EMBO MEMBER’S REVIEW

Competition—a common motif for the imprinting mechanism?

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Imprinted genes, in contrast to the majority of mammalian genes, are able to restrict expression to one of the two parental alleles in somatic diploid cells. Although the silent allele of an imprinted gene appears to be transcriptionally repressed, it often bears little other resemblance to normal genes in an inactive state. The key to the imprinting mechanism may be a form of parental-specific expression—competition between cis-linked genes and not parental-specific expression versus repression. Thus, the imprinting mechanism may be better understood if the chromosomal region containing imprinted genes is viewed as ‘active’ on both parental chromosomes.

Keywords: expression-competition/imprinting mechanism/monoallelic expression

Introduction

Imprinted genes represent ~0.1% of genes in the mammalian genome and have the special property of restricting expression to either the maternal or paternal allele in the somatic diploid cells of embryos and adults (for reviews, see Cattanach and Jones 1994; Barlow 1995; John and Surani, 1996; Leighton et al., 1996). A striking feature of imprinting is that the choice of the expressed allele is unvarying in successive generations and is predetermined by the parent of origin, suggesting that the gametes themselves can specifically modify embryonic gene expression. Furthermore, the gametic modification responsible for conferring parental-specific expression should have an epigenetic basis since imprinting can occur in mammals with genetically identical parental alleles (i.e. inbred mouse strains) as well as in outbred populations with a large degree of heterozygosity. The sheer abundance of data that correlates gene silencing with altered chromatin states has led to the expectation that the epigenetic imprinting mechanism would induce similar chromatin modifications at the silent allele of an imprinted gene. Remarkably, the emerging data do not support this expectation.

Transcriptional silence at imprinted loci

The repressed allele of an imprinted gene shows three special features. First, in all cases that have been examined, the repressed parental allele shows a drastic reduction of mRNA compared with the expressed allele. However, measurements have been made mainly of steady-state RNA pools and, although it is assumed to represent transcriptional repression, this has only been demonstrated for the H19 imprinted gene (Ferguson-Smith et al., 1993). Second, repression is not complete, and in some cases the repressed allele is active to ~5% of the level produced by the expressed allele. It is unknown whether every cell in a population is repressed to the same extent, or whether repression is stochastic and thus a small number of individual cells in a population continue to show biallelic expression. Last, monoallelic expression is not absolute and can vary in development and disease. For example, some imprinted genes show biallelic expression in the pre-implantation mouse embryo and monoallelic expression in the post-implantation embryo (Szabo and Mann 1995; Lerchner and Barlow 1997); and for some genes, monoallelic expression can be a polymorphic trait in human populations (Xu et al., 1997).

Epigenetic modifications on imprinted genes

The absence of stable mRNA from the repressed imprinted allele has obvious parallels with the tissue-specific repression of normal genes (‘normal’ describes non-imprinted genes that are biallelically expressed or repressed). Normal genes repressed in a tissue-specific manner show an absence of stable mRNA compared with abundant transcripts when the locus is expressed. They also show changes in the degree of DNA methylation, the presence of bound activator/repressor molecules and the type of chromatin in the vicinity of the gene and promoter. Transcriptionally silent chromatin is often highly condensed into heterochromatin that is typified by histone accumulation and hypoacetylation, and the DNA in heterochromatin is less accessible to nucleases and shows a delay in replication (reviewed by Hendrich and Willard, 1995; Henikoff and Matzke, 1997; Wolfe et al., 1997). Transcriptionally active genes are embedded in euchromatin which displays the reverse of these features. The repressed and expressed allele of an imprinted gene clearly share the feature of modulating steady-state mRNA levels, but many of the other features typical of heterochromatin are either unaffected or fail to show the expected correlation.

