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Review

Chromatin regulators of genomic imprinting ☆

Jamie R. Weaver, Marisa S. Bartolomei *

Department of Cell and Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, 9-123 Smilow Center for Translational Research, Philadelphia, PA 19104, USA

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ABSTRACT

Genomic imprinting is an epigenetic phenomenon in which genes are expressed monoallelically in a parent-of-origin-specific manner. Each chromosome is imprinted with its parental identity. Here we will discuss the nature of this imprinting mark. DNA methylation has a well-established central role in imprinting, and the details of DNA methylation dynamics and the mechanisms that target it to imprinted loci are areas of active investigation. However, there is increasing evidence that DNA methylation is not solely responsible for imprinted expression. At the same time, there is growing appreciation for the contributions of post-translational histone modifications to the regulation of imprinting. The integration of our understanding of these two mechanisms is an important goal for the future of the imprinting field. This article is part of a Special Issue entitled: Chromatin and epigenetic regulation of animal development.

1. Introduction

Diploid, sexually reproducing organisms inherit two sets of chromosomes, one from each parent, and so possess two copies of each autosomal gene. For the majority of these genes, both alleles are expressed, or not, as appropriate for the cell type. There are, however, genes that are monoallelically expressed in a parent-of-origin specific manner. The mammalian genome contains about 150 genes that are imprinted (a complete up-to-date list can be found at: http://www.mousebook.org/catalog.php?catalog=imprinting [1]). As a result, mammalian development requires genetic contributions from both a mother and a father [2,3].

Imprinted genes are generally found in clusters containing both maternally and paternally expressed genes, although a few singleton imprinted genes exist as well. Each imprinted cluster contains a regulatory element known as an imprinting control region (ICR). By definition, deletion of an ICR results in loss of imprinting (i.e. biallelic silencing or biallelic expression) of all genes within the cluster [4,5]. Thus, the ICR is the master regulator of imprinting in the region.

Two well-defined mechanisms of imprinted gene regulation have been described: the insulator model and the non-coding RNA (ncRNA) model. In the insulator model (Fig. 1), the ICR is bound by the insulator protein CCCTC binding factor (CTCF) on only one allele. The insulator regulates access of genes on either side of it to shared enhancers. On the allele where CTCF is bound, only the genes found between the insulator and the enhancer can be activated; the insulator prevents genes on the far side from accessing the enhancers. When CTCF is not bound, these genes can be activated by the enhancers. See also Matzat and Lei in this issue for a detailed review of recent advances in our understanding of the nature of insulators. The other major imprinting model is the ncRNA model (Fig. 1). In this case, the ICR is the promoter of a long ncRNA, which is expressed from only one allele. On this allele, expression of the ncRNA silences the rest of the genes in the domain in *cis*. On the other allele, the ncRNA is silenced and the rest of the genes are active.

Imprinted genes span a range of functions, but many are expressed during embryonic development and have roles in the regulation of cell growth and in placental function. Imprinted genes are also involved in maternal behaviors, postnatal energy homeostasis and neurological function [6,7].

There are a number of rare congenital disorders that are caused by defects in imprinting. For example, failure to express genes within the *SNRPN* imprinted domain results in Prader–Willi Syndrome (PWS) and Angelman Syndrome (AS). Genetic or epigenetic abnormalities in the *H19/IGF2* or *KCNQ1* domains result in Beckwith–Wiedemann Syndrome (BWS) or Silver–Russell Syndrome (SRS), depending on which parental allele is affected [8]. Additionally, recent studies have shown a connection between the use of assisted reproductive technologies, such as in vitro fertilization, and imprinting disorders [8].

This review will focus on the nature of the epigenetic imprinting mark and the roles of different chromatin modifications, such as DNA methylation and post-translational modification of histones, in genomic imprinting.

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* Corresponding author. Tel.: +1 215 898 9063 (office).
E-mail address: bartolom@mail.med.upenn.edu (M.S. Bartolomei).

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2. The role of DNA methylation in Imprinting

2.1. DNA methylation

Imprinted clusters contain differentially methylated regions (DMRs), which are characterized by DNA methylation of only one parental allele. DNA methylation is the covalent attachment of a methyl group to the 5-position carbon on cytosine residues. In mammals, this modification is almost exclusively found on cytosines followed by guanines (CpG sites). It is generally considered to have a repressive effect on transcription, though this is not always the case. DNA methylation is a stable mark, which is propagated through replication by the maintenance DNA methyltransferase, DNMT1 [9,10]. DNA methylation can also be established by the de novo DNA methyltransferases, DNMT3A and DNMT3B, and can be removed by either active or passive demethylation [11,12]. The mechanisms of demethylation are still under investigation, and will be discussed in further detail below. Importantly, all known ICRs are germline DMRs, meaning that differential methylation is established either during oogenesis or spermatogenesis, and this pattern is maintained after fertilization despite widespread erasure of DNA methylation during preimplantation development [13,14]. Differential methylation can also be found outside of ICRs, but it is typically established after fertilization [15]. For this reason, DNA methylation is the best candidate for the mark that designates the parental identity of each allele.

2.2. Establishing the methylation imprint in the germline

2.2.1. Erasure of prior imprints

Sex-specific methylation of ICRs is established during gametogenesis (Fig. 2A). Inherited methylation is erased and new methylation is put in place in preparation for inheritance by the next generation. This process begins as primordial germ cells (PGCs) are specified and migrate to the developing gonad. In mice, PGCs travel from the extra-embryonic mesoderm to the genital ridge between embryonic days 7.5 (E7.5) and E12.5. During this time, they undergo widespread chromatin changes. These changes include loss of both repressive and activating histone modifications, loss of DNA methylation, and reactivation of the silent allele of imprinted genes [16–18]. In humans, the process of germline reprogramming is not as well understood, but PGCs are specified and begin migrating sometime during the first four weeks of gestation, arriving in the fetal gonad between 29 and 33 days post-conception (dpc) [19].

