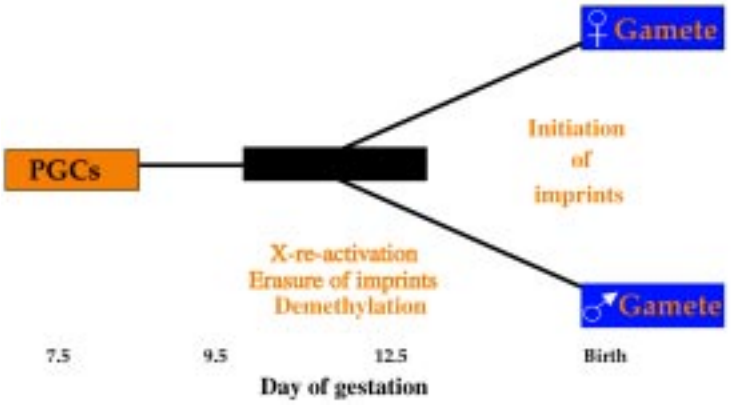


**Fig. 3. Epigenetic modifications in the germ line.** *During the proliferation of primordial germ cells (PGC) and their entry into the gonads, extensive epigenetic modifications occur. This includes erasure of the imprints, reactivation of the inactive X chromosome and demethylation of the genome. The new imprints are initiated during gametogenesis.*

differentiation, and in adult somatic tissues, only low levels are detected (Okano *et al*., 1998). Double mutants show a similar lethal phenotype at the same developmental stage as observed in Dnmt1 deficient animals (Okano *et al*., 1999). But although Dnmt3a and Dnmt3b are essential for *de novo* methylation in early embryos, the differential methylation pattern of *H19* was not affected in ES cells deficient in both Dnmt3a and 3b. Again this suggests that germ line passage is needed for the initiation of some essential epigenetic modifications.

Prior to the re-initiation of imprints during gametogenesis, there are significant global and specific epigenetic modifications in the germ line. The initial step occurs in PGCs at 11.5 dpc after the majority of PGCs have reached the genital ridge. These modifications include genome-wide demethylation (Monk *et al*., 1987), erasure of allele-specific methylation at imprinted genes (Chaillet *et al*., 1991; Szabo and Mann, 1995; Tada *et al*., 1998), and the reactivation of the inactive X chromosome in females (Fig. 3). The mechanisms responsible for these epigenetic modifications are currently unknown. There is a strong *trans*-modification activity present in the germ cell nucleus at 12.5 dpc, demonstrated by cell fusion experiments in which embryonic germ (EG) cells, derived directly from female 12.5 dpc PGCs, were fused to thymic lymphocytes (Tada *et al*., 1997). The somatic nucleus was extensively reprogrammed resulting in demethylation of paternally expressed imprinted genes, non-imprinted genes and minor satellite DNA. In addition the silent maternal allele of an imprinted gene (*Mest*) was reactivated.

In conjunction with methylation, there are other modifications that are believed to be essential for establishment and maintenance of epigenetic marks in early development and germ cells: modification of histone acetylation (Svensson *et al*., 1998), bulk chromatin conformation (Hark and Tilghman, 1998; Koide *et al*., 1994), sex-specific DNA binding proteins (Birger *et al*., 1999), and non-coding or antisense RNA molecules (Reik and Walter, 1998; Wutz *et al*., 1997) are some of the factors that have been suggested for the regulation of epigenetically controlled genes. These mechanisms may be used as an alternative to methylation, since changes occur to the epigenetic state of germ cells in a background of global hypomethylation.

The new imprints are initiated after this erasure step during gametogenesis. In the female germ line the maternal imprints are apparently introduced after the quiescent oocyte resumes growth while paternal imprints are apparently introduced in spermatogonia (Jue *et al*., 1995; Kono *et al*., 1996; Obata *et al*., 1998). While it is clear that *cis* regulatory elements are essential for the initiation of a new cycle of imprints, the mechanism of how precisely this occurs is unknown.

## A potential role for Polycomb proteins in germ cells and early embryos

An important question concerning the overall epigenetic regulation in the germ line (and preimplantation embryos) is how this is achieved at a time when the genome is apparently undermethylated. There are organisms such as *C. elegans* and *Drosophila* that are thought not to utilise DNA methylation for transcriptional regulation. One alternative mechanism for epigenetic regulation of gene expression involves the Polycomb (PcG) proteins, which are known to be implicated in all the organisms studied so far. They may also be crucial in mammals, particularly in germ cells and early embryos.

