Published in final edited form as:

Nat Genet. 2003 September ; 35(1): 11–12. doi:10.1038/ng0903-11.

An ICE pattern crystallizes

Laura Spahn and Denise P Barlow

Austrian Academy Institute of Molecular Biology, Institute of Microbiology and Genetics, Vienna University, Dr. Bohr-Gasse 9/4, A1030 Vienna, Austria. ispahn@gem.univie.ac.at or dbarlow@gem.univie.ac.at

Abstract

Identification of a new imprinting control element (ICE) on mouse chromosome 12 brings the total to five. Common features of imprinting mechanisms suggest a general model of ICE function.

Approximately 70 genes, some with important developmental roles, show parental-specific gene expression in mammals (see <http://www.mgu.har.mrc.ac.uk/imprinting/imprinting.html>). This phenomenon, called genomic imprinting, renders a diploid organism functionally haploid with respect to a gene product¹. Imprinting mechanisms do not affect the DNA sequence but are epigenetic and involve reversible modifications to DNA, such as CpG methylation and alterations to chromatin proteins^{2–4}.

ICEs are usually characterized by differentially methylated regions (DMRs) at CpG islands in which the imprinted allele is methylated and the other parental allele is unmethylated. All DMRs are candidate ICEs, but formal proof requires *in vivo* analysis of DMR deletion. The intergenic germlinederived DMR (IG-DMR) on mouse chromosome 12 is one such candidate, and on page 97 of this issue, Shau-Ping Lin and colleagues⁵ prove that it regulates imprinting of genes in the *Dlk1-Gtl2* cluster.

IG-DMR is linked to a number of characterized imprinted genes, including the paternally expressed protein-coding genes *Dlk1*, *Dio3* and *Rtl1* and the maternally expressed non-coding RNAs (ncRNAs) *Gtl2*, *Rtl3* antisense RNA and C/D snoRNAs that may all belong to one polycistronic ncRNA. To test the function of the DMR, which lies between *Dlk1* and *Gtl2*, the authors deleted the region in mice. When the methylated IG-DMR was deleted from the paternal chromosome, viable mice were produced with no change in the expression of mRNAs and ncRNAs from the paternal chromosome. Deletion of the unmethylated copy of the IG-DMR on the maternal allele, however, results in embryonic lethality and reexpression of the normally silent protein-coding genes from this chromosome, although ncRNAs were unaffected. These results clearly establish the IG-DMR as an ICE that is active when unmethylated on the maternal chromosome and inactive when methylated on the paternal chromosome.

The ICE model cometh

The report by Lin *et al.*⁵ brings to five the total number of characterized mammalian ICEs. Of these, IG-DMR and *H19*-DMD acquire a DNA methylation imprint during spermatogenesis, and PWS-SRO, *Igf2r* region2 and KvDMR1 acquire such an imprint during oogenesis^{5–9}. Despite the different parental origin of their methylation imprint, all five ICEs share similar characteristics (Table 1) and exert a similar effect on their associated imprinted cluster. Thus, we propose a general model for the mechanism of ICE action in which the ICE simultaneously silences mRNA expression and activates non-coding RNA (ncRNA) expression on the non-imprinted chromosome (Fig. 1a). On the imprinted

chromosome (Fig. 1b), a gametic DNA methylation imprint inactivates the ICE; thus, mRNA can be expressed and ncRNA is not silenced. The model does not require or exclude a role for ncRNA in silencing the mRNA. This model applies to IG-DMR, *H19*-DMD, *Igf2r* region 2 and KvDMR1 (refs. 5-8) but not to PWS-SRO, which activates multiple mRNAs and an ncRNA on the non-imprinted chromosome^{9,10}.

Because the imprinted methylated ICE is inactive, a complete ICE deletion would not be expected to change the expression pattern of an imprinted chromosome (Fig. 1b,c). In contrast, the same deletion would be predicted to convert the expression pattern of the non-imprinted chromosome to that of the imprinted one. This behavior has been called 'switching the parental epigenotype' and has been described for all five known ICEs, including PWS-SRO^{5-8,10}. Removal of the methylation imprint would also be predicted to switch the expression pattern to that of the non-imprinted chromosome, as has been shown in mice lacking parts of the DNA methylation system⁴.

ICE-y details

The *H19*-DMD potentially contradicts the common ICE model, because several deletions in this region have been shown to cause coexpression of mRNA and ncRNA from the same parental allele¹¹. We suggest that ICE deletions are incomplete when they permit coexpression of mRNA and ncRNA from the same chromosome (Fig. 1d,e). Partial ICE deletions should result in incomplete silencing and activation and relatively similar mRNA and ncRNA expression from the imprinted and non-imprinted alleles. The degree of similarity between parental chromosomes would depend on how the deletion affects the ability of the ICE to attract a methylation imprint or to regulate expression of mRNA and ncRNA.