Sasaki et al. (1992) were the first to note this apparent contradiction for the mouse Igf2 gene. Igf2 (insulin-like growth factor type 2) is a paternally expressed imprinted gene (DeChiara et al., 1991), but embryonic tissues that show paternal-specific expression have potentially active chromatin at the repressed maternal allele, as judged by hypomethylation and nuclease sensitivity. Again, in the case of Igf2r (insulin-like growth factor type 2 receptor, also known as the cation-independent mannose 6-phosphate receptor, Barlow et al., 1991), a scavenging receptor...
for IGF2, which is a maternally expressed imprinted gene, 95% of the DNA is equally methylated on both parental alleles over a 130 kb region in embryonic tissues that show maternal-specific expression. While neither Igf2 nor Igf2r show large-scale changes in methylation and chromatin organization, they do have small local patches of allele-specific methylation. This is more clearly seen in the Igf2r locus, where two distinct 2 kb patches are present, one covering the promoter of the repressed paternal allele, and the other lying in the second intron of the expressed maternal allele. Two, less distinct patches of methylation have also been detected on the expressed paternal Igf2 allele (Feil et al., 1994). H19, a maternally expressed non-coding RNA (known just by this label, Bartolomei et al., 1991), is the only other imprinted gene to be examined in the same detail and shows, in contrast to Igf2 and Igf2r, more widespread methylation and also chromatin changes. Methylation of the repressed paternal H19 allele is concentrated over the promoter and upstream region, but is also present over the body of the gene (Tremblay et al., 1995). The promoter region of the maternally expressed H19 allele has also been reported to show increased nuclease sensitivity compared with the repressed paternal allele in late gestation embryos (Ferguson-Smith et al., 1993).

The remaining 19 mammalian imprinted genes have not been examined in the same detail, and some, but not all, are also associated with local patches of allele-specific methylation. In three cases (Igf2r, H19 and Xist), one patch of methylation was shown to be inherited from one of the gametes, suggesting that it could function as a primary imprinting signal. In the remaining cases, allelic methylation imprints were present, but shown to be acquired later in embryogenesis. Interestingly, these allele-specific methylation imprints were found as often on the repressed as on the expressed allele (for reviews on methylation and imprinting, see Bird 1993; Coxon and Bestor 1993; Razin and Shemer 1995; Bestor and Tycho, 1996; Neumann and Barlow, 1996; and for a list of imprinted genes, see Lyle 1997). In addition to methylation studies, the parental alleles of imprinted loci have been shown to replicate and/or segregate asynchronously. However, in some cases, the repressed parental allele was identified as early replicating and in other cases the expressed allele was early replicating (Kitsberg et al., 1993; Knoll et al., 1994; Bickmore and Carothers, 1995).

A role for DNA methylation in imprinting

In view of the long association between hypermethylation and tissue-specific repression of normal genes, it is important to note that a role for DNA methylation in regulating imprinted monoallelic expression in vivo has been confirmed. Targeted gene disruption of the methyltransferase gene (reviewed by Jaenisch, 1997) reduced genomic methylation by >95% and induced embryonic lethality in the early post-implantation mouse embryo. Strikingly, changes in normal gene expression were not detected prior to embryonic death, but three tested imprinted genes completely lost allele-specific expression. Importantly, for two imprinted genes, a correlation was seen between the presence of a methylation imprint inherited directly from the gamete and the effect seen in the methylation-deficient embryos. For example, Igf2r, which inherits a gametic methylation imprint on the expressed maternal allele, became repressed on this allele in the absence of methylation. H19, which inherits a methylation imprint on the repressed paternal allele, became derepressed and active on this allele. Thus, methylation correlates with activity of the expressed Igf2r allele, but with inhibition of the repressed H19 allele. The third gene examined in this
work, Igf2, has not been associated with a gametic methylation imprint, and followed the behavior of Igf2r, by showing repression of the active parental allele.

Overall, this key experiment neatly demonstrated a role for methylation in imprinting, but did not show if methylation caused monoallelic expression or merely maintained this state. This review will consider two ways that methylation could act to cause monoallelic expression. The first and simplest model is that methylation could directly modify one parental allele and allow the binding of factors that would induce a heterochromatic state and thereby inhibit the binding of transcription activators on this allele. Figure 1A shows a model whereby parental-specific expression occurs when a ‘target imprinted gene’ has been methylated and repressed on one allele, but is unmodified and expressed on the other parental allele. Figure 1B shows a second model whereby methylation is not acting directly on the target imprinted gene but modifies a flanking ‘imprintor’ gene that has a regulatory role. ‘Imprintor’ here defines a gene that regulates expression of a cis-linked target gene; the imprintor gene is also proposed to lack an independent function except that of cis regulation of flanking genes. In this model termed ‘expression-competition’ (initially proposed for the Igf2–H19 gene pair, Bartolomei and Tilghman, 1992; Pfeiffer and Tilghman, 1994), an imprinted gene on one parental allele is modified by methylation and repressed, and a closely linked target imprinted gene is expressed. On the other parental allele, the imprintor is unmethylated and expressed, and this correlates with repression of the linked target. The nature of the interaction between the ‘imprintor’ and ‘target imprinted gene’ is not specified in the figure.

