

Review

Genomic imprinting: A general overview

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Usually, most of the genes are biallelically expressed but imprinted gene exhibit monoallelic expression based on their parental origin. Genomic imprinting exhibit differences in control between flowering plants and mammals, for instance, imprinted gene are specifically activated by demethylation, rather than targeted for silencing in plants and imprinted gene expression in plant which occur in endosperm. It also displays sexual dimorphism like differential timing in imprint establishment and RNA based silencing mechanism in paternally repressed imprinted gene. Within imprinted regions, the unusual occurrence and distribution of various types of repetitive elements may act as genomic imprinting signatures. Imprinting regulation probably at many loci involves insulator protein dependent and higher-order chromatin interaction, and/or non-coding RNAs mediated mechanisms. However, placenta-specific imprinting involves repressive histone modifications and non-coding RNAs. The higher-order chromatin interaction involves differentially methylated domains (DMDs) exhibiting sex-specific methylation that act as scaffold for imprinting, regulate allelic-specific imprinted gene expression. The paternally methylated differentially methylated regions (DMRs) contain less CpGs than the maternally methylated DMRs. The non-coding RNAs mediated mechanisms include C/D RNA and microRNA, which are involved in RNA-guided post-transcriptional RNA modifications and RNA-mediated gene silencing, respectively. The maintenance and reprogramming of imprinting are not significantly affected by reduced expression of *Dicer1* and the evolution of imprinting might be related to acquisition of DNMT3L (*de novo* methyltransferase 3L) by a common ancestor of eutherians and marsupials. The common feature among diverse imprinting control elements and evolutionary significance of imprinting need to be identified.

Key words: Genomic imprinting, differentially methylated regions (DMRs), non-coding RNA, imprinting evolution.

INTRODUCTION

Genomic imprinting is a germline-specific epigenetic modification of the genome that results in parent-of-origin-specific expression of a small subset of genes in offspring. The concept of genomic imprinting introduced by Metz (1938) and Crouse (1960), who coined the term in the context of the unique inheritance of sex chromosomes in the dipteran insect, *Sciara coprophila*. Zygote consisting of two maternal genomes is called gynogenones or parthenogenones and zygote, which contained

two paternal genomes is called androgenones. Neither of these two types of reconstituted zygote could develop to term but the former had better embryos, and the later, better development of placental tissues, which suggested that the parental genomes are functionally non-equivalent despite the fact that they have equivalent genetic information. This observation led to discovery of genomic imprinting, which indicate functional difference that is dictated by the parental origin of the genome (McGrath and

Solter, 1984; Surani et al., 1984). The first imprinted gene to be identified was the insulin-like growth factor 2 (*Igf2*), which is expressed exclusively from chromosome of paternal origin (Dechiara et al., 1991). Imprinted genes are involved in many versatile functions including development, growth, complex regulation of the physiology, metabolism of both embryonic and adult stages, and behavior of adult stage. The current list of imprinted genes and information about them can be obtained at <http://www.mgu.har.mrc.ac.uk/research/imprinting/> (Beechey et al., 2005). Imprinting operating at various level like genes and segments of chromosomes in mammals, whole chromosome (inactivation of paternal X-chromosome in extra embryonic tissue of mice), are the unique example of the whole haploid genome being subjected to genomic imprinting by inactivating the paternal set of chromosomes in male mealybugs (Schrader and Hughes-Schrader, 1931; Brown and Chandra, 1977). This phenomenon has been observed in flowering plant, in eutherian mammals (such as mice and humans) (Tilghman, 1999; Ferguson-Smith and Surani, 2001; Reik and Walter, 2001a) and marsupials (such as opossums and kangaroos) (O'Neill et al., 2000; Suzuki et al., 2005), but not in monotremes (egg-laying mammals such as platypus and echidnas) (Killian et al., 2000) or birds (such as chicken) (O'Neill et al., 2000; Nolan et al., 2001; Yokomine et al., 2001, 2005).

Fate of imprints during mammalian life cycle

Expression of imprinted genes requires an epigenetic marking process that allows the transcription machinery to distinguish between expressed and repressed parental alleles of imprinted genes. Parent-of-origin specific epigenetic markers are combined at fertilization in the zygote. Just after fertilization, the paternal pronucleus undergoes active DNA demethylation. It must be an active demethylation process, because it occurs before DNA replication. However, maternal pronucleus undergoes demethylation over the first several divisions, suggesting a passive demethylation process (Rougier et al., 1998). Parent specific methylation patterns in imprinted genes are retained even in the face of genome-wide demethylation by an unknown mechanism (Reik and Walter, 2001b). These parental imprints are propagated in somatic tissue during embryogenesis. Some parental imprints in the placenta are maintained by histone modifications independent of DNA methylation. In case of primordial germ cells (PGCs), PGCs migration into the genital ridge occurs at 10.5-11.5 days post-coitus (dpc). Whereas the erasure of imprint occurs identically in PGCs of developing male and female embryo between 11.5 and 12.5 dpc (Hajkova et al., 2002; Lee et al., 2002), parental imprints are re-established later in gametogenesis in a strictly sex dependent manner. In males, this process occurs between 14.5 and 18.5 dpc, while in females it occurs in growing oocytes post-nally (Ferguson-Smith and Surani, 2001; Reik and Walter,

2001b; Surani, 2001) (Figure 1). In germ cells, it is not clear whether both imprints are first erased and then the sex-appropriate imprint is established or whether the sex-appropriate imprint is retained and only the sex-inappropriate erased, though the first possibility is more likely. In PGCs, the timing of this erasure and deposition of new marks is distinct for each sex and for each gene. In male demethylation of maternal and paternal allele of *H19* is complete around 13.5 dpc, but the paternal allele is remethylated first. However, by the time the male enters meiosis, both alleles are equally methylated (Davis et al., 1999; Ueda et al., 2000). This phenomenon of asynchronous methylation of maternal and paternal alleles has also been demonstrated for the maternally methylated *Snrpn* gene in oocyte, with the maternal allele methylated first (Lucifero et al., 2004).

Expression profiles of Megs and Pegs in mammalian life cycle

Before erasure of genomic imprinting memories, both somatic cells and PGCs exhibit monoallelic expression profiles of paternally and maternally imprinted regions, which are represented as 1:1 and 1:1. After erasure (non-marked state), expression profiles change to 0:2 and 2:0; because in paternally imprinted region, paternally expressed genes (pegs) become silent and maternally expressed genes (Megs) become biallelically expressed, but in maternally imprinted region, Pegs and Megs become biallelically expressed and silent, respectively. Paternal imprinting only affects the expression profiles of paternally imprinted region producing a 2:0 and 2:0 pattern and maternal imprinting only affects the expression profiles of maternally imprinted region leading to a 0:2 and 0:2 pattern (Obata et al., 1998; Obata and Kono, 2002). It is because paternal imprint is necessary for induction of the paternally imprinted Pegs, simultaneously repressing paternally imprinted Megs, and that the maternal imprint is necessary to induce maternally imprinted Megs with simultaneous repression of maternally imprinted Pegs. The monoallelic expression profiles of Pegs and Megs are reproduced in individuals in the next generation by combining each of the parental imprinted alleles (Figure 2). The resulting reciprocal (complementary) monoallelic expression is necessary for normal mammalian development (Kaneko-Ishino et al., 2003).

DIFFERENTIAL CHARACTERISTICS OF GENOMIC IMPRINTING

Genomic imprinting not only exhibit inter and intra species difference but also between embryonic and extraembryonic tissues of an individual.

