PERSPECTIVE

Imprinting mechanisms—it only takes two

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Imprinted gene clusters contain multiple imprinted mRNA genes and at least one imprinted noncoding RNA (ncRNA). Two imprinted ncRNAs have now been shown to act as *cis*-acting domain silencers, indicating that RNA-mediated silencing may be a central feature of genomic imprinting.

The imprinting mechanism

In mammals, genomic imprinting causes identical gene sequences to be treated differently by the cells' transcription machinery, simply because of their inheritance from a maternal or paternal parent. Genomic imprinting is a *cis*-acting epigenetic mechanism that results in parental-specific expression of a few hundred of the genes in our genome. We now know that most imprinted genes are found in clusters that contain between three and 11 imprinted genes and that each imprinted cluster is regulated by one imprint control element or ICE (Spahn and Barlow 2003; Verona et al. 2003). We also know that DNA methylation acts as the imprint to repress activity of the ICE on one parental chromosome. However, nature has not chosen the simplest model whereby the imprint modifies a promoter to silence an imprinted gene. Instead, methylation imprints generally act on long-range *cis*-acting repressors that control multiple genes, often lying some distance away on the same chromosome (Bourc'his and Bestor 2006).

The majority of genes in an imprinted cluster are imprinted protein-coding mRNA genes; however, at least one is always an imprinted ncRNA. What is interesting about imprinted ncRNAs is that they show reciprocal parental-specific expression with respect to the imprinted mRNA genes in a cluster (O'Neill 2005). The parental chromosome that lacks the ICE methylation imprint expresses the ncRNA and represses the multiple mRNA genes, while the other parental chromosome carrying the ICE methylation imprint represses the ncRNA and expresses the mRNA genes. The reciprocal parental-specific expression of imprinted mRNAs and ncRNAs

has long been thought to indicate that ncRNAs play a role in silencing the mRNA genes in an imprinted cluster.

ncRNA rules!

An exciting new study from the group of Shirley Tilghman (Mancini-DiNardo et al. 2006) now shows that the imprinted *Kcnq1ot1* (potassium channel Q1 overlapping transcript1) ncRNA plays a direct role in silencing the multiple mRNA genes in the *Kcnq1*-imprinted gene cluster. The *Kcnq1* cluster is one of the largest imprinted clusters spanning 800 kb and containing nine imprinted mRNA genes that lie both upstream and downstream of the ncRNA (Fig. 1). The *Kcnq1ot1* ncRNA promoter lies in an antisense orientation in intron 10 of the *Kcnq1* gene after which it is named; thus, the ncRNA has the potential to overlap in an antisense orientation only those imprinted mRNA genes that lie upstream to *Kcnq1* in the cluster. Note that the 3' end of the *Kcnq1ot1* ncRNA has not been determined. The *Kcnq1ot1* ncRNA promoter is contained in the ICE for this cluster, which is methylated on the maternal chromosome so that the *Kcnq1ot1* ncRNA is only expressed paternally. In this study, Mancini-DiNardo et al. (2006) insert a polyadenylation signal to truncate the *Kcnq1ot1* ncRNA from 60 to 1.5 kb in mouse ES cells and use these cells to generate mice. The shortened *Kcnq1ot1* ncRNA was correctly imprinted and paternally expressed, but was unable to silence any of the flanking mRNA genes in this imprinted cluster. Since the ICE methylation imprint on the maternal chromosome silences the *Kcnq1ot1* ncRNA promoter, no effect was seen when the maternal chromosome carried the modification. These experiments very nicely show that expression of the *Kcnq1ot1* ncRNA is the silencing factor in this imprinted cluster and that the ICE methylation imprint acts to restrict its expression to the paternal chromosome.

Two types of silencing in imprinted gene clusters

The *Kcnq1ot1* ncRNA is now the second imprinted ncRNA shown to play a direct role in silencing imprinted mRNA genes. The *Air* ncRNA was shown by a