An important feature shared by all organisms is transcriptional repression in germ cells, thus preventing expression of genes that would cause entry into somatic lineages. Therefore becoming a germ cell is in part due to the lack of the cell becoming anything else. In *C. elegans,* PcG genes are amongst the key mediators of transcriptional repression in the germline (Seydoux and Strome, 1999). Mutations in these genes cause a ‘grandchildless’ (maternal-effect sterile) phenotype (Holdeman *et al*., 1998; Kelly and Fire, 1998; Korf *et al*., 1998). Mes-2 and Mes–6 represent the entire family of PcG proteins in *C. elegans* and are essential for normal proliferation and viability of the germline. The worm PcG proteins are important for gene silencing, since transgenes present in extrachromosomal arrays can be efficiently expressed in somatic cells but are silenced in the germ line. Loss of silencing of transgenes was observed in a *mes*-mutant background (Kelly and Fire, 1998). These PcG genes have homologues in *Drosophila* in the form of *Enhancer of zeste* (*E(z)*, a *mes-2* homologue), and *extra sex combs* (*esc*, homologous to *mes-6*). *E(z)* and *esc* are the two most highly conserved members of the family, with homologues in mammals and plants. They are thought to function as an evolutionarily conserved complex in embryonic development, acting earlier than other members of the PcG in *Drosophila* and mammals (Jones *et al*., 1998; Sewalt *et al*., 1998; Shao *et al*., 1999; vanLohuizen, 1998). A popular model of PcG action is that these proteins modify higher order chromatin structure, leading to a heritable repressed state.

Even more striking indications that PcG homologues connect germ line formation and epigenetic regulation come from the plant *Arabidopsis*. One *E(z)* homologue in *Arabidopsis* is called *Medea* (*Mea*). The presence of a wild-type *Mea* allele in the genome of the female gametophyte (egg and central cell which gives rise to the endosperm after fertilisation) is essential for the survival of the embryo, regardless of the presence of a wild type paternal allele (Grossniklaus *et al*., 1998). This suggests that maternal *Mea* products are essential before zygotic expression starts and are stored in the female gametophyte, or that the *Mea* locus is imprinted, with only the maternal copy expressed. In fact both are

true. Mea protein is provided maternally in the early embryo (Grossniklaus *et al.*, 1998) and *Mea* is expressed zygotically only from the maternal allele in the endosperm (Kinoshita *et al.*, 1999) and young seeds (Vielle-Calzada *et al.*, 1999). In the case of *Mea* there is another newly discovered connection to mammalian imprinting: the observed *Mea* mutant phenotype is sensitive to genome methylation (Vielle-Calzada *et al.*, 1999). In *ddm1* ('decreased DNA methylation') mutants, the genome is 70% less methylated than wild type plants. In *ddm1/Mea* double mutants a lower proportion of seeds aborted than in *Mea* mutants because the paternal *Mea* silencing breaks down.

The first evidence of a connection between other epigenetic mechanisms and PcG proteins has come from studies of *Drosophila* Mi-2, a member of the SWI2/SNF2 superfamily of ATPases. Homologous to vertebrate Mi-2, which binds preferentially to methylated DNA, dMi-2 is a component of a histone deacetylase complex. This complex binds to the transcriptional repressor hunchback and interacts genetically with Polycomb group proteins in flies, suggesting PcG repression works in conjunction with histone deacetylation (Kehle *et al.*, 1998). *In vitro* experiments on *Xenopus* extracts and mammalian cultured cells also show that Mi-2 connects DNA methylation, chromatin remodelling and histone deacetylation in vertebrates (Wade *et al.*, 1999). The mouse homologue of *esc*, *Eed*, not only interacts with *Ezh-2* (a mouse homologue of *E(z)*) but also with Histone deacetylase 2 (HDAC2) *in vitro*. It has therefore been suggested that *Eed*, *Ezh-2* and HDAC2 form one single complex (van der Vlag and Otte, 1999). Although PcG homologues have a key role in germ line formation and survival, nothing is yet known about the function of the PcG homologues in the mammalian germline. It is likely that PcG proteins may act as important epigenetic regulators in early development and germ line formation, but their precise roles in the mammalian germ line are yet to be elucidated.