Differential characteristics between plants and animals

One obvious explanation for the divergent aspects of im-

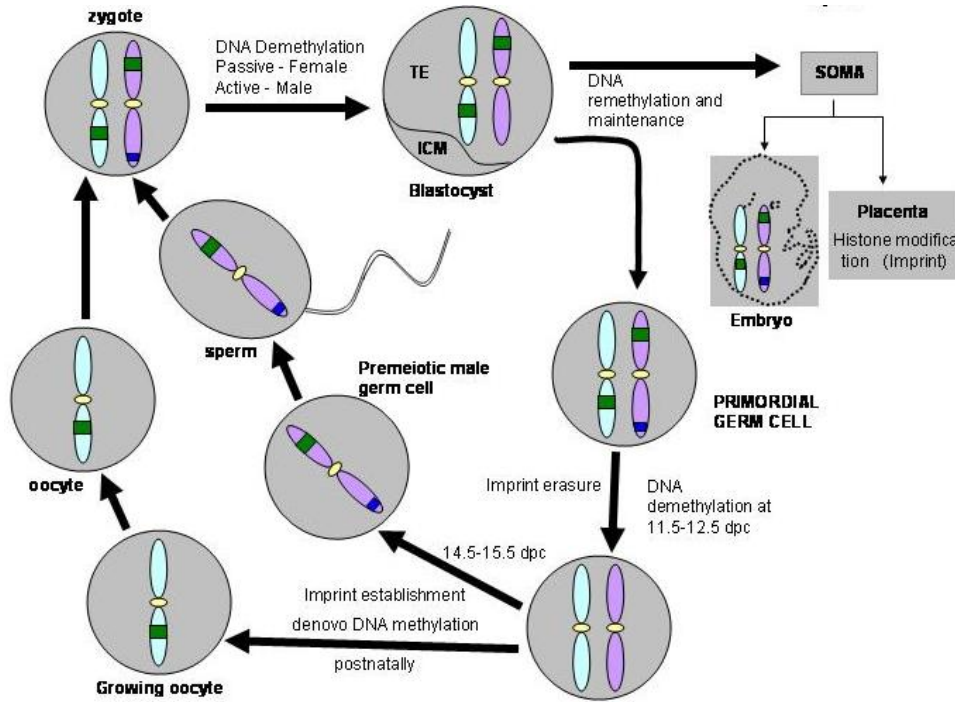


Figure 1. Ontogeny of imprint in germ cell. Gametic methylation imprint represented as green bar as well as other methylated DNA that is not retained in the early embryo as blue bar.

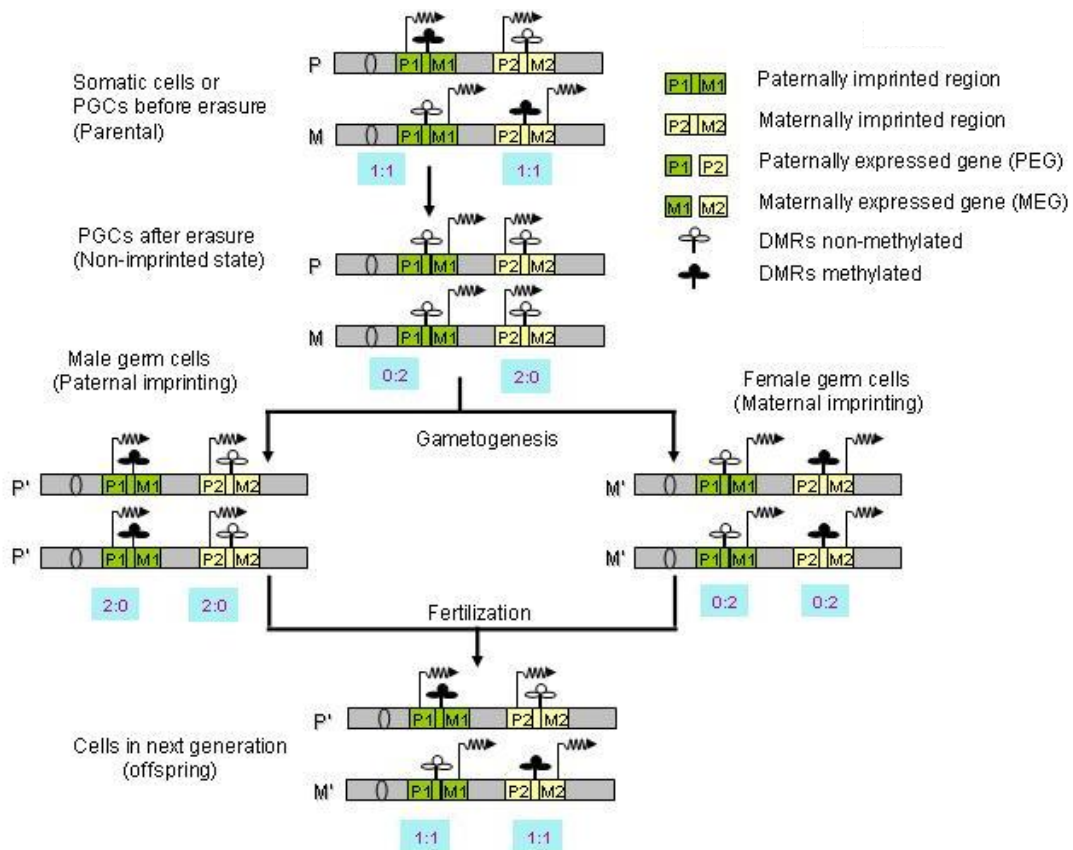


Figure 2. Pegs and Megs expression profiles during mammalian life cycle. M: Maternal chromosome, M': female germ cell chromosome, P: paternal chromosome, P': male germ cell chromosome.

printing is the difference in life history of the mammals and flowering plants (Walbot and Evans, 2003).

1. Both plants and mammals silence imprinted genes using DNA methylation and associated chromatin remodeling, but in mammals imprinted alleles are targeted for silencing, while in plants the selective activation of imprinted genes is achieved by specifically removing pre-existing methylation from the allele destined to be active (Gerhing et al., 2004; Scott and Spielman, 2004).
2. In plants imprinted gene expression is largely (or) wholly restricted to the endosperm, functional homologue of the mammalian placenta (Gehring et al., 2004), while in mammals, imprinted loci show allele specific expression across many regions of the placenta and embryo (Hu et al., 1998).
3. The need for erasing and resetting imprint mark is by passed because the cells whose descendants will eventually form gamete are participating in development throughout the life of the plant, however in mammals the germline is set aside early in embryogenesis.
4. The lack of global demethylation and resetting of imprint in plants requires maintenance but not *de novo* methyltransferase activity.

Sexual dimorphism of imprinting

Although total number of paternally expressed imprinted genes is similar to that of maternally expressed genes, the following are striking differences between maternal and paternal imprinting.

1. *De novo* methylation occur fairly late in the maturation process in growing oocyte, from midsize to metaphase II oocytes, just prior to ovulation (Lucifero et al., 2004). Methylation imprints are therefore of short duration and occur after meiotic recombination in female germline. In male, germ cell methylation mark established prior to meiosis and exists for the entire reproductive life span of the individual.
2. Most paternally repressed imprinted genes are indeed not associated with DMRs but are controlled in cis by paternally expressed nontranslated RNAs because very long term existence of germline methylation mark and large number of cell division as many as 100 times occurring in male germ cells after methylation led to high rate DMRs erosion via accumulation of C→T mutation (Crow, 2000), and the evolution of methylation independent, RNA or transcription based silencing mechanism. Whereas transient existence of germline methylation in female lead to low rate of loss of CpG sites by mutation, hence retention of methylation imprinting at DMR.
3. *Dnmt3L* is required for imprint establishment but not for transposon methylation in female germ cells, but *Dnmt3L* is required for transposon methylation and has only a minor role in *de novo* methylation at imprinted loci in male germcells.

4. Many CpG island-associated promoters are subject to maternal methylation but no known promoters are subject to paternal-specific germline methylation.

Imprinting in placenta

The genes that are specifically imprinted in trophoblast are expressed on the maternal and repressed on the paternal allele revealed by the expression studies in mice and humans. In embryos, reading of the imprinting marks relies mainly on DNA methylation (Li et al., 1993; Reik and Walter, 2001b), however histone modification seem to regulate imprinting in the placental tissues (Lewis et al., 2004; Umlauf et al., 2004). DNA methylation is probably the more stable epigenetic mark in the embryo, which may be appropriate in this case for the imprint to survive in adulthood, while histone modifications may serve purpose in the short-lived placenta that is not required after birth. Imprinting maintenance in the absence of DNA methylation was first reported for *Ascl2* on distal chromosome 7, its placenta-specific imprinting persists in *Dnmt1*-deficient embryos (Caspary et al., 1998; Tanaka et al., 1999). Silencing of *Kcnq1* paternal allele in placenta involve trimethylation of lysine 27 (K27) and dimethylation of K9 on histone H3 and histone H3 are deacetylated and show complete absence of K4 methylation.