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Figure 1. Cluster overview (placental expression pattern). Parental-specific placental expression is shown for the mouse *Kcnq1* (top) and *Igf2r* (bottom) clusters. The maternal chromosome is shown in red on top, the paternal one in blue below, for both clusters. The position and size of all genes is drawn to scale (<http://www.ensembl.org>). Promoters are indicated by arrowheads, which also show the transcription orientation; gene loci are indicated by rectangles (short genes are represented only by arrowheads). (Black) Active genes; (gray) silent genes. The similarities as well as the differences between the two clusters are listed on the right side. Three points need comment: (1) Unspliced ncRNA: The *Air* ncRNA has been described as unspliced and 108 kb long (Lyle et al. 2000). The full extent of the *Kcnq1ot1* ncRNA is not known, but it is thought to be at least 60 kb and may be unspliced (Mancini-DiNardo et al. 2006). (2) One ubiquitously imprinted mRNA gene: Only the *Cdkn1c* gene in the *Kcnq1* cluster and the *Igf2r* gene in the *Igf2r* cluster are widely imprinted in embryonic and adult tissues and may represent the primary target of the imprinting mechanism in these two clusters. (3) Escapers: The *Igf2r* cluster lacks true escapers, since at the developmental stage when *Slc22a1* is expressed (i.e., adult liver), the flanking *Slc22a2* and *Slc22a3* genes have lost imprinted expression (Zwart et al. 2001). The *Kcnq1* cluster contains true escapers, since the *Nap1/4* and *Phmx* genes are biallelically expressed in placenta when the flanking genes show imprinted expression (Umlauf et al. 2004).

similar approach to be necessary for silencing all three mRNA genes in the imprinted *Igf2r* cluster (Fig. 1; Sleutels et al. 2002). Together, these two studies (Sleutels et al. 2002; Mancini-DiNardo et al. 2006) show that imprinted ncRNAs can induce silencing in *cis* of a domain of genes. However, this mechanism does not apply to all imprinted gene clusters. The first silencing mechanism to be described (also from the group of Shirley Tilghman) (for review, see Wolffe 2000) controls parental-specific expression in the *Igf2*-imprinted cluster and is based on an insulator contained in the ICE whose activity is repressed by the DNA methylation imprint. This cluster contains the enigmatic *H19* ncRNA that shows the typical characteristic of reciprocal parental-specific expression with the two mRNA genes in this cluster (*Ins2* and *Igf2*), but lacks any direct role in their silencing (Arney 2003). While the mechanism underlying the action of the insulator in the *Igf2* cluster is well understood, we do not know yet how imprinted ncRNAs act as domain silencers.

Is it the RNA or is it transcription?

Experimental truncation of the ncRNA relieves silencing of flanking genes in the *Kcnq1*- and *Igf2r*-imprinted gene clusters. This result can be used to argue that expression of the full-length version of these ncRNAs is necessary for silencing. Both these experiments also left intact a correctly functioning ncRNA promoter, which illustrates two further features of the silencing mechanism (Fig. 1). First, that expression in *cis* of an imprinted ncRNA promoter is insufficient for silencing. Second, that the expressed ncRNA promoter can lie in an intron of an active host mRNA gene, showing that antisense transcription of the host mRNA gene does not interfere with expression of the ncRNA promoter. Since the *Air* ncRNA, and most likely the *Kcnq1ot1* ncRNA, have an

antisense transcription overlap with only one of the genes they silence, we could also reason that silencing mechanisms based on double-stranded RNA are unlikely. Indeed, the antisense transcriptional overlap resulting from *Air* expression in the *Igf2r* cluster has been shown not to be necessary for silencing the distant flanking genes (Sleutels et al. 2003).

At first glance, it may seem that the most logical model to explain the silencing action of the imprinted *Kcnq1ot1* and *Air* ncRNAs could be based on the mode of action of the *Xist* ncRNA that mediates X-chromosome inactivation in female mammals (Heard 2004). X inactivation occurs in an imprinted form in Marsupials and in extra-embryonic mouse tissues and in a nonimprinted form in embryonic mouse tissues and also in humans. Many molecular features are shared between genomic imprinting and X inactivation, most significantly, both are *cis*-acting epigenetic silencing mechanisms, and both show a positive correlation between expression of a ncRNA and silencing (Reik and Lewis 2005). Indeed, it has been suggested that X inactivation was a driving force in the evolution of genomic imprinting (Lee 2003). The *Xist* ncRNA has been shown to coat the whole 180-Mbp-long X chromosome and induce gene silencing by recruiting repressive chromatin modifications and DNA methylation. Thus, X inactivation operates by an RNA-directed targeting mechanism. Since imprinted clusters are contained in relatively short genomic regions of 100–1000 kb, the manner by which imprinted ncRNAs associate with the region they silence would clearly show some differences to *Xist* coating. Although RNA-directed targeting remains a valid model, to date, it has not been directly tested whether the *Kcnq1ot1* and *Air* ncRNAs are associated with all of the silenced genes in the imprinted cluster. Because of the relatively small size of imprinted gene clusters, this may prove difficult to test directly by fluorescent visu-

alization of the ncRNA, as used to demonstrate the association of the *Xist* ncRNA with the X chromosome (Heard 2004).