It is notable, though not incompatible with a common ICE model, that the maternal and paternal ICEs produce the same default effect (silencing mRNAs and activating ncRNA) but have different basic organization. The two paternally imprinted ICEs (*H19*-DMD, IG-DMR) are located between the regulated RNAs, approximately 80 kb downstream from the mRNA promoter and 8 kb upstream from the ncRNA promoter^{5,11}. Both the mRNA and the ncRNA have the same transcriptional orientation. The two maternally imprinted ICE (*Igf2r* region 2, KvDMR1) are located in an intron of an mRNA that they silence and seem to be part of the ncRNA promoter^{7,8}. The ncRNA has an antisense orientation and overlaps the 5' part of the mRNA. Surprisingly, the transcriptional overlap between *Air* ncRNA and *Igf2r* mRNA is not relevant for silencing¹². The intronic location may keep the ncRNA and mRNA promoter in tight linkage, and the antisense orientation of the ncRNA promoter may prevent accidental splicing from the ncRNA promoter to downstream mRNA exons.

The paternally imprinted IG-DMR and the maternally imprinted *Igf2r* region 2, KvDMR1 and PWS-SRO act bidirectionally to silence upstream and downstream mRNAs^{5,7,8,10}. The paternally imprinted *H19*-DMD is so far unique in its unidirectional action. Several laboratories have shown that the unmethylated *H19*-DMD forms an insulator that excludes downstream enhancers from interacting with the mRNA promoters on the maternal chromosome. The methylation imprint on the paternal chromosome prevents the formation of this insulator, thus allowing expression of the mRNA on only this chromosome¹³.

The role of the ncRNAs regulated by the ICEs is not fully clear, as only one maternally expressed and one paternally expressed ncRNA have been tested. The *H19*-DMD regulates expression of the *H19* ncRNA, which has no role in silencing the linked *Ins2* and *Igf2* mRNAs¹⁴. Although it may seem unexpected that the link between an ICE and non-functional ncRNA is preserved through evolution, a possible explanation is that production of the ncRNA is a side effect of an active ICE. The *Igf2r* region 2 controls expression of *Air*

ncRNA, which is involved in silencing the three linked mRNAs *Igf2r*, *Slc22a2* and *Slc22a3* (refs. 15,16). The ncRNAs activated by the IG-DMR and KvDMR1 have not yet been tested. Thus, it is too soon to know if this difference in ncRNA functionality represents a real difference between the maternal and paternal ICE mechanism.

The identification of the IG-DMR as a functional ICE by Lin *et al.*,⁵ provides us with five different ICEs from which we can identify common features. We believe that the common model for ICE function that we propose is consistent with known ICEs. We eagerly await the next step in the analysis of the imprint control elements to see if the ICE is strong enough to support the weight of this model.

Acknowledgments

We thank I. Yotova and S. Stricker for reading the manuscript.

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Figure 1.

ICE action on the non-imprinted and imprinted chromosome. **(a)** The ICE is active on the non-imprinted parental chromosome and can silence multiple mRNAs and activate ncRNA in *cis*. **(b)** The ICE is inactivated on the imprinted chromosome by the imprint, which is DNA methylation for the five known ICEs⁴. As a consequence, ncRNA is not activated and mRNAs are not silenced. **(c)** A complete ICE deletion results in the same expression pattern that is seen on the imprinted chromosome. The absence of an ICE means that this chromosome is not marked during gametogenesis and will show the same expression pattern irrespective of parental origin. **(d)** A partially deleted ICE on the imprinted chromosome will fail to attract a full methylation imprint. As a consequence, the ICE will be incompletely silenced on the normally imprinted chromosome, and this will result in partial mRNA repression and partial ncRNA activation on this chromosome. **(e)** A partially deleted ICE on the non-imprinted chromosome will fail to fully silence mRNA and to fully activate ncRNA. The resulting expression pattern will be similar to that in **d**, with reduced coexpression of mRNA and ncRNA. Solid line, expressed; dashed line, not expressed; cross-hatched line, partially expressed. Gray, mRNA; purple, ncRNA.

Table 1**ICE characteristics**

(i)	Is necessary for imprinted expression
(ii)	Is CpG-rich or CpG island
(iii)	Attracts <i>de novo</i> DNA methylation in one parental gamete
(iv)	Avoids <i>de novo</i> DNA methylation in other parental gamete
(v)	When unmethylated, silences mRNA and activates ncRNA
(vi)	When methylated, is inactive