REPETITIVE ELEMENT ROLE IN IMPRINTING

Several years ago, Mary Lyon put forward an attractive model that suggests repeated elements may function as genetic waystations that support the spreading of epigenetic inactivation mechanisms along the X-chromosome (Lyon, 1998).

Retrotransposable element

The human and mouse X chromosome possess a high content of long interspersed nuclear elements (LINE1) and Alu elements which is a member of (SINE) short interspersed nuclear elements are less frequent on the human X chromosome as compared to autosomes (Bailey et al., 2000; Waterston et al., 2002; Ross et al., 2005). It is well documented that G+C poor isochores are relatively poor in SINE and enriched in LINE elements and vice versa (Zhang et al., 2004), but it was found that the LINE and SINE content was not correlated to the relative G+C content of the analyzed regions. The overall content and also the relative distribution of retrotransposable elements is characteristic features of imprinting clusters and X chromosome. It is based on observations that the SINE depletion in imprinted regions is most evident in intergenic regions (Ke et al., 2002; Allen et al., 2003; Luedi et al., 2005) and pronounced depletion of SINEs is observed near imprinting control centers in the Beckwith-wiedemann syndrome (BWS) region, that is, bet-

ween genes that exhibit consistent tissue independent imprinting (Greally, 2002).

Subfamilies of primate Alu elements were shown to be hypomethylated in sperm as compared to oocytes and somatic tissues (Hellmann-Blumberg et al., 1993; Rubin et al., 1994). In contrast, LINE1 elements are hypermethylated in sperm and somatic tissues (Howlett and Reik, 1991; Lane et al., 2003). LTRs are not significantly over- or under-represented in imprinted regions hence it is less likely that they are important elements for the germline specific marking of imprinted regions. Since the imprinted genes and retrotransposed elements became *de novo* methylated during similar time in female and male germ cells respectively, they might involve similar mechanistic machineries for both types of DNA elements. Since Dnmt3a and Dnmt3l act in the male as well as in the female germlines, germline-specific protein might bind specifically to DMRs and/or repetitive elements, thereby either inducing or inhibiting epigenetic modifications. In a nutshell, retrotransposable element might create an environment for allele specific marking in the germline by recruiting epigenetic machineries to specific genomic locations.

Tandem repeats

It has been hypothesized that tandemly repeated DNA elements in or close to DMRs are involved in regulation of imprinting (Neumann et al., 1995). Tandem repeat arrays in imprinted regions in animals can be divided into two large subclasses long repeat motifs that encode small RNAs (snoRNA and miRNA) and direct tandem repeats of variegating length.

Long repeat motifs

The long repeat motifs of more than 70 bp length have sequence encoding snoRNA and miRNA. These repeat motifs are separated by unique sequence or retrotransposed elements so they may cover more than 100 kb long genomic segments. The major examples of such repeats are the snoRNA clusters in the Prader-Willi/Angelman syndrome region, and miRNAs and snoRNA in the *Dlk1/Gtl2* region.

Direct tandem repeats

The motifs range between 5 and 400 bp in length are arranged in direct head to tail order. At least 23 imprinted genes contain direct tandem repeats in or close to DMRs. Almost all the most prominent DMRs, the so called imprinting centers (ICs) that regulate mono-allelic expression of numerous neighboring genes, possess direct tandem repeats.

In contrast to the positional conservation individual sequence motifs and the surrounding DMR sequence are poorly conserved, prominent examples are the IG-DMR upstream of *Gtl2* and the imprinting centers IC1 and IC2

in the BWS region (Paulsen et al., 2001, 2005). The IC1 upstream of *H19* regulates reciprocal imprinting of the maternally expressed *H19* gene and the neighboring paternally expressed *Igf2* gene. The function of the IC1 depends on a high concentration of bound CTCF protein that can be achieved by the array of repeated binding motifs. The repeated nature of direct tandem repeat motifs may be required directly or indirectly to control the establishment and function of an allele-specific chromatin structure.

Tandem repeat motifs are among the hallmarks of imprinted genes. It is postulated that both, the local effects caused by direct tandem repeats and the large genomic effects promoted by the retrotransposon signatures may be needed to create a suitable environment for the establishment and maintenance of imprinting. In addition to these, rather general features of imprinted genes, other locus and gene specific structures like non-coding RNAs are very likely to also have a strong influence. It seems very likely that the mix of such general and locus-specific signatures promotes imprinting of genes.

Paoloni-Giacobino et al. (2006) hypothesized a model in which the primary role of tandem repeats of primary DMDs is most likely to act as highly effective epigenetic maintenance signal (to maintain the gametic methylation pattern indefinitely following fertilization). According to this model, during the fourth embryonic S phase (8 cell embryo) hemimethylated tandem repeats of the DMD attract Dnmt1o maintenance methyl transferase, this lead to its catalytic activity and inheritance of DMD sequence methylation. Dnmt1o could be attracted to the hemimethylated DMD due to the arrangement of CpG dinucleotides, the imperfect repetitiveness, and a specific chromatin structure induced by the hemimethylated DMD.

EPIGENETIC MODIFICATIONS

In this section, we discussed epigenetic modification, which will help in better understanding of genomic imprinting regulation. It is defined as heritable and reversible instructions superimposed over the DNA (Jenuwein and Allis, 2001). Epigenetic mechanisms can operate either at the level of DNA or at the level of chromatin. DNA in the form of chromatin has greatly enhanced range of possible epigenetic modification.

DNA methylation

A key attribute of genomic imprinting is the inheritance of expression potential from the gamete through all of prenatal development, and cytosine methylation is the only mark known to be transmitted by mitotic inheritance; there is no evidence of a mechanism that would mediate the inheritance of states of histone modification (Goll and Bestor, 2002). Palindromic or symmetric cytosine that lie directly 5' of guanine residue (CPG's) are the predominant target of DNA methylation in mammals and plants.

Cytosine within CNG and CNN are also commonly methylated in plants and filamentous fungi (Selker and Stevens, 1985). The methylation reaction is catalysed by DNMTs using S-adenosyl methionine as the methyl donor. Methylation associated gene silencing is achieved either by blocking access of transcription factors to DNA, or through the recruitment of methyl-CpG binding domain (MBD) protein, which form complexes with histone deacetylases, histone methyltransferase or chromatin remodeling proteins to generate transcriptionally refractory chromatin (Li, 2002). DNA methylation is not exclusively associated with silencing, for example the paternal allele of insulin-like growth factor type 2 (*Igf2*) is expressed by virtue of methylation within an adjacent imprinting control region which prevent binding of the enhancer-blocking zinc-finger protein CCCTC binding factor (CTCF) (Bell and Felsenfeld, 2000; Hark et al., 2000). Genomic imprint should be inherited after they are established in the gamete. Consistent with this, the methylation patterns at several DMDs have been shown to be perpetuated in pre implantation embryos and later during fetal development.

Factors regulating methylation of imprinted gene

Cis acting DNA signal for methylation

The cis-acting signals for DNA methylation have been identified at four imprinted loci discussed below.

***Igf2r*:** *Igf2r*, a maternally expressed gene located on mouse distal chromosome 17 (Barlow et al., 1991) contain a DMD within intron 2 (region 2) that acquires maternal allele methylation. A 6-bp sequence required to protect the paternal allele from being methylated, at the 5' end of 113-bp fragment of region 2 identified by mutation is a region referred to as the allele discriminating signal (ADS). Similarly, mutations identified an 8-bp sequence at 3' end of 113-bp fragment referred to as the *de novo* methylation signal (DNS), sufficient for acquiring DNA methylation in either parental pronucleus. ADS and DNS function in methylation establishment but not for methylation maintenance.

***H19*:** The *H19* DMD contains sequence needed for methylation maintenance but not for methylation establishment. The CTCF binding sites within the *H19* DMD are needed to prevent maternal allele methylation.

***Snrpn*:** It is paternally expressed imprinted gene on chromosome 7. Two DNS signal and one ADS signal identified using a human transgene in mouse may function to establish methylation (Kantor et al., 2004). The 10 and 7-bp MPI sequences required for maintenance of paternal imprint is termed maintenance of paternal imprint (MPI).

***Rasgrf1*:** The DMD and repeats together constitute a binary switch that regulates imprinting at *Rasgrf1*. The repeated sequence element is required for establishment of DMD methylation in the male germline at the endogenous

locus (Yoon et al., 2002). Repeats are also required for maintenance of methylation at *Rasgrf* in the pre-implantation embryo but are dispensable for maintenance of methylation after embryonic day 5.