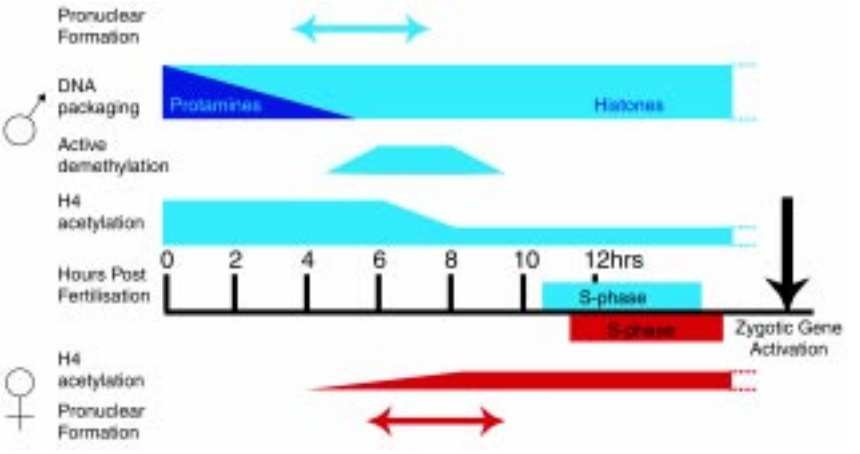


**Fig. 4. Early events in preimplantation mouse development.** *Some of the earliest events in development, prior to the onset of S-phase, are shown in this schematic diagram. Events specific to the male pronucleus are represented above the time-line in blue, with protamines in dark blue and histones in pale blue. Female-specific events are shown in red. Zygotic gene activation occurs in both the maternal and paternal pronucleus following S-phase in the zygote, with the paternal pronucleus demonstrating a greater transcriptional competency. Approximate timings for events have been taken from the following sources: Hogan et al., 1994 (Pronuclear formation); Nonchev and Tsanev, 1990 (Histone/protamine exchange); Adenot et al., 1997 (Histone H4 acetylation); Mayer et al., 2000a (Demethylation of the paternal genome); Ferreira and Carmo-Fonseca, 1997 (Timing of S-phase).*

## Epigenetic regulation in preimplantation embryos

There are some similarities between epigenetic modifications in the germ line and early embryos. In particular there is genome-wide demethylation in early embryos although the imprints are not erased at this time, unlike in the germ line. Recent studies have shown that a somatic nucleus transplanted into an oocyte can be re-programmed resulting in full term development (Wakayama *et al.*, 1998). This phenomenon shares some similarities with the reprogramming of a somatic nucleus exposed to the germ cell environment in the somatic-germ cell hybrids described above (Tada *et al.*, 1997).

Even before the onset of the first S-phase in the fertilised oocyte significant changes occur to the male and female pronuclei, the most dramatic of which happen to the male pronucleus. A schematic diagram illustrating some of the epigenetic events in early embryogenesis is shown in Fig. 4. The sperm genome is more highly methylated relative to the oocyte, which appears to be globally hypomethylated (Monk *et al.*, 1987). There is passive genome-wide demethylation throughout preimplantation development (Howlett and Reik, 1991; Kafri *et al.*, 1993), which is thought to be essential for ensuring totipotency in the embryo, followed by de novo methylation following implantation. It was recently shown by immunofluorescent staining for methylated cytosine residues that there is a rapid and apparently active demethylation of the paternal pronucleus, between 6 to 8 hours after fertilisation and prior to the first S-phase (Mayer *et al.*, 2000b), illustrated in Fig. 4. This occurs in addition to the passive process that affects both the maternal and paternal genomes. Some CpG sites at imprinted loci escape this global demethylation, including CpGs in the imprinting centre upstream of *H19* which are maintained in the methylated state (Olek and Walter, 1997; Tremblay *et al.*, 1995).

In addition to demethylation, the paternal pronucleus experiences changes in histone H4 acetylation immediately after fertilisation (Adenot *et al.*, 1997). Both the sperm genome and the chromosomes of the oocyte in meiotic arrest do not appear to be hyperacetylated as judged by immunostaining for acetylated lysine 5 on H4, the last residue of the histone to be acetylated. Following fertilisation, and prior to S-phase, the paternal genome gains an intense stain, while the female pronucleus remains unstained (Adenot *et al.*, 1997). By S/G2 phase the two pronuclei have equilibrated and show identical patterns of acetylation, (see Fig. 4), although the male pronucleus has greater transcriptional competency at this point (Aoki *et al.*, 1997; Ram and Schultz, 1993; Wiekowski *et al.*, 1993). It should be noted that these results relate only to H4 lysine 5, and may not reflect changes in acetylation at other residues. It has been suggested that this transient rise in acetylation is linked to the replacement of sperm protamines with histones from the oocyte pool, which would become acetylated for chromatin assembly.

