The role of DNA methyltransferase 1 in maintaining [illegible] methylated regions associated with imprinted genes

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Introduction to Genomic Imprinting

Some mammalian genes have monoallelic expression where either only the paternal allele or only the maternal allele is expressed (Figure 1). This mammalian phenomenon is known as genomic imprinting. Differential methylation of DNA on the cytosine of a CpG dinucleotide determines the expression of imprinted genes where the methylated allele is typically silenced and the unmethylated allele is expressed. These differentially methylated regions (DMRs) are known to be maintained by DNA methyltransferase 1 (Dnmt1). Primary (1°) DMRs are established in the germ cells and are consistently maintained throughout growth and development while secondary (2°) DMRs are acquired during embryogenesis and are more variable.

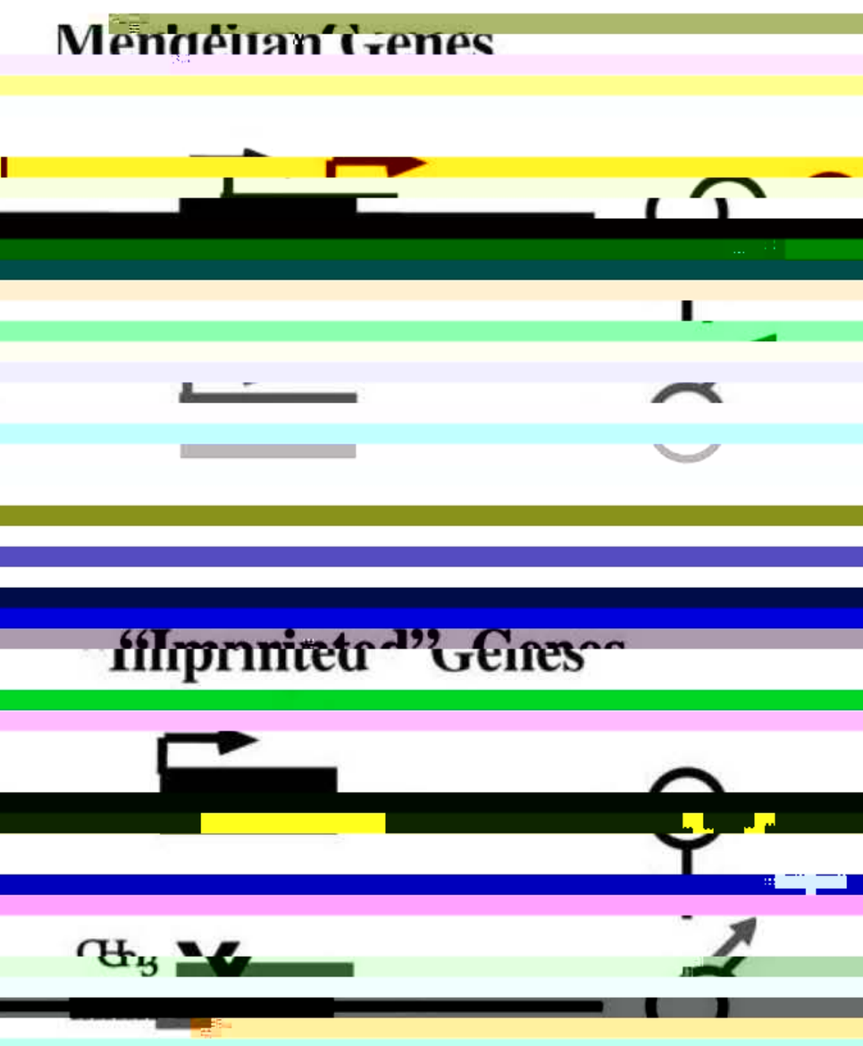

Figure 1. Mendelian genes vs. imprinted genes. Arrows represent expression; X represents lack of expression. Source: Vrana (2007) Journal of Mammalogy 88(1):5–23.

The Role of Dnmt1 in maintaining methylation at secondary DMRs

Maintaining differential methylation is essential for [illegible] genes. Failure to maintain methylation can result in [illegible] Silver-Russell and Beckwith-Wiedemann syndromes, which affect growth and development. We hypothesize that [illegible] DMRs is not well maintained and hence may need to be re-acquired, involving multiple DNA methyltransferases. To test this hypothesis, we studied the role of Dnmt1 by exploring the methylation patterns of primary and secondary DMRs at imprinted genes in mice bearing a loss of function mutation in *Dnmt1* [illegible] compromised methyltransferase activity (P allele). The P mutation [illegible] substitution of the mouse-specific sequence with the rat-specific sequence in the intrinsically disordered domain (IDD) of *Dnmt1* (Figure 2). When homozygous, the mutant P allele results in late embryonic lethality, likely as a result of a dramatic reduction in global methylation. In contrast, primary DMRs showed a far less drastic reduction in methylation when compared to WT and P/+ embryos, suggesting that *Dnmt1* may function differently at different sequences (Shaffer *et al.*, 2015).