The protein factors, other than DNMTs, which collaborate with the DNMTs to direct them to target DNA sequence that acquire methylation are discussed below.

Polycomb group proteins

EED is a member of polycomb group, that has been shown to be part of multimeric protein complex that has histone methyl transferase (HMT) and histone deacetylase (HDAC) activity (van der Vlag and Otte, 1999; Czermin et al., 2001). Different mechanisms for imprinted methylation and expression operate at different loci because *Eed* mutant affect only a subset of imprinting.

Chromatin modifying factor

The trans acting factors important for histone modifications, especially histone H3 lysine 9 methylation (H3mK9) and HP1, which binds H3mK9, are critical for normal DNA methylation in *Neurospora* (Tamaru and Selker, 2001), *Arabidopsis* (Jackson et al., 2002) and mice (Lehnertz et al., 2003). Though the connection between several forms of DNA methylation and histone methylation is clear, it is ambiguous how imprinted DNA methylation is affected.

Chromatin remodeling factor

DDM1 (decrease in DNA methylation 1) encodes a member of SWI/SNF2 family, which reposition nucleosome in an ATP dependent manner affect DNA methylation (Jeddeloh et al., 1999). It is also required to maintain histone H3 methylation pattern (Gendrel et al., 2002), providing evidence for mutual interactions among histone modifications, DNA methylation and chromatin remodeling in human mutation in *ATRX*, which encode an SWI/SNF-like protein exhibit decreased DNA methylation at repetitive DNA (Gibbons et al., 2000).

Regulation of CTCF

The switching from CCCTC binding factor (CTCF) to BORIS (testis specific paralog of CTCF) expression and down regulation of CTCF (present in male and female germline) may be critical for allele specific methylation of *H19*. Another explanation for how CTCF may regulate allele-specific methylation is poly ADP-ribosylation of CTCF by PARP1 seems to regulate CTCF in its role as an insulator. Reale et al. (2005) showed that PARP1 can form a complex with *Dnmt1* *in vivo* and possibly protect the unmethylated states of CpG islands.

Putative ADS/DNS binding protein

A specific protein present only in androgenetic ES cells bind to ADS of *Igf2*. Similarly, specific DNS binding factor

is present in both androgenetic and gynogenetic ES cells extracts. These specific proteins involved in the binding to the ADS and DNS is yet to be shown to play a role in regulating methylation.

Histone modifications

The n-termini of histones, the histone-tails, protrude out of the nucleosome and the amino acid residues at the tails are sites for various post-translation modifications, like phosphorylation, sumoylation, ubiquitination, methylation and acetylation (Li, 2002). The importance of chromatin remodelling and covalent modification of histones resulting in unique 'histone code' in maintaining the development fate of cellular lineages is being increasingly recognized. The histone code hypothesis proposes that transcriptionally active and silent regions have characteristic patterns of histone-tail modifications (Jenuwein and Allis, 2001). Transcriptionally active genes are characterized by methylation of histone H3 lysine 4 and acetylation of H3 and H4. Common hallmarks of silent genes are H3K9me, H3K27me and H4K20me (Jenuwein and Allis, 2001; Sarma and Reinberg, 2005). The complexity is further increased by mono, di and tri-methylation of lysine residues. MacroH2A, a variant of H2A, is associated with transcriptionally inactive X chromosome (Heard, 2004; Sarma and Reinberg, 2005).

The nuclear periphery is thought to contain chromosomal domain that are less transcriptionally active than domains located in the nuclear center (Kosak and Groudine, 2004).

Interactions of epigenetic modifications

Lewis and Reik (2006) predicted a model for how maternal germline DNA methylation can lead to a paternal postzygotic methylation mark. The IC2 and *Igf2r/Air* clusters contain a maternally methylated imprinting centre (IC), non-coding RNAs and postzygotic paternally methylated DMRs. The germline DMR leads to allele-specific expression of a non-coding RNA. This RNA may be responsible for recruiting polycomb protein and H3 lysine 27 methylation at pre-implantation stages. After implantation, the lysine 27 methylation recruits lysine 9 methylation. The histone methylation then target DNA methylation of a postzygotic DMR (Figure 3). In the *Igf2/H19* locus, the maternal germline methylation is necessary to create other paternal, postzygotic DMRs. On the maternal chromosome, CTCF binds to the unmethylated IC at an unknown time in early development. This leads to the establishment of allele-specific higher-order chromatin structures which then protect the IC and other DMRs from *de novo* DNA methylation. The epigenetic stages involved in the establishment of the postzygotic DMRs are unknown but a possible pathway involves histone methylation as proposed by Lewis and Reik (2006)

(Figure 3). Heterochromatin spread occurs due to recruitment of the Suvar methylases by Hp1, methylation of adjacent nucleosome followed by Hp1 binding and so on (Hall et al., 2002).

REGULATORY MECHANISM

Most of the imprinted gene clusters are under control of discrete DNA element called imprinting centres (ICs). The molecular and cellular mechanism by which ICs control other gene and regulatory regions in the cluster involves insulation of gene and non-coding RNAs including microRNAs (miRNAs) (O'Neill, 2005). The miRNAs may repress translation, induce degradation of mRNA, or be involved in chromatin remodeling. Although, the whole complex nature of the imprint itself remains to be fully elucidated, the key player of the imprint involve one or more differentially methylated regions displaying an allele-specific DNA methylation pattern, which determine the expression status of the imprinted genes (Murrell et al., 2004). In addition, the differential histone modifications associated with some imprinted genes are thought to regulate chromatin organization and gene expression (Grewal and Moazed, 2003).

Imprinting centre and its characteristics

An imprinting centre is defined as one or more discrete DNA elements, which regulate imprinted gene expression and epigenotype throughout an imprinting cluster. It regulates in cis imprint resetting and imprint maintenance in the whole domain. Genes within imprinting clusters share many regulatory elements and often have similar developmental and tissue specific patterns of expression.

The first IC to be genetically characterized was the differentially methylated region (DMR) upstream of H19, which is also called differentially methylated domain (DMD) or ICR (Imprint control region). DMRs are the region in many imprinted genes, which show parent-of-origin-dependent DNA methylation patterns on one parental allele but not on the opposite allele. There are two classes of DMR, primary DMRs acquire gamete-specific methylation in either spermatogenesis or oogenesis and maintain the allelic methylation difference throughout development. Secondary DMRs establish differential methylation pattern after fertilization, most probably through the influence of primary DMRs (Lopes et al., 2003). So far, 15 primary DMRs have been identified, among which 12 are maternally methylated and 3 are paternally methylated. Loss of the primary DMRs often result in aberrant expression of associated imprinted gene and such DMRs are called imprint control regions (ICRs) (Wutz et al., 1997; Thorvaldsen et al., 1998; Fitzpatrick et al., 2002; Yoon et al., 2002; Lin et al., 2003; Williamson et al., 2004; Liu et al., 2005).