What alternatives are there for ncRNA-mediated silencing if the logical “RNA-directed targeting” model was not chosen by nature? The release of silencing by experimental truncation of the imprinted *Kcnq1ot1* and *Air* ncRNAs cannot distinguish between a role for the ncRNA per se and a role for transcription of the ncRNA across the length of its locus. Thus, it is possible that the actual transcription of ncRNAs could induce silencing of genes laying several hundred kilobase pairs upstream and downstream. How could this come about?

Two possibilities could be considered (Fig. 2). First, ncRNA transcription could activate a domain repressor contained within the ncRNA transcription unit. A domain repressor is defined here as a DNA element able to induce repression of multiple genes in *cis*; it should be noted that this type of element has not yet been identified in the mammalian genome. Domain repressors are envisaged to attract the accumulation of repressive chromatin modifications to the locus that would then spread bidirectionally over the whole cluster. The repressive effect would be limited to the imprinted cluster by flanking boundary elements or the absence of sequences on autosomal chromosomes that promote the spreading of repressive chromatin. This type of transcription model contrasts to X-chromosome inactivation since repressive chromatin, but not the ncRNA, would be predicted to coat the whole imprinted cluster.

Second, ncRNA transcription could repress a domain activator contained within the ncRNA transcription unit. Domain activators are imagined to be analogous to Locus Control Regions or LCRs (Dean 2006). ncRNA transcription could repress a domain activator and so

silence all genes under its control. We envisage that the act of ncRNA transcription through this domain activator would physically displace interacting proteins. This type of model would not require coating of the imprinted cluster by either the ncRNA or by repressive chromatin; instead, it would be predicted that only the silenced gene promoters would be affected.

Both of these transcription-based models would require that transcription of the ncRNA is qualitatively different from that of the imprinted mRNA genes. This can be deduced from the finding that a shortened ncRNA promoter does not suffer any effects from antisense transcription of the host mRNA gene, while ncRNA expression is able to silence mRNA genes (Fig. 1). To date, there are no indications that ncRNA transcription is different compared with transcription of the silenced mRNA genes; however, there is also little information available on the general transcriptional and post-transcriptional properties of imprinted ncRNAs. The ideal “eureka” experiment that would clearly distinguish between a transcriptional versus an RNA-targeting model in imprinted gene clusters is proving frustratingly elusive. Nevertheless, experiments that would test for the presence of the ncRNA and of repressive chromatin modifications across the entire imprinted cluster would clearly help to determine how the imprinted *Kcnq1ot1* and *Air* ncRNAs induce regional gene silencing.

It only takes two

So far, the silencing mechanism has been determined for only three imprinted clusters (the *Igf2*, *Igf2r*, and *Kcnq1* clusters). Note that a full list of all imprinted clusters and primary references can be found at <http://www.mgu.har.mrc.ac.uk/research/imprinting>. It is interesting to note that the six well-characterized imprinted clusters can be divided into two groups (Regha et al. 2006). Type I has four members (the *Kcnq1*-, *Igf2r*-, *Pws*-, and *Gnas*-imprinted clusters) that all carry maternal methylation ICE imprints and contain a ncRNA that has an antisense orientation with respect to one of the silenced mRNA genes. Type II has two members (the *Igf2* and *Dlk1* cluster) that both carry paternal methylation ICE imprints and contain a ncRNA with no transcription overlap. With the publication of this latest paper from the group of Shirley Tilghman (Mancini-DiNardo et al. 2006), we now know that two of the Type I maternally imprinted clusters share a common ncRNA-dependent silencing mechanism, while the single Type II paternally imprinted cluster examined uses a different, insulator-dependent model. The results from other imprinted clusters are eagerly awaited to see whether this indicates there are only two types of basic imprinting mechanisms in mammals, one for paternally imprinted clusters and the other for maternally imprinted clusters.

The imprint remains

Genomic imprinting has been the focus of intense interest since the discovery of the first imprinted genes in

Figure 2. Transcription-dependent silencing models. The domain repressor model (*top*) and the domain activator model (*bottom*) are shown for the paternal chromosome in the *Kcnq1*-imprinted cluster. ncRNA transcription is envisaged to activate the domain repressor and induce it to accumulate repressive chromatin (stop signals). In contrast, ncRNA transcription through the domain activator is proposed to repress this element and prevent its interaction with enhancers (gray X) needed to drive expression of the genes in the imprinted cluster. More details are given in the text.

