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; Wolffe *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

Fig. 1. Two models of the imprinting mechanism. (A) Direct allelic-specific modification of the target imprinted gene. (B) Expression-competition by allele-specific modification of a *cis*-linked 'imprinter' gene. Solid black, target imprinted gene; black dots, imprinter gene; arrow, expressed gene; T bar, repressed gene; gray circle, methylation imprint. Only one pair of chromosome homologs is illustrated.

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). It should be noted, however, that many experiments assaying parental-specific DNA methylation, nuclease accessibility and replication timing have not examined the relationship of this behavior to the onset of monoallelic expression. Thus, although it is proposed in this review that the absence of stable RNA at the repressed allele of an imprinted gene is not associated with the same chromatin changes that are associated with tissue-specific repression of normal genes, more extensive analysis needs to be undertaken on a larger number of imprinted genes to test this proposal fully.

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 'impressor' gene that has a regulatory role ('impressor' here defines a gene that regulates expression of a *cis*-linked target gene; the impressor 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 impressor 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 impressor is unmethylated and expressed, and this correlates with repression of the linked target. The nature of the interaction between the 'impressor' 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) impressor 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

Imprinting by expression-competition

Fig. 2. Expression-competition at two imprinted loci. (A) *Igf2*–*H19* on mouse chromosome 7. (B) *Igf2r*–AS-RNA on mouse chromosome 17. Paternally expressed genes are written in blue above the chromosome; and maternally expressed genes are written in red below the chromosome. Solid black, coding mRNAs; black dots, non-coding RNAs. *Igf2* and *H19* are separated by 100 kb and have the same transcription orientation; Ins (insulin) is 10 kb upstream of *Igf2*. The AS-RNA is contained in an intron of the *Igf2r* gene and has the opposite transcription orientation compared with *Igf2r*.

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* impressor 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-impressor 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 *Igf2*–*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 likely to affect more than one *cis*-linked target gene and, vice versa, that target imprinted genes may be influenced by more than one *cis*-linked imprintor gene. Second, that expression-competition between the imprintor and target imprinted gene may be shifted in both directions. For example, while it has already been shown that deletions of ‘imprintors’ such as *H19* and the *Igf2r*-associated AS-RNA cause derepression of their linked target imprinted genes (Leighton *et al.*, 1995; Wutz *et al.*, 1997), it might be expected that deletions of the target imprinted genes (respectively *Igf2* and *Igf2r*) should allow derepression of their linked imprintor.

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 imprinted 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

Fig. 3. Solid black, coding mRNAs; black dots, non-coding RNAs. The *KvLQT* gene generates imprinted non-coding RNAs and a non-imprinted coding mRNA in heart tissue. Blue text shows paternally expressed genes; red text, maternally expressed genes. Brackets, normal genes. Numbers indicate approximate distance in kb. BWSR1, the Beckwith–Wiedemann syndrome critical translocation breakpoint cluster region 1. p57KIP2 (cyclin-dependent kinase inhibitor protein 2), *KvLQT* (a putative potassium channel protein involved in the long QT heart arrhythmia syndrome), HASH2 (human homolog of mouse Mash2 named for mammalian achaete-scute homologue), TH (tyrosine hydroxylase), INS (insulin), *IGF2* (insulin-like growth factor type 2). See text for details.

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 *Kvlt* 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 imprinted 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. *PARI*, *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

Fig. 4. The PWS/AS region on 15q11–13. The critical regions that have been genetically defined as containing the PWS and AS genes are marked. Paternally expressed genes colored in blue are: *ZNF127* (zinc finger 127); *SNRPN* (small nuclear riboprotein-associated polypeptide N); *PAR5*, *PARSN*, *PARI* (Prader–Willi Angelman region transcripts); *IPW* (imprinted in Prader–Willi transcript), *ASR1*, *ASR2* (Angelman syndrome region transcript). Maternally expressed transcripts are in red: *UBE3A* (ubiquitin protein ligase). The *SNRPN* gene contains 5' exons (BD exons) that produce an alternative splice variant that lacks an open reading frame; transcripts using exon α contain an open reading frame. 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 *ZNF127* genes and are found in PWS patients, these define the PWS-ICR (Prader–Willi imprinting control center). See text for details.

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 (Sutcliffe *et al.*, 1994; Saitoh *et al.*, 1995; Dittrich *et al.*, 1996; Ning *et al.*, 1996; Wevrick and Francke, 1997).

