—Review—

Genomic Imprinting: Mechanisms, Significance and Evolution

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Introduction

Although the genetic information inherited from one parent is basically equivalent to that inherited from the other parent, their functions are not always equal. Some genes are regulated according to their parental origin and such genes are called "imprinted genes". Genomic imprinting in mammals was first demonstrated by several elegant developmental and genetic experiments [1–3]. In short, abnormal development was observed in uniparental (parthenogenetic, gynogenetic or androgenetic) mouse embryos and certain uniparental disomies. Thus it was shown that both a paternal and a maternal set of chromosomes are required for normal development. Since then, about 60 imprinted genes have been discovered. The imprinting marks regarding the parental origin (imprints) are established during gametogenesis and maintained precisely in somatic cells of the offspring throughout the whole life [4]. Many imprinted genes have been shown to be involved in growth regulation of embryos. In this review, we summarize the recent findings on the mechanisms, significance and evolution of genomic imprinting.

DNA Methylation and Imprinted Gene Expression

DNA cytosine 5-methylation is one mechanism that primarily regulates genomic imprinting [5], although it is obviously not the only mechanisum [6]. A majority of imprinted genes possess differentially methylated regions (DMRs) in and/or around the genes. Many imprinted genes, including *Igf2, Peg1, Peg3, Snrpn, Igf2r, Kvlqt1* and *p57*, have maternally methylated DMRs [7–10], whereas paternally methylated DMRs have been identified only in or around *H19, Rasgrf1* and *Dlk1/Gtl2* [11–13]. How does DNA methylation regulate the monoallelic expression of imprinted genes? We can categorize the proposed mechanisms into four simple models: a) prevention of sense transcription, b) prevention of antisense transcription, c) regulation of silencers and d) regulation of insulators (Fig. 1) [14].

DNA Methylation Reprogramming in Mouse Development

There are global DNA demethylation and remethylation processes in mouse development. After fertilization, both the paternal and maternal genomes become demethylated in cleavage stage embryos. The genomes are then remethylated in lineage-specific ways after implantation. Methylation imprints inherited from gametes somehow escape the demethylation process and serve as the epigenetic memories regarding the paternal origin. Once the lineage-specific methylation patterns are established, they are maintained in the descendant cells.

The reprogramming of DNA methylation in germ cells occurs in a way quite different from that in somatic cells (Fig. 2). A small population of primordial germ cells (PGCs), which arise from epiblasts, migrate into the genital ridge at E10.5–E11.5. Not only the lineage-specific methylation patterns but also methylation imprints are completely erased in PGCs, and then they are remethylated to establish the new sex-specific methylation pattern (including the methylation imprints) (see below).

Recently, the methylation imprints of cloned mouse embryos produced from PGCs were studied in detail

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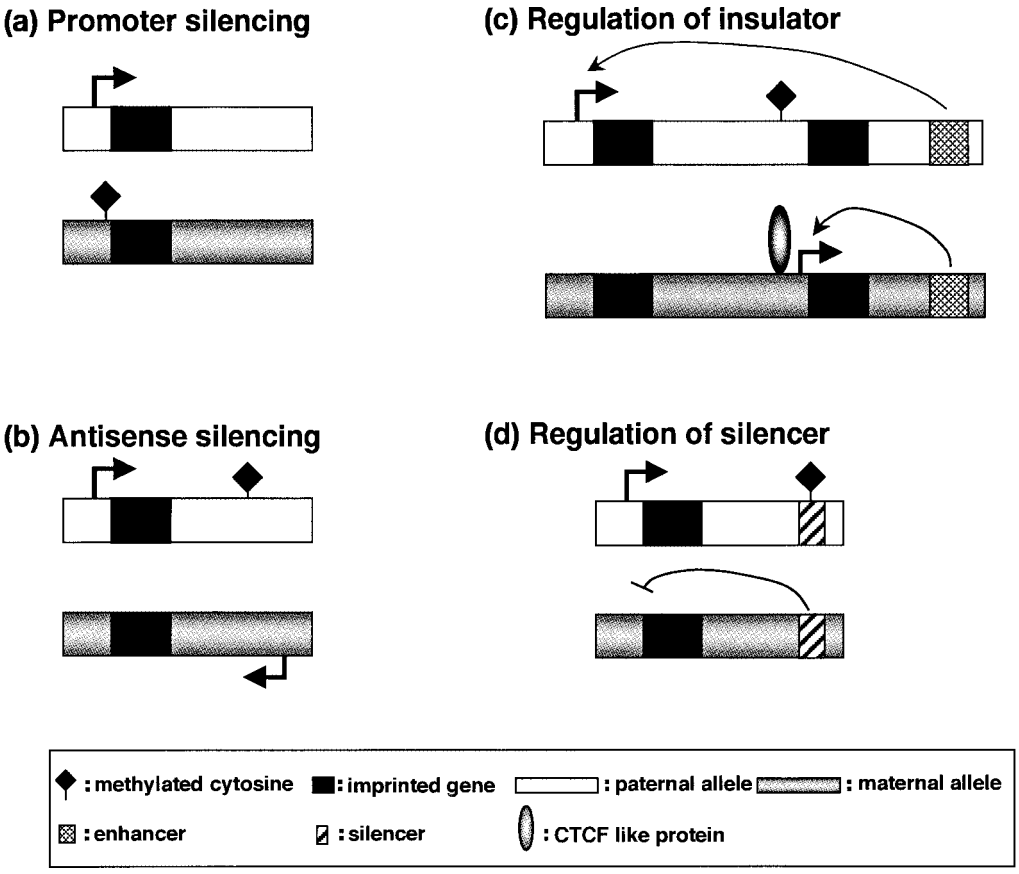

