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Maternal regulation of imprinting

Parental imprinting is the process by which the expression of the alleles at some genetic loci depends on the parent-of-origin. A central question since the discovery of parental imprinting concerns the mechanism, which underpins the "imprinting" event that causes the allele-specific expression during development (for reviews see Metz 1938; Hughes-Schrader 1948). Exploring this question in insects and mammals has shown that there are a relatively greater number of genes that are imprinted during oocyte development, when compared to spermatogenesis. At one extreme, in certain soft-scaled insects such as *Pulvinaria hydrangeae* imprinting is entirely regulated by the oocyte (Nur 1963, 1972). As described by Nur, parthenogenetically-derived females are usually produced by *P. hydrangeae* ($2n = 16$). However, parthenogenetic males could sometimes be found in percentages ranging from 1 to 60. They could be easily identified because one haploid set of 8 chromosomes becomes heterochromatic during early embryonic stages and remains so thereafter. Careful cytogenetic analyses showed that female meiosis is normal in this species and resulted in the expected outcome of two polar bodies and a haploid nucleus. Of particular significance was the observation that this egg nucleus divides once again and the daughter nuclei fused to form a diploid zygote substitute that gives rise to completely homozygous progeny. More importantly, it was observed that in eggs destined to become male, imprinting of one of the two daughter nuclei occurs in the brief interval between the division of the egg nucleus and the subsequent fusion of the daughter nuclei to form the diploid zygote substitute. It is in this brief interval that one of the daughter nuclei moves into a cytoplasmic region where it undergoes imprinting. In eggs destined to become female, either the egg cytoplasm is not "conditioned" to make male embryos, or, that neither daughter nucleus moves into the requisite region of the cytoplasm. This scheme would suggest that the egg cytoplasm can be compartmentalized and that the paternal genome is extensively remodeled after entry into the maternal ooplasm (Chandra and Brown 1975).

These essentially descriptive analyses have been greatly extended by molecular studies in mammals, which have described a mechanism for the "imprinting" event that marks imprinted genes in the developing oocyte. In mammals, there are nearly 50 imprinted genes and the imprinting mechanism involves DNA methylation of differentially methylated regions (DMRs) that results in allele-specific expression of imprinted genes (Li *et al* 1993). Most imprinted genes acquire their methylation imprint in female germ cells (Obata *et al* 1998). Two recent papers have addressed the issue of how methylation imprints are laid down in developing oocytes (Bourc'his *et al* 2001; Hata *et al* 2002). How imprinted genes are specifically methylated is unknown because the mammalian *de novo* methylases (*Dnmt3a* and *Dnmt3b*) and the maintenance methylase (*Dnmt1*) lack any sequence specificity beyond the recognition of the CpG dinucleotide (Okano *et al* 1999). Since CpG's are scattered throughout the genome the problem is how the methyltransferases know which CpG's to methylate. This problem of specificity was recognized some time ago and led to the suggestion that "imprintors" (which can be proteins or RNA) target *de novo* methylases to CpG's associated with imprinted genes so that they can be methylated (Singh 1994; Singh and James 1995). The studies of Bourc'his *et al* (2001) and Hata *et al* (2002) have defined the first "imprintor" as Dnmt3L. The *Dnmt3L* gene shares homology with *Dnmt3* family of methyltransferases but lacks the methyltransferase activity of the other members, *Dnmt1*, *Dnmt3a* and *Dnmt3b*. Strikingly, mutation of *Dnmt3L* results in the loss of maternal methylation imprints and the abnormal expression of maternally-inherited imprinted genes during development. Biochemical evidence indicates that regulation of methylation by Dnmt3L is achieved by its association with and targeting of *de novo* methyltransferases, *Dnmt3a* and *Dnmt3b*, to DMR sequences within imprinted genes.

It is not clear whether Dnmt3L is involved in targeting CpG methylation imprints in humans. In a parallel study to those of Bourc'his *et al* (2001) and Hata *et al* (2002), Judson *et al* (2002), describe a human female that had lost the ability to methylate imprinted genes in the oocyte. However, there was no identifiable mutation in the *DNMT3L* gene in this individual, indicating that other mechanisms for targeting CpG methylation may be operating. One possibility, drawing from the earlier work on insects, is that heterochromatin complexes might target *de novo* methyltransferases (Singh 1994).

Little is known about the mechanism(s) of how specific genes are imprinted in the male germ-line in mammals. The evidence that CpG methylation imprints acquired during spermatogenesis (Tremblay *et al* 1995) cause the CpG methylation that is necessary for maintenance of allele-specific expression in the embryo and the adult is, strictly speaking, circumstantial. This uncertainty arises because it is not possible to specifically modify single CpG dinucleotides without changing DNA sequence. It seems the possibility remains that, as with the situation in *P. hydrangeae*, paternal imprints could be placed after fertilization and be under maternal control. This may not be as far-fetched as it seems, for work in mice shows that the oocyte cytoplasm contains factors that can cause epigenetic changes with phenotypic consequences later in development (Babinet *et al* 1990). Such factors have all the characteristics for regulating imprinting in that short space of time between fertilization and syngamy, where the parental genomes are separate and the paternal genome is being remodeled in the developing male pro-nucleus by the ooplasm.

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