The mechanism by which DNA methylation is lost in PGCs is not completely understood. Demethylation occurs fairly rapidly between E10.5 and E11.5, at a time when most PGCs appear to be in the G2 phase [18]. This would seem to support an active method of demethylation. The recent discovery of the TET dioxygenases, which convert mC to hydroxymethylcytosine, suggests one possible method of demethylation. *Tet1* and *Tet2* are expressed in PGCs [20,21] and could function in this capacity. Whereas *Tet1*$^{-/-}$ PGCs are reported to undergo normal demethylation [22], defects in a small number of imprinted genes were reported in Tet1$^{-/-}$; Tet2$^{-/-}$ double knockout mice, suggesting at least a partial role of hydroxymethylation in DNA demethylation [23]. Base excision repair proteins, such as PARP1, APE1, and XRCC1, have also been implicated in active demethylation during PGC reprogramming [20]. Finally, since PGCs are still undergoing mitosis during the period of demethylation, passive demethylation (where the newly synthesized strand is not methylated during S phase) could be involved. Saitou and colleagues recently showed that PGCs undergo mitosis more rapidly than previously realized, with a doubling time of approximately 12 h between E9.5 and E12.5 [24]. In addition, they found that PGCs at this time express little to no *Dnmt3a*, *Dnmt3b*, or *Dnmt3L*, and DNMT1 is not localized to replication forks. Taken together, these results support a combination of hydroxymethylation and passive demethylation as the best explanation for loss of methylation at imprinted loci in PGCs. The mechanisms of DNA demethylation will be discussed below in the context of the preimplantation embryo.

2.2.2. Timing of de novo methylation

New methylation imprints are subsequently established in females postnatally (Fig. 2B), while in males they are established beginning prenatally (Fig. 2C). Methylation of ICRs is established in the female germline during oocyte growth and is completed by the time oocytes arrest at the metaphase II (MII) stage [14,25]. Trasler and colleagues [25] analyzed the methylation status of multiple imprinted loci in young female mice, when a large number of follicles mature simultaneously. They found that primary stage oocytes from mice at 1 day post partum (dpp) completely lacked DNA methylation. DNA methylation began to accumulate on maternally methylated DMRs around 10 dpp. The *Peg3* and *Snrpn* DMRs gained methylation first, followed by the *Igf2r* DMR and finally the *Peg1* DMR. The amount of methylation correlated with the size of the oocyte. To determine whether the degree of methylation at maternally methylated ICRs was determined primarily by the age or size of the oocyte, Kono and colleagues collected oocytes from female mice at 10, 15, and 20 dpp, as well as from adults, sorted them by size, and examine methylation of multiple imprinted loci [26]. In adolescent mice, they found that the degree of methylation depended on the size of the oocyte, regardless of age. In adult mice, methylation also proceeded as oocytes grew, but oocytes of a particular size from adults generally were slightly less methylated than oocytes of the same size from adolescent mice.

Interestingly, Trasler and colleagues also found that the maternally inherited copy of the *Snrpn* gene was methylated earlier in the course of oocyte development than the paternally inherited copy [25]. This is very similar to what was observed at the *H19*/*Igf2* ICR during spermatogenesis, where the paternal allele acquired methylation in mitotically arrested prospermatogonia between E14.5 and E15.5, while the maternal allele only began acquiring methylation at E18.5 and was not completely methylated until after the onset of meiosis, postnatally [13,27]. These results indicate that the chromatin at imprinted loci carries some history of its previous methylation status, possibly in the form of other chromatin modifications, despite reprogramming in PGCs.

2.2.3. De novo methyltransferases

Methylation is accomplished by the de novo methyltransferases, DNMT3A, DNMT3B, and DNMT3L. Evidence points to DNMT3A as the major de novo methyltransferase involved in imprinting. Embryos derived from oocytes conditionally deleted for *Dnmt3a* die during embryogenesis and lack methylation of all maternally methylated germline DMRs [28]. Conditional deletion of *Dnmt3a* in the male germline results in sterility. In spermatogonia, prior to failure of spermatogenesis, methylation was absent at two of the three germline DMRs examined [28]. In contrast, DNMT3B is largely uninvolved in methylating imprinted domains. *Dnmt3b*$^{-/-}$ oocytes are fully viable and display normal methylation of all maternal germline DMRs [28]. The methylation status in *Dnmt3b*$^{-/-}$ sperm was likewise unaffected, and null sperm produced viable offspring [28]. Instead, DNMT3B is more important for the methylation of repetitive DNA, such as the centromeric minor satellite repeats [11].

The de novo methylation of germline DMRs must be targeted specifically to imprinted loci and needs to be established at different locations in males and females. Multiple mechanisms have been proposed to target de novo methylation to the correct loci. DNMT3L is an enzymatically inactive co-factor that stimulates methylation by DNMT3A and DNMT3B [29]. It is required for acquisition of DNA methylation at imprinted loci in gametes [30]. *Dnmt3L*$^{-/-}$ females were viable and produced oocytes, but the oocytes lacked all methylation at maternally methylated ICRs. If *Dnmt3L*$^{-/-}$ oocytes were fertilized, the resulting embryos died in utero. The loss of methylation appeared to be specific to imprinted loci, as genome-wide methylation was unaffected [30,31]. However, more recently, whole-genome bisulfite sequencing

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