Fig. 1. Monoallelic expression of imprinted genes by DNA methylation.

[15, 16]. It was demonstrated that, in PGCs, the differential methylation of imprinted genes is erased between E11.5 and E12.5. The timing of erasure is identical in the male and female PGCs. By contrast, the timing of remethylation is different in the two sexs. In males, methylation imprints are established in gonocytes (or prospermatogonia) before entering meiosis [17, 18]. In females, methylation imprints are established after birth, in growing oocytes in the meiotic prophase [19, 20].

Mechanisms of Methylation Imprinting in Gametogenesis

So far, three mammalian **DN**A **m**ethyl**t**ransferase genes, *Dnmt1, Dnmt3a* and *Dnmt3b*, have been identified. The products of all these genes show methyltransferase activity to unmethylated DNA, although Dnmt1 has a strong preference for hemi-methylated DNA.

Dnmt1 is necessary for maintaining the genome methylation patterns, including the methylation imprints. Once the methylation imprints are lost in *Dnmt1*-deficient ES cells, overexpression of exogenous *Dnmt1* by cDNA transfection cannot recover the imprints. Germ-line transmission of the genome is necessary to reestablish the methylation imprints [21]. Targeting of *Dnmt1o*, an oocyte-specific form of *Dnmt1*, shows that it is necessary for the maintenance but not for the establishment of the methylation imprints [22]. There is no evidence that *Dnmt1* is involved in methylation imprinting.

Dnmt3L (**DN**A **m**ethyl**t**ransferase **3 L**ike) protein possesses a PHD-like domain, which is conserved between Dnmt3a, Dnmt3b and a protein encoded by the X-linked ATRX gene, in its N-terminal domain. Its C-terminal domain is related to the catalytic domains of Dnmt3a and Dnmt3b, although it lacks the critical amino acid residues for DNA methyltransferase activity. The restricted expression of *Dnmt3L* in ES cells, chorion and gonads, suggests that *Dnmt3L* may regulate DNA methylation specifially in these cells. Interestingly,