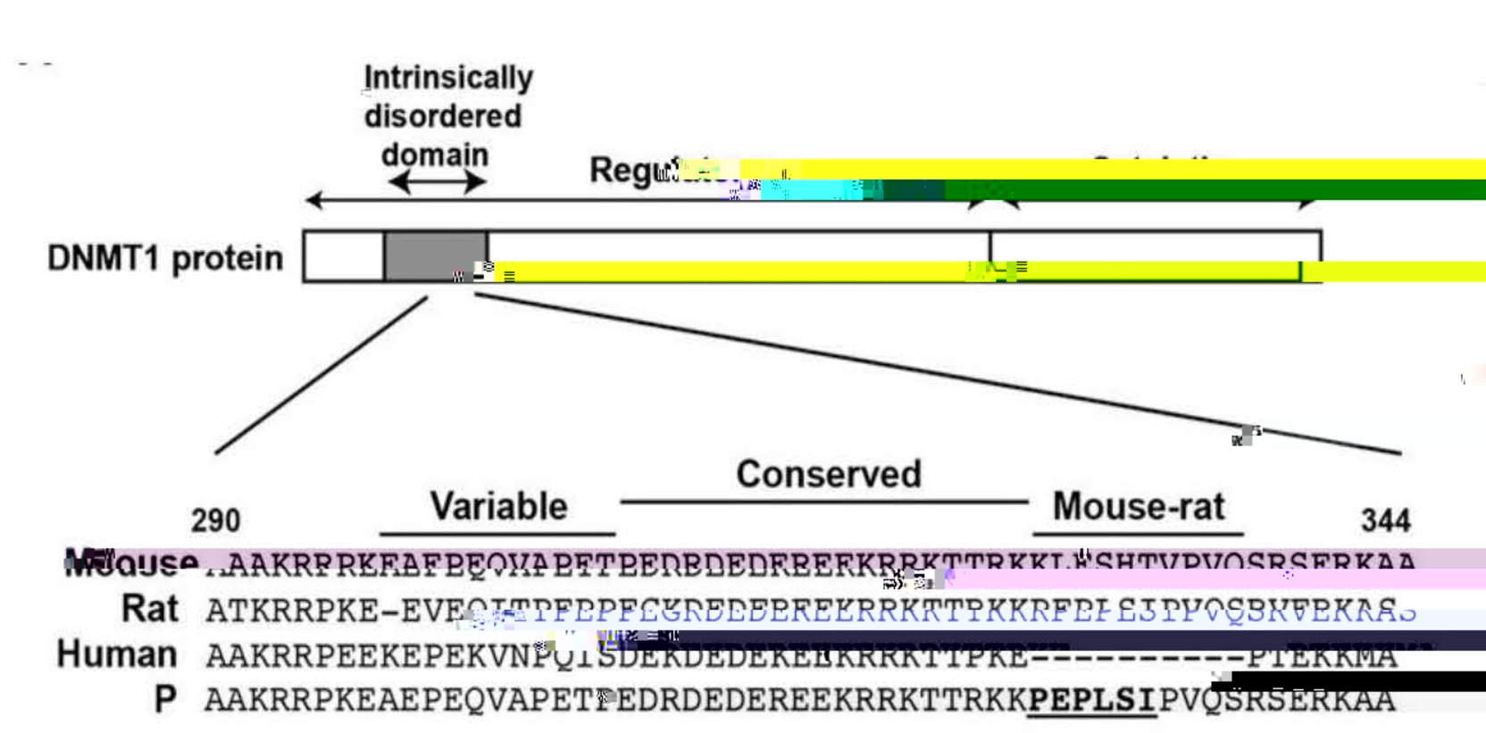

Figure 2. A section of the IDD of Dnmt1 in different mammals and the position of the P allele mutation. [illegible] Shaffer et al. (2015). Genetics [illegible]

Figure 3. [illegible] dpc old embryos with placentae from a cross between two mice heterozygous for the P allele [illegible] embryos and placentae are smaller in size as compared to their WT and P/+ littermates [illegible]

A comparison of methylation patterns in [illegible] heterozygous [illegible] maintaining DMRs and [illegible] our understanding of the regulation of imprinted genes, especially how different epigenetic domains interact to regulate expression at clusters of imprinted genes. We studied homozygous mutant embryos using bisulfite mutagenesis to determine how methylation patterns change over time at DMRs. We investigated the methylation patterns at a [illegible] of 16 DMRs – 7 primary DMRs and 9 secondary DMRs (summarized in Table 1). For 7 DMRs, we analyzed P/P embryos at 12.5 days post coitum (dpc) as well as WT and P/+ controls using bisulfite mutagenesis and sequencing of individual subclones. [illegible] analyzed 15 DMRs in 15.5 dpc WT and P/P embryos using bisulfite mutagenesis and Next Generation Amplicon Sequencing.