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 *ZNF127* and *PARI* 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 that *SNRPN* is a 'target imprinted gene' whose loss allows derepression of a hypothetical *cis*-linked 'impritor gene'. It would thus follow that abnormal expression of this putative impritor

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.

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.

Conclusions

This review argues that imprinted expression may not result from one active and one silent parental allele, but instead may result from a parental-specific expression-competition between an impritor 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 β globin locus (Choi and Engel, 1988); more recently it has been used to explain how targeted gene disruptions containing an active neomycin gene inhibit expression of flanking genes (Fodde *et al.*, 1994; Fiering *et al.*, 1995; Olson *et al.*, 1996). As a general concept to explain allele-specific expression, the expression-competition model (first proposed by Bartolomei and Tilghman, 1992) is by no means proven, but it has the advantage of explaining many puzzling aspects of imprinting. First, the lack of widespread repressive chromatin features and the presence of very localized epigenetic modifications would now be predicted if both parental alleles of an imprinted gene are active, with one allele expressing the target imprinted gene while the other expresses the impritor gene. Second, the preponderance of imprinted non-coding RNAs can also be explained if impritor genes only function as *cis* repressors, and thus have no coding requirement. In support of the argument that impritor genes exist, it is striking that derepression of *cis*-linked flanking genes does occur in the three cases where imprinted non-coding RNAs have been deleted (*H19*, Leighton *et al.*, 1995; *Xist*, Marahrens *et al.*, 1997; *Igf2r*, Wutz *et al.*, 1997). The third feature, that not all imprinted genes retain their imprinted status at other chromosomal sites (Lee *et al.*, 1993), follows from the prediction that target genes will lose their imprinted status if separated from their impritor

gene. As modeled in Figure 1, only imprintor 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 imprintor 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 imprintor 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