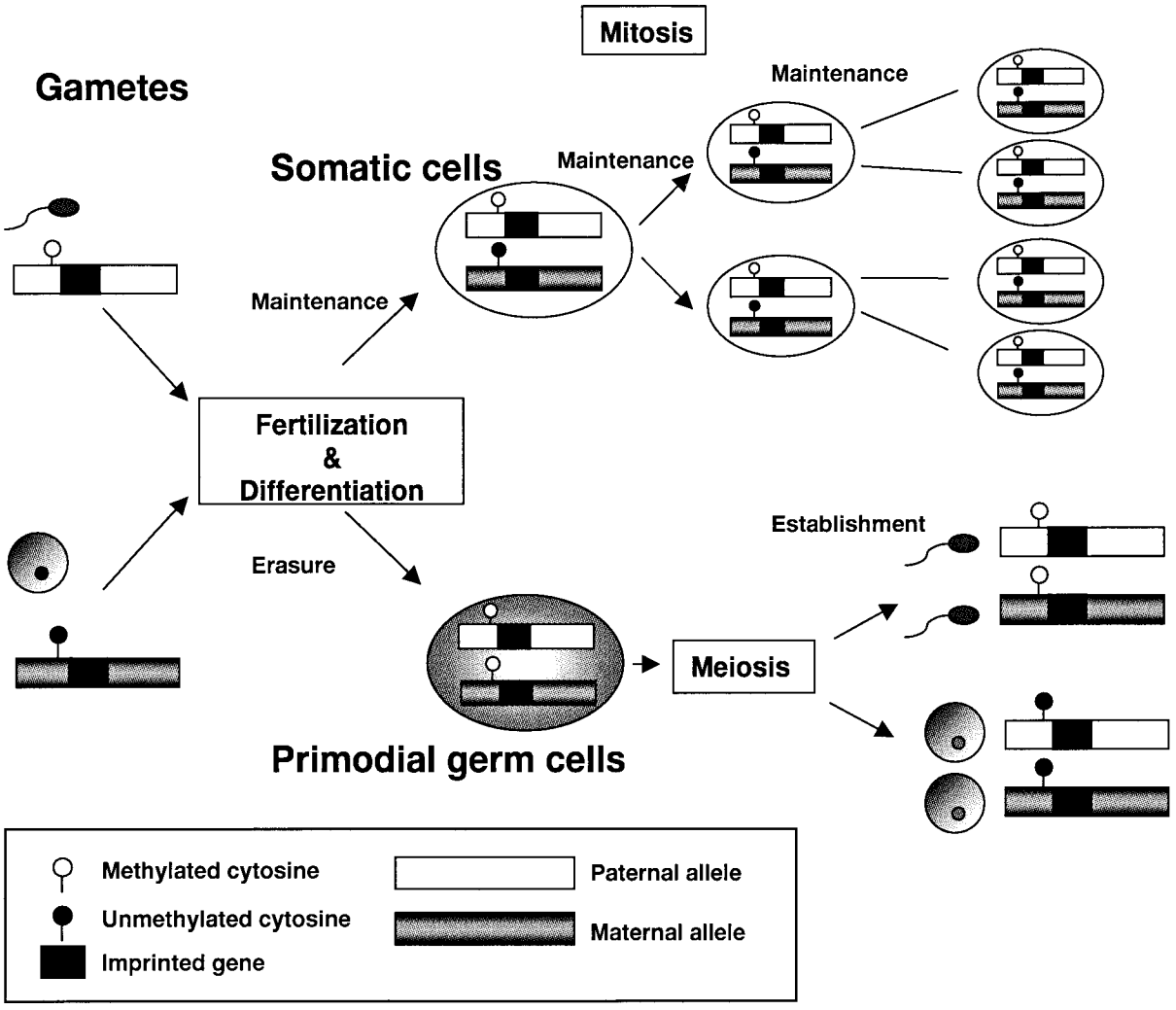

Fig. 2. Maintenance and reprogramming of DNA methylation imprints.

embryos derived from *Dnmt3L* homozygous mutant mothers lack the maternal methylation imprints even though these embryos have a normal copy of *Dnmt3L* inherited from their fathers [23, 24]. Overexpressed Dnmt3L protein can interact with overexpressed Dnmt3a or Dnmt3b in the nuclei of transfected cells. Furthermore, embryos derived from a [*Dnmt3a-/-*, *Dnmt3b+/-*] ovary lack the maternal methylation imprints [24]. These data clearly show that *Dnmt3L* is essential for methylation imprintig in oogenesis and that *Dnmt3a* and/or *Dnmt3b* may cooperate with *Dnmt3L*.

Reasons for the Evolution of Genomic Imprinting

Although imprinted genes are autosomal, they are functionally hemizygous since one of the alleles is always silenced. Obviously this increases the risk of functional loss of a gene function by mutations. Then, why have mammals adopted and still maintain imprinting as a gene regulation strategy?

Several theories have been postulated. One model explains that genomic imprinting evolved to prevent parthenogenesis or aneuploidy [25]. Other theories say that imprinting is a defense against foreign DNA [26, 27] or against invasion of the uterus by the trophoblast [28].

The last theory, which is most popular, is refered to as the “conflict theory” [29]. Functional analysis of many imprinted genes has revealed that the majority of the genes regulate fetal growth (Table 1). Generally, paternally expressed genes tend to make the fetus larger and maternally expressed genes work negatively

Table 1. Expression/DNA methylation pattern of imprinted genes and phenotypes of mutants

Gene	Expression allele	Methylation allele	Phenotypes of mutant mice
Peg1/Mest	Pat	Mat	Fetal growth retardation Abnormal maternal behavior
Peg 3	Pat	Mat	Fetal growth retardation Abnormal maternal behavior
Rasgrf1	Pat	Pat	Postnatal growth retardation?
H19	Mat	Pat	Fetal overgrowth
Igf2r	Mat	Pat	Fetal overgrowth
$P57^{kip2}$	Mat	Mat	Placental hypertrophy
Kvkqt1	Mat	Mat	Abnormal inner ear Gastric hyperplasia

on fetal growth. The theory predicts that, if there is a possibility of polyandry, a father's gene would serve to make the offspring larger (to increase the survival rate) whereas a mother's gene (which is likely to be shared by other offsprings) would try to save the maternal resource for future pregnancy. Thus, a growth-promoting gene, for example, would be selected to be expressed only from the paternal copy during evolution. Genomic imprinting is observed in both euthelians and marsupials (diverged ~130 million years ago) but not in monotremes (diverged ~145 million years ago) [30] or chickens (diverged ~300 million years ago) [31]. Thus, intrauterine gestation could indeed be an important driving force for imprinting evolution.

Outlook

Recent progress in epigenetics suggests that multiple mechanisms, including DNA methylation and histone modifications, regulate the genomic function cooperatively. The *in vitro* differentiation system reviewed in this issue should be of greate help in elucidating the epigenetic mechanisms of genomic imprinting. To understand the evolutional reasons for imprinting, we need to collect more information on the function of the imprinted genes. Comparative studies on other vertebrate species such as chickens, monotremes or marsupials should also shed light on these fundamental questions.

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