Since genomic imprinting is a cis-directed process, cer-

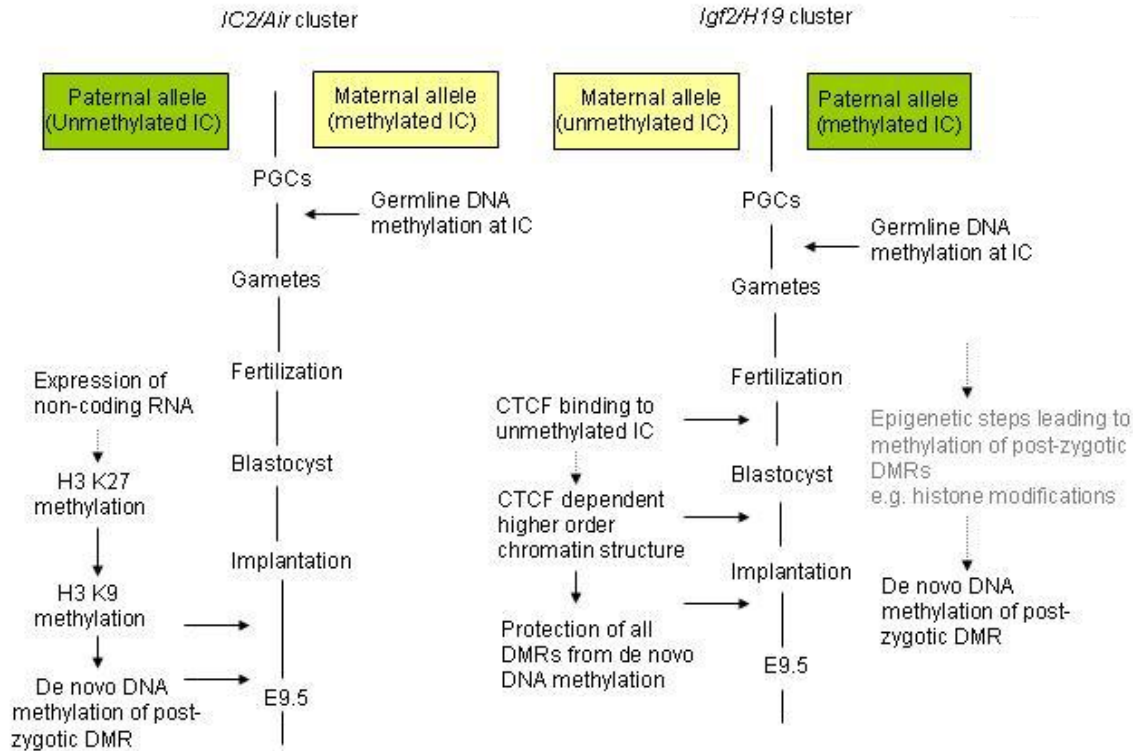


Figure 3. The predicted order of establishment of epigenetic modifications at imprinting clusters in early embryonic development. Hatched arrows indicate progression for which there is no experimental evidence. Light grey text indicate predicted steps which have not been demonstrated.

tain structural features of DMDs might indeed be the cis-acting signals directing correct establishment and maintenance of genomic imprints (Rand and Cedar, 2003). Consistent transgene imprinting was only obtained when 130 kb of genomic sequence surrounding the H19 DMD or when 300 kb of contiguous *Igf2r* sequence including DMD2 were used to generate transgenic lines (Wutz et al., 1997). It was hypothesized that large amount of contiguous genomic sequence of an imprinted locus are essential for its imprinting at an ectopic genomic location. This implies that there are two likely general genomic arrangements of the cis-acting imprinting signals. Either the signals are very large or there are multiple small signals scattered throughout the genomic region defined by the size of consistently imprinted mouse transgenes. DMDs play crucial role in both the differential expression and differential epigenetic marking of the parental alleles of imprinted genes.

Kobayashi et al. (2006) determined the extents of 15 primary DMRs in 12.5-dpc mouse embryo by sulfite sequencing. They found that DMRs have more CpGs than the whole mouse genome, but in general, CpGs are less than the non imprinted CpG islands. However, three (*Peg3*, *Snrpn* and *H19*) out of 15 DMRs has no CpG islands. They found that some DMRs had sharp boundaries and others had transition zones. One possible explanation for less CpG content in DMRs than non

imprinted CpG islands was due to mutation susceptibility of primary methylated DMRs CpG dinucleotides, so they gradually lost CpGs and inevitably accumulate C/T transition in successive generation during evolution. It is well known that methylated cytosine is mutable to thymine (C/T transition) by spontaneous deamination (Holliday and Grigg, 1993).

Kobayashi et al. (2006) found that the average G+C content of the paternally methylated DMRs was significantly smaller than that of the maternally methylated DMRs and paternally methylated DMRs contain less CpGs than the maternally methylated DMRs. The difference in CpG content between paternally and maternally methylated DMRs was due to longer persistence of methylation imprints in male than female germline based on observation that in male paternal methylation imprints are established in gonocyte in the fetal testis and persist in the germline throughout the reproductive life of the male (Davis et al., 1999; Ueda et al., 2000; Li et al., 2004). By contrast, the maternal methylation imprints are imposed in growing oocytes after birth (Li et al., 2004; Lucifero et al., 2004). In addition, male germline divides many times after methylation imprints are established but female germcell do not. It is also possible, however that the CpG content is one of the features recognized by the *de novo* methylation machinery. CpG islands are generally free of methylation but weak or small CpG islands

may lose protection from methylation in one of the germ-lines and could behave as DMRs. DMRs are present in imprinted gene promoters, including antisense RNA genes, in chromatin boundaries, in silencer and activator sequences, suggesting their role in regulating monoallelic expression. The following are characteristics of imprinting centre:

1. Some large imprinting clusters can be further subdivided into domains containing separate ICs whose functions are limited to the domains.
2. All ICs contain germline (primary) DMRs. In addition to germline DMRs there are also post-zygotic (secondary) DMRs that are established after fertilization (Kierszenbaum, 2002).
3. In primordial germ cells, DNA methylation in both types of DMR is erased before the parental specific methylation is established.
4. With respect to DNA methylation, the parental alleles of imprinted genes have different levels of CPG methylation in DMD that are located at specific sites within or surrounding the gene, one parental allele is methylated on the majority of CPG dinucleotides within a DMD and the opposite parental allele is methylated on a small percentage of CPG dinucleotides or not methylated at all.
5. In addition to differential DNA methylation, ICs also show allelic differences in chromatin structure, namely DnaseI hypersensitivity and covalent modifications of histone tails.
6. In general, repressive histone modifications such as methylation at histone H3 lysine 9 and lysine 27 are found at ICs and other DMRs on the methylated allele, whereas activating histone modifications such as H3 and H4 acetylation and H3 lysine 4 methylation are found on the unmethylated allele.
7. The allele-specific difference in histone modifications mark promoter, exonic and intergenic regions (whole of the ICs cluster) in extraembryonic tissue, whereas in embryonic tissue only the DMRs are marked (Lewis et al., 2004; Umlauf et al., 2004).
8. Imprinting centre act as a chromatin insulator. An insulator is defined as a sequence of DNA that blocks enhancers from interacting with gene promoter when positioned between the two and/or acts as a barrier to the spread of transcriptionally repressive condensed chromatin.
9. The CCCTC binding factor (CTCF) is known to bind to insulators and mediate their enhancer blocking activity. There are also non-CTCF dependent insulators at imprinting loci. It is possible that YY1 or unknown proteins may bind to other ICs to give them insulator activity in a similar manner to CTCF.
10. Noncoding RNA transcribed in antisense to that of target gene, have promoters within or near ICs.
11. Methylation of DMRs are exceptional due to fact that they escape the genome-wide demethylation that takes place during the first cleavages in embryo development. They also avoid the global *de novo* methylation that takes

place post implantation (Kafri et al., 1992; Razin and Shemer, 1995).

Hence, currently the defining properties of ICs appear to be that they *cis* regulate other imprinted genes within the same clusters. Differential DNA methylation is established in germlines and maintained in somatic tissues of the offspring. Differential histone modifications also mark allelic differences. They act as chromatin insulator and contain promoter for non-coding RNAs.

Different mechanisms involved in regulation

Imprinting regulation probably at many loci involves insulator protein dependent and higher-order chromatin interaction (looping); non-coding RNAs mediated mechanisms; and mechanisms involving both (Dual role of ICs).

For co-ordination of epigenotype across an imprinting cluster, the first model proposed was linear spreading of DNA methylation (Turker and Bestor, 1997). However, after examining regional control of DNA methylation in the *Igf2/H19* region, Lopes et al. (2003) proposed an alternative model: long range chromatin interactions (or) looping.

Looping model

The two possible mechanisms by which chromatin looping could be involved in the epigenetic regulation of imprinting clusters was revealed by the following examples.