Despite the fact that the expression-competition model increases the level of complexity of the imprinting mechanism, four features of imprinted genes point in the direction of expression-competition between cis-linked genes. These are: (i) imprinted genes often have generally active chromatin on both alleles (with some exceptions, see the caveat above); (ii) closely linked imprinted genes often show reciprocal parental-specific expression, for example Igf2 is paternally expressed while H19 is maternally expressed; (iii) imprintor genes have no coding requirement, and imprinted non-coding RNAs (such as H19) are often closely linked to imprinted coding mRNAs; (iv) the model offers an explanation for the behavior of imprinted genes in methylation-deficient mutant mice whereby some imprinted genes such as Igf2 and Igf2r were silenced, but H19 and Xist became active on both parental alleles.

The Igf2–H19 gene pair—a paradigm for expression-competition

The isolation of the maternally expressed imprinted H19 gene which lies 90 kb downstream from the paternally expressed imprinted Igf2 gene (Bartolomei et al., 1991) drew attention to the fact that the physical grouping of imprinted genes may reflect aspects of the imprinting mechanism. A model was proposed (Bartolomei and Tilghman, 1992) whereby Igf2 and H19 are imprinted because they compete in cis for a shared set of regulatory elements. In this model, maternal H19 expression is driven by the shared regulatory elements and Igf2 is inactive (see Figure 2A), but, on the paternal chromosome, H19 is modified and inactive and Igf2 now has access to the regulatory elements. A methylation imprint upstream of the H19 gene on the paternal allele is predicted to be the modification that blocks access of H19 to the shared regulatory elements (Tremblay et al., 1995). The importance of these upstream sequences has been demonstrated recently by showing that paternal expression of H19 transgenes can be restored if this element is deleted (Elson and Bartolomei, 1997). Since the basis of the model is competition, co-expression of Igf2 and H19 in cis, i.e. on the same chromosome, would not normally occur. However, biallelic expression of Igf2 has been demonstrated in adult tissues of mice and humans and in disease conditions, suggesting that the competition between H19 and Igf2 for the shared regulatory elements can be overridden in some circumstances. For example, it has been shown that a different promoter, presumed to be independent of the shared regulatory elements, drives biallelic expression of IGF2 in human adult liver (Vu et al., 1994).

The expression-competition model (Bartolomei and Tilghman, 1992) provided a satisfactory explanation for the silencing of Igf2 and the gain of biallelic expression of H19 in the methylation-deficient mice described above. If H19 functions only as a cis imprintor of Igf2, other predictions can also be tested. The first of these, that deletion of H19 will derepress Igf2 on the maternal chromosome, has been confirmed by gene deletion experiments (Leighton et al., 1995). The second prediction, that H19 has no independent function aside from repressing Igf2, is supported, though not proven, by the observation that deletions of H19 generate no additional phenotype in mice that also carry a targeted disruption of Igf2 (Leighton et al., 1995), and by the observation that transgenes expressing high amounts of H19 do not induce a defect (Pfeiffer et al., 1996).

The Igf2r–AS-RNA a new target–imprintor pair?

Maternal-specific expression of the Igf2r gene is associated with a maternal-specific gametic methylation imprint that
is present on a CpG island lying in the second intron (Figure 2B). The interpretation that Igf2r expression depended on methylation to inhibit the binding of a repressor (Stöger et al., 1993) was supported by the complete loss of expression seen in mice lacking genomic methylation (Li et al., 1993). Recently, we have used yeast artificial chromosome (YAC) transgenes to examine the role of chromosome location and the role of the intronic CpG island in Igf2r imprinting (Wutz et al., 1997). These results showed that the chromosome location played no role, but imprinting of Igf2r was completely dependent on the intronic CpG island. Deletion of this intronic CpG island from the YAC transgenes caused derepression of the paternal Igf2r allele, mirroring the observation in methylation-deficient mice. Interestingly, a novel paternally expressed non-coding RNA that originates from this intronic CpG island (named antisense RNA or AS-RNA, O.W.Smrzka, unpublished data) is no longer expressed following deletion of the intronic CpG island from these transgenes (Wutz et al., 1997). These results, which suggest that Igf2r imprinting also depends upon a form of expression-competition between the Igf2r promoter and the intronic CpG island AS-RNA promoter, have strong parallels with the imprinting of the Igf2r–H19 gene pair (Figure 2).