- Albrecht, U., Sutcliffe, J.S., Cattanach, B.M., Beechey, C.V., Armstrong, D., Eichele, G. and Beaudet, A.L. (1997) Imprinted expression of the murine Angelman syndrome gene *Ube3a*, in hippocampal and Purkinje neurons. *Nature Genet.*, **17**, 75–78.
- Barlow, D.P. (1995) Gametic imprinting in mammals. *Science*, **270**, 1610–1613.
- Barlow, D.P., Stöger, R., Herrmann, B.G., Saito, K. and Schweifer, N. (1991) The mouse insulin-like growth factor type 2 receptor in imprinted and closely linked to the *Tme* locus. *Nature*, **349**, 84–87.
- Bartolomei, M.S. and Tilghman, S.M. (1992) Parental imprinting of mouse chromosome 7. *Semin. Dev. Biol.*, **3**, 107–117.
- Bartolomei, M.S., Zemel, S. and Tilghman, S.M. (1991) Parental imprinting of the mouse *H19* gene. *Nature*, **351**, 153–155.
- Bestor, T.H. and Jøenisch, R. (1993) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell*, **69**, 915–926.
- Bestor, T.H. and Tycko, B. (1996) Creation of genomic methylation patterns. *Nature Genet.*, **12**, 363–366.
- Bickmore, W.A. and Carothers, A.D. (1995) Factors affecting the timing and imprinting of replication on a mammalian chromosome. *J. Cell Sci.*, **108**, 2801–2809.
- Bird, A.P. (1993) Imprints on islands. *Curr. Biol.*, **3**, 275–277.
- Cattanach, B.M. and Jones, J. (1994) Genetic imprinting in the mouse: implications for gene regulation. *J. Inherited Metab. Dis.*, **17**, 403–420.
- Choi, O.-R.B. and Engel, J.D. (1988) Developmental regulation of β -globin switching. *Cell*, **55**, 17–26.
- Coxon, A. and Bestor, T.H. (1993) The pros and cons of DNA methylation. *Curr. Biol.*, **3**, 384–385.
- DeChiara, T.M., Robertson, E.J. and Efstratiadis, A. (1991) Parental imprinting of the insulin-like growth factor II gene. *Cell*, **64**, 849–859.
- Dittrich, B. *et al.* (1996) Imprint switching on human chromosome 15 may involve alternative transcripts of the *SNRPN* gene. *Nature Genet.*, **14**, 163–170.
- Elson, D.A. and Bartolomei, M.S. (1997) A 5' differentially methylated sequence and the 3' flanking region are necessary for *H19* transgene imprinting. *Mol. Cell. Biol.*, **17**, 309–317.
- Feil, R., Walter, J., Allen, N.D. and Reik, W. (1994) Developmental control of allelic methylation in the imprinted mouse *Igf2* and *H19* genes. *Development*, **120**, 2933–2943.
- Ferguson-Smith, A.C., Sasaki, H., Cattanach, B.M. and Surani, M.A. (1993) Parental origin-specific epigenetic modification of the mouse *H19* gene. *Nature*, **362**, 751–755.
- Fiering, S. *et al.* (1995) Targeted deletion of the 5' HS2 of the murine β -globin LCR reveals that it is not essential for proper regulation of the β -globin locus. *Genes Dev.*, **9**, 2203–2213.
- Fodde, R.W. *et al.* (1994) A targeted chain termination mutation in the mouse *Apc* gene results in multiple intestinal tumors. *Proc. Natl Acad. Sci. USA*, **91**, 8969–8973.
- Hendrich, B.D. and Willard, H.F. (1995) Epigenetic regulation of gene expression: the effect of altered chromatin structure from yeast to mammals. *Hum. Mol. Genet.*, **4**, 1765–1777.
- Hennikoff, S. and Matzke, M.A. (1997) Exploring and explaining epigenetic effects. *Trends Genet.*, **13**, 293–295.
- Jaenisch, R. (1997) DNA methylation and imprinting: why bother? *Trends Genet.*, **13**, 323–329.
- John, R.M. and Surani, M.A. (1996) Imprinted genes and regulation of gene expression by epigenetic inheritance. *Curr. Opin. Cell Biol.*, **8**, 348–353.
- Kishino, T., Lalande, M. and Wagstaff, J. (1997) *UBE3A/E6-AP* mutations cause Angelman syndrome. *Nature Genet.*, **15**, 70–73.
- Kitsberg, D., Selig, S., Brandeis, M., Simon, I., Keshet, I., Driscoll, D.J., Nicholls, R.D. and Cedar, H. (1993) Allele-specific replication timing of imprinted gene regions. *Nature*, **364**, 459–463.
- Knoll, J.H.M., Cheng, S.D. and Lalande, M. (1994) Allele specificity of DNA replication timing in the Angelman/Prader-Willi syndrome imprinted chromosomal region. *Nature Genet.*, **6**, 41–46.
- Lalande, M. (1997) Parental imprinting and human disease. *Annu. Rev. Genet.*, **30**, 173–195.
- Lee, J.E., Tantravahi, U., Boyle, A.L. and Efstratiadis, A. (1993) Parental imprinting of an *Igf-2* transgene. *Mol. Reprod. Dev.*, **35**, 382–390.
- Lee, M.P., Hu, R.J., Johnson, L.A. and Feinberg, A.P. (1997) Human *KvLQT1* gene shows tissue-specific imprinting and encompasses Beckwith-Wiedemann syndrome chromosomal rearrangements. *Nature Genet.*, **15**, 113–115.
- Lerchner, W. and Barlow, D.P. (1997) Paternal repression of the imprinted mouse *Igf2r* locus occurs during implantation and is stable in all tissues of the post implantation mouse embryo. *Mech. Dev.*, **61**, 141–149.
- Leighton, P.A., Ingram, R.S., Eggenschwiler, J., Efstratiadis, A. and Tilghman, S.M. (1995) Disruption of imprinting caused by deletion of the *H19* gene region in mice. *Nature*, **375**, 34–39.
- Leighton, P.A., Saam, J.R., Ingram, R.S. and Tilghman, S.M. (1996) Genomic imprinting in mice—its function and mechanism. *Biol. Reprod.*, **54**, 273–278.
- Lyle, R. (1997) Gametic imprinting in development and disease. *J. Endocrinol.*, **155**, 1–12.
- Marahrens, Y., Panning, B., Dausman, J., Strauss, W. and Jaenisch, R. (1997) *Xist*-deficient mice are defective in dosage compensation but not in spermatogenesis. *Genes Dev.*, **11**, 156–166.
- Matsuura, T., Sutcliffe, J.S., Fang, P., Galjaard, R.-J., Jiang, Y.-H., Benton, C.S., Rommens, J.M. and Beaudet, A. (1997) *De novo* truncating mutations in *E6-AP* ubiquitin-protein ligase gene (*UBE3A*) in Angelman syndrome. *Nature Genet.*, **15**, 74–77.
- Neumann, B.J. and Barlow, D.P. (1996) Multiple roles for DNA methylation in gametic imprinting. *Curr. Opin. Genet. Dev.*, **6**, 159–163.
- Ning, Y., Roschke, A., Christian, S.L., Lesser, J., Sutcliffe, J.S. and Ledbetter, D.H. (1996) Identification of a novel paternally expressed transcript adjacent to *SNRPN* in the Prader-Willi syndrome critical region. *Genome Res.*, **6**, 742–746.
- Olson, E.N., Arnold, H.H., Rigby, P.W.J. and Wold, B.J. (1996) Know your neighbors: three phenotypes in null mutants of the myogenic bHLH gene *Mrf4*. *Cell*, **85**, 1–4.
- Panning, B. and Jaenisch, R. (1996) DNA hypomethylation can activate *Xist* expression and silence X-linked genes. *Genes Dev.*, **10**, 1991–2002.
- Pfeifer, K. and Tilghman, S.M. (1994) Allele-specific expression in mammals—the curious case of the imprinted RNAs. *Genes Dev.*, **8**, 1876–1874.
- Pfeifer, K., Leighton, P.A. and Tilghman, S.M. (1996) The structural *H19* gene is required for transgene imprinting. *Proc. Natl Acad. Sci. USA*, **93**, 13876–13883.
- Razin, A. and Shemer, R. (1995) DNA methylation in early development. *Hum. Mol. Genet.*, **4**, 1751–1755.