The first example involves *Igf2/H19* locus, the ICs of the unmethylated maternal chromosome of this locus interacts with the DMR1 region of *Igf2* and a downstream matrix attachment region (MAR3) in a CTCF dependent manner. The binding of CTCF to the IC creates an inactive chromatin domain surrounding *Igf2* which is specific to the unmethylated maternal allele, which prevents downstream enhancers directly interacting with *Igf2* promoter region. In the case of imprinted *Dlx5* locus, the methylation sensitive binding of MeCP2 (methyl binding protein) to a DMR form an inactive chromatin structure on the paternal chromosome and an active structure on the maternal allele. There is redundancy between methyl binding domain proteins (MBDs) at imprinted loci. Another method of forming discrete domain of active or silent chromatin, in large scale could involve tethering of imprinted regions to fixed structures in the nucleus. In the case of paternal allele of *Igf2/H19* locus, the chromatin structure in absence of CTCF binding due to methylation of IC seems to depend on matrix attachment regions (MARs), which attach the paternal allele to the nuclear structure. MARs on both parental alleles have also been found in large number between the two imprinting clusters on the mouse distal chromosome 7 (Purbowasito et al., 2004). Thus, MARs may also act as boundary elements to block the spreading of chromatin modifications or to form separate chromatin or loops. Labrador and Corces (2002)

proposed a model in which boundary or insulator elements throughout the genome establish chromatin domains, organizing the chromatin fibres into local compartment of condensed (inactive) chromatin and decondensed (active) chromatin. This model can be extended to imprinted genes, thus an unmethylated insulator may bind to a nuclear structure dividing the imprinting cluster into two and dividing promoters and enhancers into separate compartments. If the same insulator is inactivated by methylation, they cannot form two separate domains and therefore enhancers and promoters will interact.

Mechanism involving non-coding RNAs

The best characterized mammalian non-coding RNA is *Xist*, which coats the future inactive 'X' and triggers the events which lead to gene silencing along the length of the X chromosome (Heard, 2004). In two well characterized clusters (IC2 and *Igf2r/Air*), these RNAs are expressed from the paternal allele, which silence the surrounding genes paternally. In the *Igf2r/Air* IC, the critical element is the *Air* non-coding RNA transcript. It is not known, how the *Air* RNA mediates silencing of the surrounding genes. However, possible models include an RNAi based mechanism, transcription through specific DNA elements or a chromosome coating mechanism as in X chromosome inactivation. The *Kcnq1ot1* non-coding RNA from the IC2 cluster has many similar features of *Air* and *Xist* RNA. Its transcription is initiated from the unmethylated paternal IC in the IC2 cluster and all the flanking genes are paternally silenced.

General features of Non-coding RNAs: The presence of non-coding RNA genes, often (but not always) transcribed in the opposite orientation to protein-coding genes, is a recurrent theme in imprinted domains.

a. C/D RNA: C/D type small RNAs (C/D RNA) are metabolically stable, 60-to 300-nucleotide-long, and present in eukaryotes and archaeobacteria. In eukaryotic cells, they reside in nucleus, either in the nucleolus (they are called C/D snoRNAs for C/D small nucleolar RNAs) or in the cajal bodies (they are called scaRNAs for small cajal body-specific RNAs). In vertebrate, vast majority of C/D RNA genes are located within the intron of protein coding gene, more often and also within the introns of non-coding RNA genes. Most of the C/D RNAs appear to be processed from the host gene introns through exonucleolytic degradations of the debranched lariat. The human C/D RNA sequences are available at ([http://www-snorna.biotoul.fr/](http://www.snorna.biotoul.fr/)). C/D RNAs owe their names to canonical structural motifs, the C-box (consensus 5'-PuUGAUGA-3') and the D-box (consensus 5'-CUGA-3') present close to their 5' and 3' termini, respectively. They also contain more degenerate C' and D' boxes that occupy an internal position within the RNA sequence. Many C/D RNAs contain conserved antisense elements (8 to 21 nucleotide

long) positioned upstream from the D- and/or the D'-motifs. C/D RNAs act by pairing with RNA targets on which they guide ribose methylation at specific ribonucleotide (the modified nucleotide is always paired to the fifth nucleotide upstream from the D or D' box) (Figure 4a). C/D snoRNAs modify the Pol I transcribed pre-rRNAs or the Pol III-transcribed U6 spliceosomal snRNA while scaRNAs modify the Pol II-transcribed U1, U2, U4 and U5 spliceosomal snRNAs (Figure 4a). A broad proportion of C/D RNAs-including most of the imprinted C/D RNAs is devoid of any specific RNA target, so it might target other cellular transcript (including mRNA?) or might play a different role in RNA modification guiding in which no pairing with a target RNA is needed.

b. microRNA (miRNA): It constitute the largest eukaryotic small RNA family discovered so far, they are 21-to 23-nucleotide-long single-stranded RNA molecules that are processed from one arm of an irregular 60-to 70-nucleotide-long hairpin structure called pre-miRNA. The pre-miRNA genes exhibit different genomic organization. They can be transcribed from their own promoters either as independent entities or as polycistrons, or they can be included in larger transcript units of coding or non-coding genes (Kim, 2005). A pre-miRNA gene is first transcribed by RNA pol II as a several kb long pri-miRNA (the miRNA primary transcript). Then, the RNase-III type enzyme Drosha assisted by DGCR8 make pair of cuts on the large pri-miRNA precursor to give rise to the pre-miRNA, this cut establishes one end of the miRNA. The pre-miRNA is then translocated to the cytoplasm by exporting 5-mediated export, where another multidomain RNase-III Dicer enzyme create other extremity of the miRNA, to yield a short RNA duplex. This duplex is unwound upon loading on the RISC (the mature miRNA-containing ribonucleoparticle), giving rise to the single-stranded mature miRNA. The mouse and human miRNA sequences are available at <http://www.sanger.ac.uk/Software/Rfam/mirna>.

Two pathways can be adopted for miRNAs mediated gene silencing at the post-transcriptional level, by basepairing with a target RNA (Figure 4b): (i) if perfect (or almost perfect) complementarity is shared, the target RNA is directed for cleavage; (ii) If the miRNA presents a partial complementarity with an mRNA (generally in its 3' UTR part), then the translation of the target mRNA is made non-productive by still poorly characterized mechanisms. The most 5' part of the miRNA (2nd to 8th nucleotide, also called the 'seed') plays a critical role for target recognition (Doench and Sharp, 2004). Plant miRNA with the full complementarity to its target can also act at the translation level (Aukerman and Sakai, 2003). In plant systems, miRNA has been shown to direct asymmetric DNA methylation within the gene it targets (Bao et al., 2004). Whether such a mechanism operating in the nucleus at the DNA level can also take place in mammals is unknown. By computational predictions of miRNA target, about 10 to 30% of mRNA populations are potentially