Table 1. Summary of DMRs analyzed [illegible]

Name	Type		Studied
[illegible]	[illegible]	[illegible]	[illegible]

Methodology

Studying methylation pattern using bisulfite mutagenesis

Figure 4. (A) Chemistry of bisulfite mutagenesis. [illegible] Cytosine is converted into uracil. When amplified by PCR, uracil is replaced by thymine. [illegible]

Embryonic DNA extracted and genotyped

Polymerase Chain Reaction (PCR) amplifies region of interest

Region of interest ligated to plasmid vector

[illegible] subcloning and competent cells transformed

Individual subclones sequenced and analyzed to determine methylation

Next Generation Amplicon Sequencing

[illegible] 15.5 dpc [illegible] DMRs compared to [illegible] DMRs with some exceptions

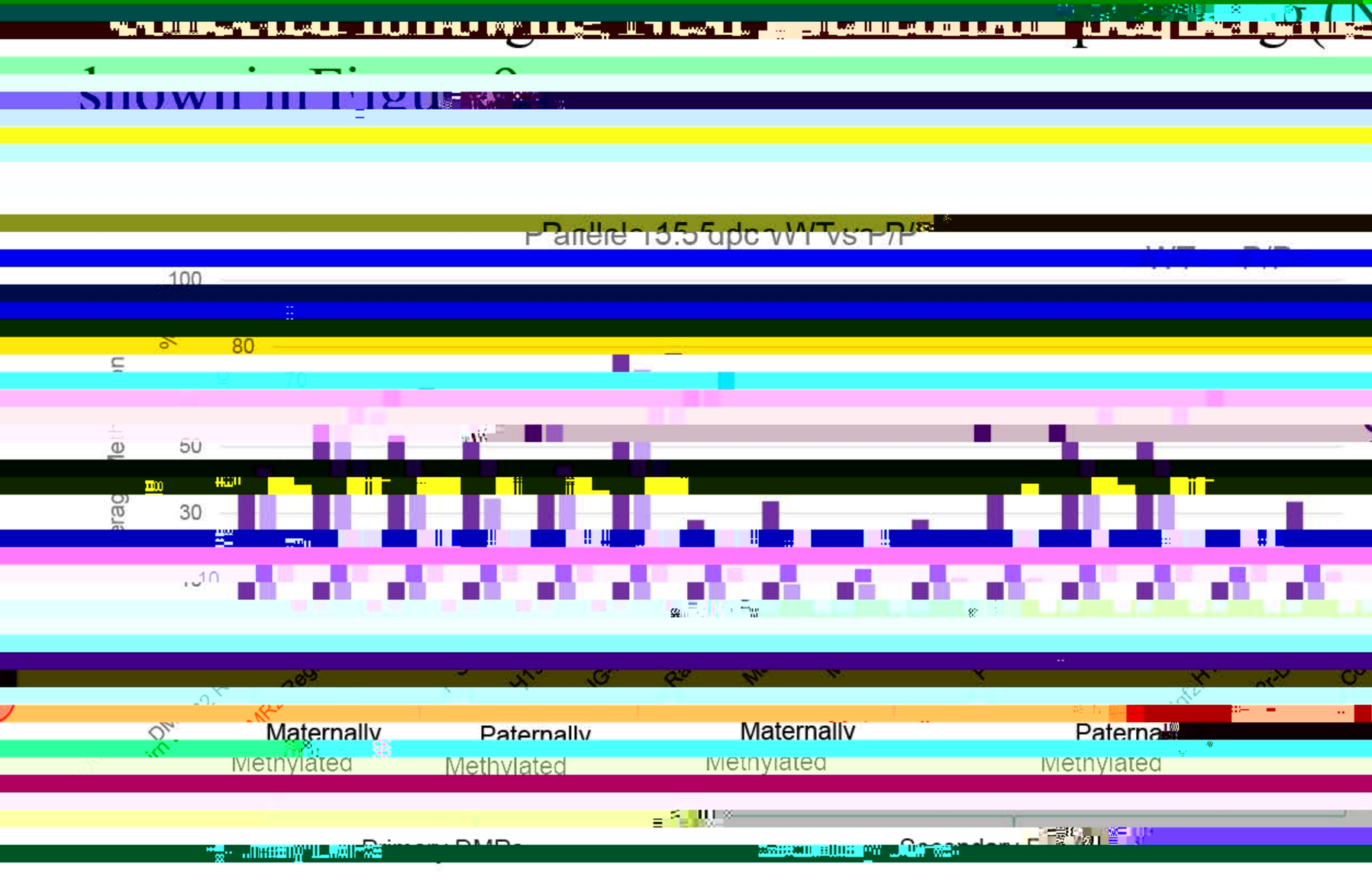

Figure 9. Average methylation at the 15 analyzed DMRs in WT vs. P/P 15.5 dpc embryos.

Data is a randomly extracted 2% subset of total NGS data. [illegible] The absence of circles represents anomalous data.

In general, the data from 15.5 dpc embryos showed that most [illegible] DMRs have drastic reduction in methylation [illegible]

Methylation at [illegible]

[illegible]

Acknowledgements

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