**Imprintors and target genes**

The two examples in Figure 2 show an ‘imprintor and target imprinted gene’ pair. However, it is already clear from the H19–Igf2 locus that deletions of H19 not only relieve repression of the 100 kb distant Igf2 gene, but also derepress insulin which lies 10 kb further upstream (Figure 2). Thus, the organization of imprinted genes is likely to be more complex than a simple one-to-one relationship. The three examples discussed below (the human Beckwith–Wiedemann syndrome, the Prader–Willi/Angelman syndrome and X inactivation) are interpreted in terms of the expression-competition and suggest two further features of this model. First, that imprintors are involved in concert. In contrast to BWS, paternal deletions of the target imprinted gene should lead to derepression of the latter. Instead, two distinct translocation clusters are seen that lie 0.7 and 5 Mb upstream of Igf2. Only the proximal cluster breakpoints have been isolated, and all of them are contained within a known gene called KvLQT which is mutated in the long QT heart arrhythmia syndrome (Lee et al., 1997). Interestingly, with respect to the imprintor gene model, non-coding, maternally expressed KvLQT RNA isoforms are produced in most tissues (the functional KvLQT mRNA is biallelically expressed in heart, thus long QT is not an imprinted syndrome). The correlation between distant maternal translocation breakpoints and elevated Igf2 suggests that distant genes, as well as the closely linked H19 gene, are imprintors of Igf2. However, this prediction needs to be tested by targeted gene deletion of the KvLQT gene in mice.

**Beckwith–Wiedemann syndrome (BWS)**

This is a human fetal overgrowth condition associated with increased tumor predisposition (reviewed by Lalande, 1997; Reik and Maher, 1997). BWS maps to human chromosome 11p15 to the region containing the IGF2 and H19 loci, whose imprint status is conserved between mice and humans (Figure 3). BWS mainly arises from two types of chromosome anomaly, paternal duplications and maternal translocations, both of which are associated with elevated Igf2 levels. Since IGF2 is paternally expressed, paternal duplications will increase IGF2 levels. The maternal-specific translocations do not, however, physically separate H19 from IGF2. In analogy with the mouse model, translocations that separate an imprintor from the target imprinted gene should lead to derepression of the latter. Instead, two distinct translocation clusters are seen that lie 0.7 and 5 Mb upstream of IGF2. Only the proximal cluster breakpoints have been isolated, and all of them are contained within a known gene called KvLQT which is mutated in the long QT heart arrhythmia syndrome (Lee et al., 1997). Interestingly, with respect to the imprintor gene model, non-coding, maternally expressed KvLQT RNA isoforms are produced in most tissues (the functional KvLQT mRNA is biallelically expressed in heart, thus long QT is not an imprinted syndrome). The correlation between distant maternal translocation breakpoints and elevated Igf2 suggests that distant genes, as well as the closely linked H19 gene, are imprintors of Igf2. However, this prediction needs to be tested by targeted gene deletion of the KvLQT gene in mice.

**Prader–Willi syndrome (PWS) and Angelman syndrome (AS)**

These syndromes affecting respectively hypothalamic (PWS) and general CNS (AS) function, map to human chromosome 15q11–13 and provide another example whereby multiple imprinting genes appear to be regulated in concert. In contrast to BWS, paternal deletions of 15q11–13 generate the distinct PWS phenotype while maternal deletions generate the AS phenotype. This suggests the existence of two imprinted genes, one paternally and one maternally expressed, that encode distinct neural functions (reviewed by Lalande, 1997). A transcription map spanning breakpoints for both PWS and AS contains nine transcripts described as imprinted (Figure 4). Many of these (e.g. IPW) produce stable non-coding transcripts, however, some non-coding RNAs (e.g. PAR1, PAR5, PARSN and ASR1.2) have only been identified by RT–PCR assays and may not represent stable RNAs. The SNRPN gene spans 360 kb of DNA and produces both coding and non-coding imprinted transcripts. The exons at the 3’ part of the gene generate a coding mRNA when spliced with exon α, and non-coding RNAs when spliced...
with the 5’ BD exons. Interestingly, with respect to the expression-competition model, deletion of exon α is associated with loss of expression of the flanking paternally expressed genes and is found in PWS patients. In contrast, mutations close to the BD exons are found in AS patients and define the AS-ICR (Angelman syndrome imprinting control center). Deletions of exon α in SNRPN are associated with complete loss of expression of SNRPN and the flanking IPW and ZN127 genes and are found in PWS patients, these define the PWS-ICR (Prader–Willi imprinting control center). See text for details.