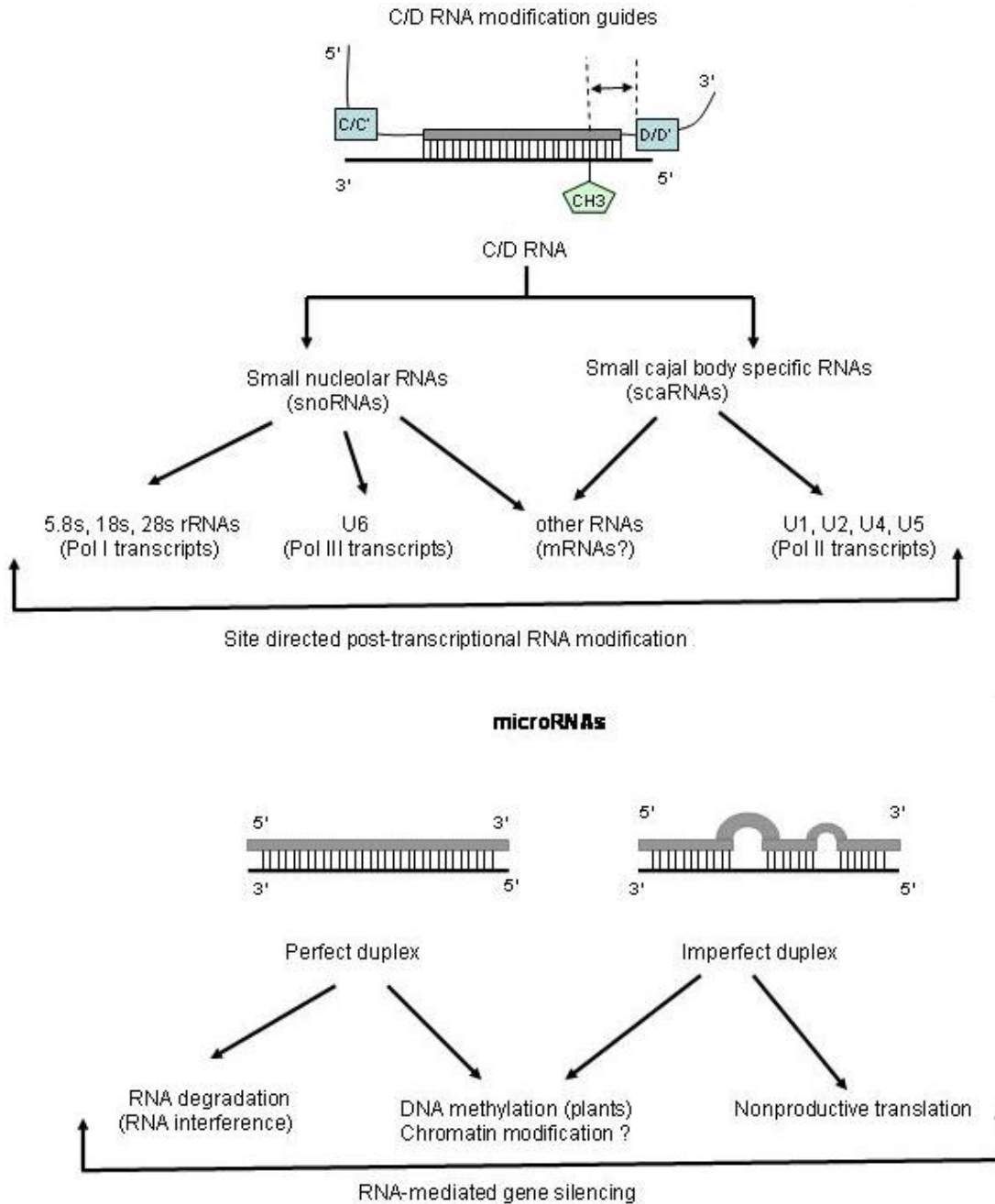


Figure 4. Function of (a). C/D RNA modification guides and (b). miRNAs. The sequence complementary to cognate target RNA are depicted as grey region of the strand.

targeted by miRNAs. But concretely, few genetic data about the biological role of vertebrate miRNA are available.

Imprinted small RNA genes: More than 100 C/D RNA and miRNA genes were predicted by use of computational methods and systematic DNA cloning strategies in two imprinted loci of mammals: human 14q32 (so called callipyge domain) and 15q11-q13 (so-called

prader-willi domain). They seem to share several structural and functional characteristics: (i) they are grouped into clusters of homologous repeated gene copies, with most embedded within introns of large non-coding genes subject to alternative splicing; (ii) They are not found in non-eutherian mammals; (iii) they display a tissue specific expression pattern with prepondent expression in adult brain; (iv) they lack an obvious functional antisense element against a cellular transcript except few and their molecular and biological functions remain highly elusive.

The target identification for small RNA is difficult task because imprinted small RNAs are specific to eutherian species (they were not detected in chicken or even opossum brain). Which considerably limits the use of comparative sequence analysis for target identification, and functional parts of the small RNAs involved in target specificity (C/D snoRNA sequence upstream from the D/D' boxes or the 5' seed for miRNAs) can vary notably from one copy to the other, suggesting that they may target different RNA species. The miRNAs and RNAi related processes play a key role in neuronal function, brain morphogenesis as well as stem cell self-renewal and cell fate decisions and behavioural phenotypes. A miRNA (miR-32) is at the basis of an antiviral defense mechanism in human cells (Le Cellier et al., 2005). In *Schizosaccharomyces pombe*, Dicer produced siRNAs derived from centromeric repeats are incorporated into RITS (RNA-induced transcriptional silencing complexes), a RISC-related complex, to direct heterochromatin formation and gene silencing at the transcriptional level (Matzke and Birchler, 2005). Several lines of evidence in mammalian system support the existence of nuclear RNA-mediated gene silencing mechanisms: (i) small RNA-mediated RNA degradation is active in the nucleus (Robb et al., 2005); (ii) DNA methylation and histone H3K9 methylation can be triggered by small interfering RNAs (Kawasaki and Taira, 2004; Morris et al., 2004), although this may not occur in all experimental systems; (iii) Dicer-deficient mouse ES cells show reduced levels of DNA and histone H3K9 methylation of centromeric DNA, high levels of centromeric repeats (Kanellopoulou et al., 2005). A conditional loss of functional mutant of Dicer in hybrid human-chicken cells also causes accumulation of transcript from alpha-satellite sequences and leads to abnormal mitotic cells, presumably due to a defect in the formation of centromeric heterochromatin (Fukagawa et al., 2004; Kanellopoulou et al., 2005). Thus, Dicer-related RNAi machinery is required for the silencing of centromeric heterochromatin in vertebrates. Repeated sequence are known to attract silencing and considering the involvement of small RNAs and the RNAi machinery in the construction of repressive chromatin in mammals, it is legitimate to propose that imprinted small RNAs-and more especially the large miRNA gene at 14q32 could account for certain epigenetic regulation at the basis of genomic imprinting. In many RNA-mediated gene silencing mechanism, like X chromosome inactivation (mammals), RNA-directed DNA methylation (plants) or heterochromatinization at the sexual maternal locus (*S. pombe*), the RNA and/or the RNAi machinery is mainly required to initiate the epigenetic state. Hence, miRNAs might guide epigenetic marking early during development (in the germline), but once the imprints are installed on gamete haplogenomes, they might be dispensable for the subsequent maintenance and reading of the imprints. DNA methylation plays a central role in genomic imprinting. First, there is interest to investigate

their potential involvement in DNA methylation, including non-canonical contexts as observed in plants (on cytosine in CpNpG and asymmetric sequence contexts). Additionally, miRNAs could help to install an allele-specific chromatin state through recruitment of histone modification complexes.

RNA induced methylation: RNA directed DNA methylation (RdDM) was first demonstrated by Wassenegger et al. (1994) and has been extensively studied in plants (Chan et al., 2005). RdDM is carried out by double stranded RNA (dsRNA), which may be produced from transcription through inverted repeats. Double stranded RNA is then cleaved by the RNAase III family enzyme Dicer into small interfering RNAs (siRNAs) of 21-26 length which are able to direct methylation of homologous sequences. The involvement of histone modifiers and DNMTs in RdDM indicate a complex system of regulation of DNA in plants. siRNAs have since been shown to direct DNA methylation in human cells (Kawasaki and Taira, 2004; Morris et al., 2004). These findings suggest that, a similar mechanism may act at imprinted loci, in which dsRNA could be formed by transcription through inverted repeats often present in it or simultaneous transcription of sense and antisense transcript. Martienssen (2003) proposed a mechanism by which tandem repeat can continuously produce siRNAs via the use of RNA dependent RNA polymerase (RdRP). RdRP is identified in RNA viruses, yeast and plants, but it is yet to be identified in mammalian systems. From the common appearance of tandem repeats at imprinted loci and at heterochromatinized regions elsewhere in the genome, it is tempting to speculate that such repeats regulate imprinted methylation by an RNA dependent mechanism. At first, miRNAs were identified as translational repressors by binding to the 3' UTRs of target genes but recent evidence also supports a role for miRNAs in DNA methylation (Bao et al., 2004).

Dual role of ICs

The *IC2/Kcnq1* and the *Igf2r/Air* clusterS contain placental-specific imprinted genes on one side of the IC and genes imprinted in both embryonic and extra embryonic lineages on the other. In the mouse *IC2/Kcnq1* cluster these two sets of genes are clearly regulated by IC in two different ways. One involves CTCF binding to the insulator on the unmethylated paternal IC and using higher-order chromatin structures to restrict enhancer access. On the maternal chromosome CTCF binding is blocked by DNA methylation. This mechanism is active in all lineages and results in stable imprinting of genes on the telomeric side of the locus. The other mechanism occurs only in the extraembryonic lineage but may begin in the pre-implantation embryo. This involves the *Kcnq1ot1* RNA,

possibly coating the chromosome and recruiting repressive histone modifications to the placentaly imprinted genes on the centromeric side of paternal allele. The histone modifications are recruited to genes on both sides of the IC but are probably irrelevant on the telomeric side since the stronger, looping-based mechanism takes precedence. The RNA/histone methylation mechanism is much less stable, is not maintained through the later stages of gestation. The *Air* RNA is important for imprinting in both embryonic and extraembryonic line-ages in *Igf2r/Air* cluster (Sleutels et al., 2002) but there are clear differences between the placental specific imprinted genes which have no associated DMRs and *Igf2r* which exhibits methylation dependent imprinting in many tissues.

