CellPress

Review

Causes and Consequences of Multi-Locus Imprinting Disturbances in Humans

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Eight syndromes are associated with the loss of methylation at specific imprinted loci. There has been increasing evidence that these methylation defects in patients are not isolated events occurring at a given disease-associated locus but that some of these patients may have multi-locus imprinting disturbances (MLID) affecting additional imprinted regions. With the recent advances in technology, methylation profiling has revealed that imprinted loci represent only a small fraction of the methylation differences observed between the gametes. To figure out how imprinting anomalies occur at multiple imprinted domains, we have to understand the interplay between DNA methylation and histone modifications in the process of selective imprint protection during pre-implantation reprogramming, which, if disrupted, leads to these complex imprinting disorders (IDs).

Trends

Imprinted DMRs represent a small minority of the methylation differences between gametes, but somatic protection of these elements is essential to avoid developing imprinting disorders (IDs).

A subset of patients with IDs have methylation defects at single disease-associated imprinted differentially methylated regions, but other individuals may have MLID affecting additional imprinted regions.

The frequency and loci involved in MLID varies between IDs, with patients with Beckwith–Wiedemann syndrome presenting with the highest and most severe MLID cases, while this phenomenon has not been reported in patients with Angelman or Temple syndrome.

To date, mutations in three *trans*-acting factors (ZFP57, NLRP2, and NLRP5) have been associated with MLID.

Imprinting and Methylation

Imprinted genes are only transcribed from one parental allele, leading to parent-of-origin-specific expression, with allelic expression directly controlled by allelic methylation [1]. To date, all imprinted domains contain at least one differentially methylated region (DMR) that acquires methylation during gametogenesis that is maintained throughout development. Curiously, most maternally methylated DMRs encompass CpG-rich sequences, referred to as CpG islands, which are often intragenic alternative promoters, whereas paternally methylated DMRs have a significantly lower CpG content and are intergenic. Furthermore, some imprinted loci also contain DMRs that become allelically methylated in the embryonic diploid genome, termed 'somatic DMRs', which are under the hierarchical influence of germline DMRs [2] (Figure 1, Key Figure).

Life Cycle of Imprints

During early development, primordial germ cells (PGCs), which ultimately give rise to the gametes, erase the somatic cell epigenetic profile from which they originate. This demethylation includes imprints and is achieved in a two-step process involving both passive and active demethylation. PGCs initially undergo rapid expansion, ensuring replication-dependent global demethylation [3] that is aided by the transcriptional silencing of the *de novo* DNA methyltransferases [4] and the key cofactor Uhrf1 [3]. A totally unmethylated state is ensured after a second wave of demethylation [5] facilitated by activation-induced cytidine deaminase (Aid/Apobec) [6] and the ten-eleven translocation (TET) family enzymes. Tet1 and 2 are expressed in PGCs during the reprogramming window [7] and catalyze the oxidization of 5mC to 5hmC [8], which is lost during replication since it is inefficiently recognized by DNMT1 [9]. Once devoid of

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 http://dx.doi.org/10.1016/j.tig.2016.05.001

Key Figure

The Life Cycle of Epigenetic Changes at Imprinted Loci in Mice

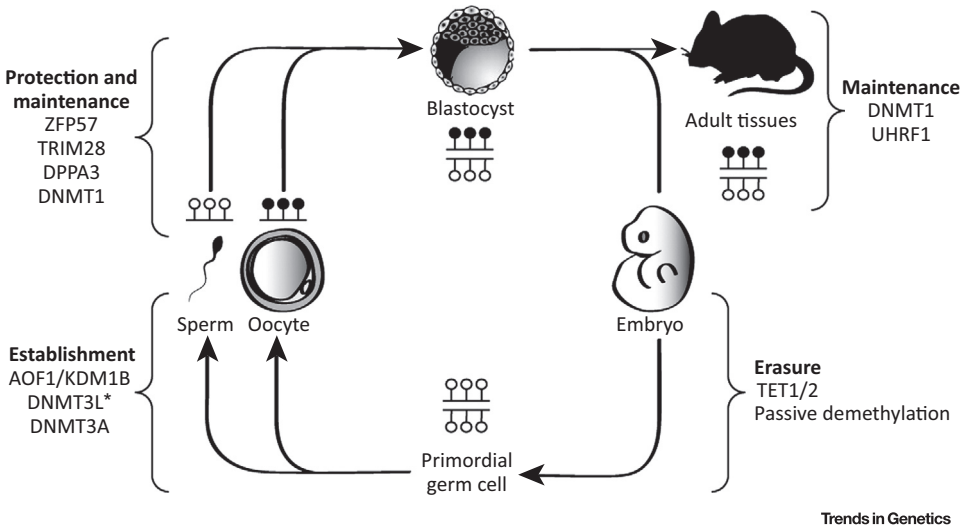

Figure 1. Regions of differential methylation are established in the germline and protected from preimplantation reprogramming by the maintenance factors DNMT1, ZFP57, and DPPA3. The allelic methylation is then preserved by the semiconservative action of DNMT1-UHRF1. In primordial germ cells of developing embryos, the DNA methylation at imprinted DMRs is erased so that the new profiles can be established according to the sex of the embryo. This complex procedure involves histone demethylation of H3K4 and the subsequent recognition and DNA remethylation by the DNMT3L-DNMT3A complex. *Note that *DNMT3L* is not expressed in human oocytes, suggesting different recruiting methods between species.

methylation, sex-specific remethylation in PGCs occurs, giving rise to the methylation profile of the gametes that includes germline imprints. Studies in mice reveal that *DNMT3L* regulates the *de novo* methylation activity of DNMT3A on DMRs by stimulating its enzymatic activity and facilitating binding to unmodified H3K4 (H3K4me0) [10–14]. During pre-implantation development, imprinted methylation is protected against **embryonic epigenetic reprogramming** (see Glossary). Both maternal and zygotic Dnmt1 are required for maintaining methylation imprints [15,16]. Several other proteins have also been implicated in the maintenance of the maternal and paternal DNA methylation at DMRs: DPPA3 (also known as PGC7/Stella) and the KRAB zinc finger protein ZFP57 protein, both of which bind conserved recognition sequences in mice and humans [17–19] (Figure 2). During postimplantation development and in adult tissues, imprinted methylation is maintained by DNMT1-UHRF1 [20]. Converse to the demethylation wave in the preimplantation embryo, there is a *de novo* DNA methylation wave at the time of implantation from which the unmethylated alleles of DMRs require protection, which has been shown to involve CTCF, OCT4, and the permissive histone modification H3K4me2/3 [2,21,22].

Imprinting Disorders and Aberrant DNA Methylation

Alterations in any of the above processes can lead to aberrant imprinting, which can result in either the reactivation of the original silent allele or the silencing of the previously active allele. Since methylation profiles are faithfully copied during replication, an abnormal imprinted methylation profile will be maintained through somatic development and be present in multiple tissues. If methylation defects occur in only a few cells of the preimplantation embryo, then somatic mosaicism will result [23].

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