Conclusions

This review argues that imprinted expression may not result from one active and one silent parental allele, but instead may result from a parent-specific expression-competition between an imprintor gene and a flanking target imprinted gene. Competition is not a new gene regulatory mechanism in mammals, and was first used to model sequential gene expression at the X inactivation in female placental mammals

X inactivation occurs by expression of the XIST transcript from the inactive chromosome (reviewed by Solter and Wei, 1997) and has many similarities to imprinting, including: (i) the mechanism inactivates one of the two X chromosomes in a diploid cell; (ii) X inactivation is paternal specific in placental tissue although random in embryonic tissue; (iii) the Xist product is a non-coding RNA that acts in cis; and (iv) Xist represents the fourth example of a gene whose expression is known to be regulated by DNA methylation in vivo (Panning and Jaenisch, 1996). At present, the similarities between X inactivation and imprinting cannot be drawn too far, but for the purposes of this review it is sufficient to allow that there are precedents in mammals for expression of a non-coding RNA to be associated with repression of a large number of cis-linked genes.

The AS gene has been identified as UBE3A, a ubiquitin protein ligase that functions in protein turnover (Kishino et al., 1997; Matsuura et al., 1997). UBE3A lies 450 kb downstream of SNRPN and was shown to contain small mutations in AS patients who lacked other chromosome anomalies. Maternal-specific expression of UBE3A has been demonstrated recently in some regions of the brain (Albrecht et al., 1997). The PWS gene has not yet been identified, although SNRPN remains a candidate (Wevrick and Francke, 1997). With the caveat that the argument below is employed only as a test of the general applicability of the expression-competition model, the following scenario could be envisaged. First, that maternal-specific transcripts from the UBE3A locus are regulated in cis by expression of the BD non-coding exons from the paternally expressed SNRPN gene. This argument is supported by the finding that mutations in the AS-imprinting control center (ICR) (Figure 4) map to the region containing the BD exons (Dittrich et al., 1996). In contrast, mutations in the PWS-ICR (Figure 4) are linked to deletions of SNRPN exon α. These deletions not only cause loss of SNRPN expression but are also accompanied by loss of expression of the flanking paternally expressed ZN127 and PAR1 genes (Sutcliffe et al., 1994; Saitoh et al., 1995). The effects of loss of SNRPN expression on flanking genes could be explained by proposing that SNRPN is a ‘target imprinted gene’ whose loss allows derepression of a hypothetical cis-linked ‘imprinter gene’. It would thus follow that abnormal expression of this putative imprintor gene on the paternal chromosome would lead to repression of genes flanking SNRPN. This model of the PWS/AS region in terms of expression-competition has no experimental support at this time and it should be noted that the ‘imprinting control center’ model (Sutcliffe et al., 1994; Saitoh et al., 1995; Dittrich et al., 1996) whereby the 5’ part of SNRPN is proposed to contain two long-range cis-acting imprinting control centers (AS-ICR and PWS-ICR, Figure 4), is a viable alternative model.
gene. As modeled in Figure 1, only imprinter genes would carry the gametic imprint and only they would retain their imprinted status at other chromosomal locations. The puzzle that now remains is how expression of the imprinter gene acts to inhibit expression of cis-linked genes and, in particular, how the competition between the two promoters is upset in disease conditions that result in a loss of imprinted expression. There are many possibilities that can be tested experimentally. Recent gene replacement experiments (Ripoche et al., 1997) have suggested that the H19 gene sequence is not important for function. However, similar experiments at the Xist locus have shown that function is lost by targeted replacement of the body of the gene (Marahrens et al., 1997). Thus, it is possible that imprinter genes could act in different ways to interfere with expression of flanking genes, and for Igf2r it is possible that the most simple model of promoter occlusion by the AS-RNA could be the basis of imprinting this gene.

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References


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