The PWS/AS cluster IC is described as bipartite. Establishment of imprinting on the paternal chromosome requires the PWS-IC in cis whereas establishment of imprinting on the maternal chromosome requires the AS-IC. In addition, the tissue-specific maternal expression of *Ube3a* requires the *Ube3a* antisense RNA. Thus there may be as many as three mechanism regulating imprinting in this cluster.

Role of dicer in imprinting

In fission yeast, Dicer and Argonaute are required not only for RNAi but also for transcriptional silencing of centromeric repeats by chromatin modifications (Hall et al., 2002; Volpe et al., 2002). Dicer generated siRNA form the RNA induced initiation of the transcriptional gene silencing (RITS) complex, which contains Argonaute protein and is required for heterochromatin silencing (Noma et al., 2004). Dicer is required for centromeric heterochromatin silencing also in chicken and mouse cell (Fukagawa et al., 2004; Kanellopoulou et al., 2005). In mammals, although recent papers have reported that the introduction of siRNAs complementary to the promoter regions of some genes induces DNA methylation (Kawasaki and Taira, 2004; Morris et al., 2004), the native target of transcriptional gene silencing induced by siRNA have been unclear. In addition, an RNAi-related phenomenon called cosuppression was observed for an imprinted gene, *U2af1-rs1* (Hatada et al., 1997). The mechanism underlying cosuppression is very similar to those of RNAi because there are common RNA intermediates and also similar genes are required in the silencing pathway (Hamilton and Baulcombe, 1999; Hannon, 2002).

Fukasawa et al. (2006) suggested that the reduced expression of *Dicer1* did not affect the genomic imprinting and a very low level of Dicer enzyme would be sufficient for silencing of functional genes. With respect to this, maintenance of imprinting requires a very low level of DNA methylase activity because imprinting continues during the early embryonic stages when the DNA methylase activity is reduced to a minimum. *Dicer1* was not required for the maintenance of transcriptional silencing at pericen-

centromeric satellite sequence, the maintenance of DNA methylation and X chromosome inactivation in female cells, and the stable shutdown of developmentally regulated gene (Cobb et al., 2005). The similarity between X chromosome inactivation and genomic imprinting indicate that, *Dicer1* may not be required for maintenance of genomic imprinting.

HYPOTHESIS FOR EVOLUTION OF GENOMIC IMPRINTING

Conflict hypothesis or kinship theory

According to this theory, the situation of direct codependence results in competition for resources between foetus and mother. The paternal expressed gene like *Igf2* will favour growth of each individual foetus at the expense of all other foetuses and mother, resulting in larger progeny that have a competitive advantage over those of other males. But the maternal genome will favour an equal distribution of resources among all foetuses and preservation of itself for future pregnancies (Haig and Graham, 1991; Moore and Haig, 1991). Though it is plausible and intellectually pleasing, all imprinting phenomena cannot be explained (Hurst and Mc Vean, 1997; Iwasa, 1998). This theory has been challenged by the lack of phenotype observed in knockout mice of the maternally imprinted small nuclear ribonucleoprotein N (*Snrpn*), which was expected to show an overgrowth phenotype and paternal expressed Zn finger and C2 domain protein (*Zac*) has growth inhibitory effect. A possible prediction by Haig (2006) is that inhibitors of demands located on the X-chromosome and enhancers of demand located on autosomes, because X-linked loci are maternally derived two-thirds of the time by contrast to autosomal loci which are maternally derived half of the time. The prediction that the X-chromosome should express a bias towards inhibiting the demands that offspring impose on their mothers is called the hypothesis of X-Linked Inhibitory Bias (XLIB).

Complementation hypothesis

It is proposed from the mechanistic point of view, imprinting regulation in somatic and germ cells by Lee et al. (2002) and Kaneko-Ishino et al. (2003). It argues that genomic imprinting is essential for mammalian development as a mechanism regulating complementary or reciprocal expression profiles of paternal and maternal genomes, because Pegs and Megs cannot be expressed from the same chromosome simultaneously, even when the parental imprints are completely erased. In addition, it also rescues Pegs and Megs involved in development and growth from catastrophic situation, in which the expression of either half of the imprinted gene was lost.

Barrier to parthenogenesis in mammals

Genomic imprinting prevents accidental or unexpected parthenogenesis, which is life threatening and undesirable in females, because food and environmental factors like temperature and climatic conditions suitable for breeding is seasonal. It requires genetic contribution from both parents, and is evolutionarily advantageous in producing variation by mixing genetic information. The “paternal dual barrier theory” state that two sets of coordinately imprinted genes, *Igf2-H19* and *Dlk-Gtl2*, function as a critical barrier to parthenogenetic development in order to render paternal contribution obligatory for the descendants of mammals (Kono, 2006).

Host defense mechanism

According to this proposal, genomic imprinting arose as an accessory system by which mammalian genome represses exogenous DNA sequences using DNA methylation. However, it fails to explain why imprinting occurs exclusively in mammals because DNA methylation and retrotransposons are not unique to mammals. It also does not explain why all imprinted genes are not methylated.

Ovarian time bomb theory

Genomic imprinting by placing control of placental development on the paternal genome would have a protective effect from trophoblastic tumorigenesis in females, which could become malignant in the absence of genomic imprinting. But it does not explain imprinting of neither the paternal genomes nor why genes which are not involved in placental development are still imprinted.

Novel placental hypothesis

The significant relationship between placental formation and genomic imprinting in mammals and observation that most imprinted gene are expressed in placenta lead to proposal of this hypothesis by Kaneko-Ishino et al. (2003). It is assumed that imprinted gene is regulated to ensure appropriate expression in placental tissue, which enabled the ancestral mammal to form placental structures.

EVOLUTIONARY LINK BETWEEN DNMT3L AND IMPRINTING

One of the important functions of DNA methylation in mammals is to regulate genomic imprinting. In mammals, DNMT3A and DNMT3B are so-called *de novo* methyltransferase, which create new methylation patterns on non-methylated DNA (Bestor, 2000). DNMT1 (DNA Methyltransferase1) is a maintenance methyltransferase,

which methylates the newly synthesized hemimethylated DNA strand after DNA replication. *de novo* methyltransferase 3A (DNMT3A) and a related protein with no methyltransferase activity, DNMT3L, have been shown to be essential for the establishment of germline specific methylation imprints associated with imprinted genes (Bourc'his et al., 2001; Hata et al., 2002; Kaneda et al., 2004). After fertilization, DNMT1 propagates the methylation patterns and regulates parent-of-origin-specific gene expression in somatic tissues of embryo and adults (Li et al., 1993).

A possible link is obtained between the existence of this protein and the evolution of genomic imprinting from the observation that DNMT3L is present in eutherians and marsupials but likely to be absent in birds and fish. Thus, DNMT3L is a key regulator of genomic imprinting, and acquisition of this gene via gene duplication in a common ancestor of eutherians and marsupials may have been a critical event in the evolution of imprinting. The original function of DNMT3A is probably essential to the survival and/or development of vertebrates but a mechanism evolved only in placental mammals utilized this enzyme to establish the parent-of-origin-specific methylation imprints.

CONCLUSION

The possession of DNMT3L and placenta during mammalian evolution are probable key events for evolution of imprinting in placental mammals. In essence, the presence and consequently also the absence of distinct types of repetitive elements may influence the accessibility of DMRs to the specific epigenetic modification machineries in the parental germlines. Although, the whole nature of imprinting mechanisms needs to be unraveled, the higher order chromatin (looping) and non-coding RNAs may play significant role in imprinting. It has been proven that low level of Dicer did not affect imprinting. Even though several theory has been proposed, there is need for unified hypothesis for imprinting evolution that encompasses all imprinted gene.

Future research

We are at initial stages of this phenomenon, despite being the tremendous amount of knowledge we have amassed just over two decades. Ingenious and insightful experiment will be required to show the whys and hows of imprinting. In germ cell, the query of whether all parental imprint are erased and established freshly or sex specific imprint are retained, while other nonspecific imprint are erased need to be solved. Elucidation of various mechanisms involved in regulation of imprinting, enlighten other scientific area like stem cell research, cloning and imprinting disorder. There are exciting question for future research on the evolutionary origins of autosomal imprinting in the placenta and its link to imprinted X inactivation.

Although the role of cis acting DNA signal like ADS and DNS is attributed to DNA methylation, the *in vivo* function need to be established. There is need for unified hypothesis for imprinting evolution that encompasses all imprinted gene.

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