Immunofluorescence studies have also shown that the maintenance methyltransferase Dnmt1 is actively excluded from the nuclei

of preimplantation embryos (Cardoso and Leonhardt, 1999), although a transient entry of the protein into the nucleus can be detected at the eight cell stage (Carlson *et al.*, 1992). This suggests a mechanism by which passive demethylation of the genome may occur prior to implantation. It is likely that the methylation changes in preimplantation development are much more complex, as Dnmt 3a and 3b have recently been implicated as the *de novo* methyltransferases in mouse development (Okano *et al.*, 1999). It will be interesting to compare the expression patterns and subcellular distribution of these three methyltransferases in early development, and to investigate the balance between methylation and demethylation at this time. Methyltransferases are well-documented, yet little is known about precisely how demethylation occurs or whether there are active demethylases involved in this process. Given the recently discovered mechanistic link between histone deacetylation and DNA methylation (Fuks *et al.*, 2000), it seems plausible that there might be a similar direct link between the proteins effecting DNA demethylation and histone acetylation. The concurrent active demethylation and rise in histone acetylation of the male pronucleus shortly after fertilisation may be indicative of such a mechanism.

Recent studies have added another dimension to the question of how male and female chromosomes are recognised and organised in the early embryo (Mayer *et al.*, 2000b). Male and female-derived genomes are found to be segregated in distinct territories in the early embryo, at least until the four cell stage. Further experiments involving differential labelling of male and female centromeric sequences in interspecific hybrid embryos also indicate that this nonrandom distribution of maternal and paternal chromatin is present in two and four cell embryos, but begins to break down at later stages. Recent data also demonstrates that prior to the onset of meiosis in the germline, the replication "clock" of imprinted alleles is reset (Simon *et al.*, 1999). Both alleles become synchronously replicating, with imprinted loci in male gametes replicating early in S-phase compared to the same loci in female gametes. These newly reset differences in replication timing are maintained in preimplantation development, coincident with a general preference for the male pronucleus to replicate earlier than the female in the one cell embryo (Adenot *et al.*, 1997; Ferreira and Carmo Fonseca, 1997). It is possible that the two genomes are still undergoing sex-specific modifications at this point, requiring them to be spatially separated. For example, the genomes may be 'indexed' into active and inactive regions in an independent fashion, perhaps dependent on replication events, which may be important for maintenance of imprinted or other gene expression patterns later in development.

Further work is required to delineate the exact order in which these epigenetic changes are occurring in the embryo and which factors are involved in mediating them. In addition, the mechanism(s) by which genomic imprints are protected within the male pronucleus during the early stages of embryogenesis are unknown, and whether these are similar to the factors responsible for protecting the female genome at this time. Since imprints are erased in primordial germ cells, this suggests that such protection mechanisms must not be functional in these cells, or can be modified.

## Conclusions

Mammalian development exhibits some unique features that may have arisen during the course of evolution and progression towards viviparity. A significant aspect is the development of the trophectoderm lineage in mammals that has resulted in changes in events involving early development and patterning of the pluripotent epiblast cells. Genomic imprinting and the functional differences between the parental genomes during development is another unusual aspect that results in the monoallelic expression of imprinted genes. These imprinted genes serve a variety of disparate functions and the implications of why this should be so is puzzling.

Genomic imprinting is a reversible epigenetic phenomenon and the mechanism of this process is being unravelled gradually. Undoubtedly, *cis* control elements play a crucial role in conferring this property on imprinted genes through the employment of specific heritable epigenetic modifications. Many of the critical steps associated with imprinting occur in the germ line where much of the focus of research needs to be directed. These specific epigenetic modifications need to be examined in the context of the origin of the overall properties of the germ cell lineage itself, including how the germ cells are established in the absence of germ line determinants prevalent amongst other model organisms. Transcriptional regulation when the genome is relatively undermethylated in germ cells and early embryos in mammals also requires consideration. This important role may be served by an alternative system involving PcG proteins.

Finally, understanding of the mechanism of genomic imprinting and in particular how the epigenetic modifications are induced and erased could add a significant insight to the general understanding of epigenetic mechanisms in a broader context. Amongst these are the phenomena which show that the somatic and pluripotent epigenetic states are potentially reversible although there is little understanding of the underlying mechanisms. The advent of human pluripotent ES and EG cells has raised the prospects for their use in cell therapy in humans by induction of differentiation into specific cell types such as neurons, pancreatic and liver cells. Understanding of the epigenetic mechanisms that underlie such different phenotypic manifestations is crucial for the efficient manipulations of these cells, as well as for understanding what role these mechanisms play during normal mammalian development.

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