Evolution of imprinting mechanisms: the battle of the sexes begins in the zygote

Imprinted genes in the mammalian genome are those genes for which one of the parental alleles is repressed, whereas the other one is transcribed[1,2]. Forty imprinted genes are currently known in the mouse (excluding antisense transcripts), and maternally and paternally repressed genes are represented equally[3]. Almost all imprinted genes have differentially methylated regions (DMRs); some DMRs are methylated differently in egg and sperm, and these differences can be inherited in the somatic tissues of the offspring[1,2]. DNA methylation is normally associated with gene silencing[4]; however, 7 of 18 imprinted genes have DMRs that are methylated on the active allele[3]. In mouse embryos that fail to maintain methylation because of a deficiency in DNA methyltransferase I, some imprinted genes (*H19*, *Snrpn*) are expressed from both parental alleles, but others (*Igf2*, *Igf2r*) are silenced on both parental alleles[5,6]. Imprinted genes can be silenced 'epigenetically' by mechanisms such as promoter methylation, or 'genetically' by mechanisms involving, for example, antisense RNA, silencers or chromatin boundaries[1] (Fig. 1). Significantly, in this case the allele is silent in the absence of epigenetic modification, and the methylation of such silencing elements is required for the expression of the imprinted gene. Here we show that, for most genes from either category, the methylation imprint is derived from the oocyte, and attempt to provide an explanation for this intriguing asymmetry.

Table 1 lists imprinted genes together with their antisense transcripts (if identified) and the methylation patterns that most likely reflect their germline imprint[3]. It is striking that all antisense transcripts discovered so far (seven) are maternally repressed (this excludes the X-linked gene *Xist*, which has a paternally repressed antisense transcript[7]). By contrast, with two exceptions (*H19* and *Rasgrf1*), the currently known germline methylation imprints are imposed in the maternal germ line (12 maternal, 2 paternal). Thus, maternally repressed genes are mostly 'epigenetically' silenced by maternal methylation (Fig. 1). By contrast, of the seven paternally repressed genes (Table 1), five have maternally repressed antisense transcripts, and four have maternal methylation patterns that are thought to be germline imprints. Significantly, no protein-coding gene has been found that is paternally repressed and paternally methylated (Table 1). It is evidently more common for paternally repressed genes to be silenced 'genetically' by means of paternal antisense (or other mechanisms) than 'epigenetically' by paternal methylation (Fig. 1). Functional analyses using nuclear transplantation confirm that the acquisition of maternal imprints in oocytes is important for the silencing of maternally repressed genes (*Peg1*, *Peg3*, *Snrpn*) as well as for the expression of maternally expressed genes[8] (*Igf2r*, *p57Kip2*), but the acquisition of paternal imprints is important only for the repression of the paternal copy of *H19* (ref. 9).

We suggest that this asymmetry has arisen because of the active and genomewide demethylation of the paternal genome in the mouse zygote[10,11]. This would make difficult the inheritance of paternal methylation in DMRs, as observed for *Igf2* (ref. 10). Paternal demethylation does not occur in the zebrafish[12] (which does not have imprinting), but occurs in other mammalian species with imprinting (W. Dean *et al.*, unpublished data). Demethylation of the paternal genome can be viewed as a reprogramming mechanism by which the egg (that is, the maternal genome) strips off paternal imprints when the paternal genome is at its most vulnerable, consistent with proposals of the genetic conflict theory of imprinting[13]. One can therefore speculate that if paternal demethylation evolved after imprinting, the primordial imprinting mechanism might have been a simple one in which maternal methylation led to maternal repression (as it does now) and paternal methylation led to paternal repression. Alternatively, imprinting might have arisen after paternal demethylation.
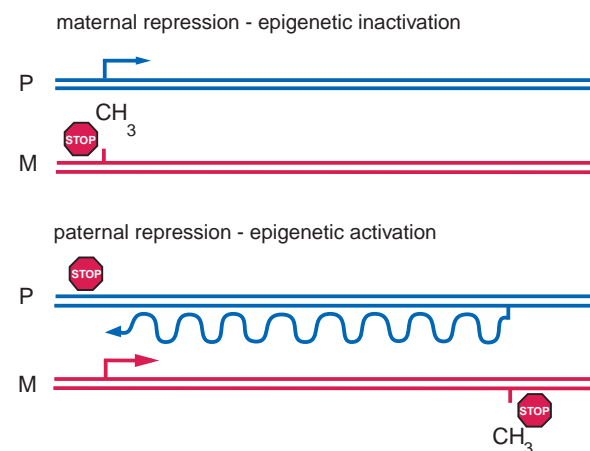

Fig. 1 Epigenetic silencing and epigenetic activation. A maternally repressed gene is maternally methylated and thereby silenced. A paternally repressed gene is silenced by an antisense transcript (or other mechanisms). The maternal copy of the antisense transcript is silenced by DNA methylation and the sense transcript is therefore expressed.

Table 1 • Methylation and antisense transcripts in imprinted genes

	Repression	Methylation	Repression of antisense
H19	PAT	PAT	
Rasgrf1	MAT	PAT	
Igf2	MAT		MAT
Kcnqt1	PAT	MAT	MAT
p57Kip2	PAT	(MAT?)	
Peg1/Mest	MAT	MAT	
Peg3	MAT	MAT	
Nnat	MAT	MAT	
Snrpn	MAT	MAT	
Znf127	MAT	MAT	MAT
Ube3a	PAT		MAT
Igf2r	PAT	MAT	MAT
*U2afbp-rs*1	MAT	MAT	
Nesp	PAT	MAT	MAT
Impact	MAT	MAT	
Ndn	MAT	MAT	
Copg2	PAT		MAT
Total	10 MAT 7 PAT	12 MAT 2 PAT	7 MAT

Data are from ref. 3. The methylation of *p57Kip2* is shown in brackets because although the gene shows paternal methylation (in mouse only and not in humans), functional analyses indicate that the imprint is acquired during oogenesis[8].

correspondence

Subsequent to the emergence of paternal demethylation, paternally repressed genes evolved the 'genetic' mechanisms of silencing and the 'epigenetic' mechanism of activation. That there is significant evolution of imprinting mechanisms is supported by the recent finding in the opossum that the *Igf2r* gene is paternally repressed (as in the mouse), but the mechanism does not involve a maternally methylated DMR in intron 2 (ref. 14).

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