- Reik,W. and Maher,E.R. (1997) Imprinting in clusters: lessons from the Beckwith–Wiedemann syndrome. *Trends Genet.*, **13**, 330–334.
- Ripoche,M.A., Kress,C., Poirier,F. and Dandolo,L. (1997) Deletion of the H19 transcription unit reveals the existence of a putative imprinting control element. *Genes Dev.*, **11**, 1596–1604.
- Saitoh,S., Rogan,P.K., Buiting,K., Schwartz,S., Cassidy,S.B., Glenn,C.C., Driscoll,D.J., Horsthemke,B. and Nicholls,R.D. (1995) Minimal definition of the imprinting center and fixation of a chromosome 15q11–13 epigenotype by imprinting mutations. *Proc. Natl Acad. Sci. USA*, **93**, 7811–7815.
- Sasaki,H., Jones,P.A., Chaillet,J.R., Ferguson-Smith,A.C., Barton,S.C., Reik,W. and Surani,M.A. (1992) Parental imprinting: potentially active chromatin of the repressed maternal allele of the mouse insulin-like growth factor II (Igf2) gene. *Genes Dev.*, **6**, 1843–1856.
- Solter,D. and Wei,G. (1997) Ends Xist, but where are the beginnings? *Genes Dev.*, **11**, 153–155.
- Stöger,R., Kubicka,P., Liu,C.-G., Kafri,T., Razin,A., Cedar,H. and Barlow,D.P. (1993) Maternal-specific methylation of the imprinted mouse Igf2r locus identifies the expressed locus as carrying the imprinting signal. *Cell*, **73**, 61–71.
- Stutcliffe,J.S., Nakao,M., Christian,S., Orstavik,K.H., Tommerup,N., Ledbetter,D.H. and Beaudet,A.L. (1994) Deletions of a differentially methylated CpG island at the SNRPN gene define a putative imprinting control region. *Nature Genet.*, **8**, 52–58.
- Szabo,P.E. and Mann,J.R. (1995) Allele-specific expression and total expression levels of imprinted genes during early mouse development—implications for the imprinting mechanism. *Genes Dev.*, **24**, 3097–3108.
- Tremblay,K.D., Saam,J.R., Ingram,R.S., Tilghman,S.M. and Bartolomei,M.S. (1995) A paternal specific methylation imprint marks the alleles of the mouse H19 gene. *Nature Genet.*, **9**, 407–413.
- Vu,T.H. and Hoffman,A.R. (1994) Promoter-specific imprinting of the human insulin-like growth factor type 2 gene. *Nature*, **371**, 714–717.
- Wevrick,R. and Francke,U. (1997) An imprinted mouse transcript homologous to the human imprinted in Prader–Willi syndrome (IPW) gene. *Hum. Mol. Genet.*, **6**, 325–332.
- Wolffe,A.P., Wong,J., Pruss,D. (1997) Activators and repressors: making use of chromatin to regulate transcription. *Genes to Cells*, **2**, 291–302.
- Wutz,A., Smrzka,O.W., Schweifer,N., Schellander,K., Wagner,E.F. and Barlow,D.P. (1997) Imprinted expression of the Igf2r gene depends on an intronic CpG island. *Nature*, **389**, 745–749.
- Xu,Y., Grundy,P. and Polychronakos,C. (1997) Aberrant imprinting of the insulin-like growth factor II receptor gene in Wilm’s tumor. *Oncogene*, **14**, 1041–1046.

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