1991 (Barlow et al. 1991; Bartolomei et al. 1991; DeChiara et al. 1991). While questions on the function and evolution of genomic imprinting still await conclusive answers, extensive progress has been made in the intervening 15 yr toward understanding the different epigenetic mechanisms controlling imprinted expression. The group of Shirley Tilghman has long been at the forefront of this progress; e.g., through the discovery of many new imprinted genes including the *H19* ncRNA (Bartolomei et al. 1991), the demonstration that imprinted genes were clustered (Zemel et al. 1992), the identification of imprint control elements (Leighton et al. 1995), the discovery of the insulator-based silencing mechanism at the *Igf2* cluster (Hark et al. 2000), and this latest discovery of ncRNA-mediated silencing at the *Kcnq1*-imprinted cluster that indicates a common silencing mechanism may exist at maternally imprinted clusters (Mancini-DiNardo et al. 2006). The impact of these contributions is enormous and the practical consequence is that, today, genomic imprinting provides one of the best models of domain epigenetic gene silencing in mammals. The decision of Princeton University in 2001 to elect Shirley Tilghman as President made a big impact on the epigenetics community. While this study from Mancini-DiNardo et al. (2006) may turn out to be the final performance, the talented scientists who were mentored by Shirley Tilghman and are now at the forefront of the imprinting field will ensure that her imprint will remain.

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References

- Arney, K.L. 2003. *H19* and *Igf2*—Enhancing the confusion? *Trends Genet.* **19**: 17–23.
- Barlow, D.P., Stoger, R., Herrmann, B.G., Saito, K., and Schweifer, N. 1991. The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the *Tme* locus. *Nature* **349**: 84–87.
- Bartolomei, M.S., Zemel, S., and Tilghman, S.M. 1991. Parental imprinting of the mouse *H19* gene. *Nature* **351**: 153–155.
- Bourc'his, D. and Bestor, T.H. 2006. Origins of extreme sexual dimorphism in genomic imprinting. *Cytogenet. Genome Res.* **113**: 36–40.
- Dean, A. 2006. On a chromosome far, far away: LCRs and gene expression. *Trends Genet.* **22**: 38–45.
- DeChiara, T.M., Robertson, E.J., and Efstratiadis, A. 1991. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* **64**: 849–859.
- Hark, A.T., Schoenherr, C.J., Katz, D.J., Ingram, R.S., Levorse, J.M., and Tilghman, S.M. 2000. CTCF mediates methylation-sensitive enhancer-blocking activity at the *H19/Igf2* locus. *Nature* **405**: 486–489.
- Heard, E. 2004. Recent advances in X-chromosome inactivation. *Curr. Opin. Cell Biol.* **16**: 247–255.
- Lee, J.T. 2003. Molecular links between X-inactivation and autosomal imprinting: X-inactivation as a driving force for the evolution of imprinting? *Curr. Biol.* **13**: R242–R254.
- 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.
- Lyle, R., Watanabe, D., te Vrugte, D., Lerchner, W., Smrzka, O.W., Wutz, A., Schageman, J., Hahner, L., Davies, C., and Barlow, D.P. 2000. The imprinted antisense RNA at the *Igf2r* locus overlaps but does not imprint *Mas1*. *Nat. Genet.* **25**: 19–21.
- Mancini-DiNardo, D., Steele, S.J.S., Levorse, J.M., Ingram, R.S., and Tilghman, S.M. 2006. Elongation of the *Kcnq1ot1* transcript is required for genomic imprinting of neighboring genes. *Genes & Dev.* (this issue).
- O'Neill, M.J. 2005. The influence of non-coding RNAs on allele-specific gene expression in mammals. *Hum. Mol. Genet.* **14** Spec No 1: R113–R120.
- Regha, K., Latos, P.A., and Spahn, L. 2006. The imprinted mouse *Igf2r/Air* cluster—a model maternal imprinting system. *Cytogenet. Genome Res.* **113**: 165–177.
- Reik, W. and Lewis, A. 2005. Co-evolution of X-chromosome inactivation and imprinting in mammals. *Nat. Rev. Genet.* **6**: 403–410.
- Sleutels, F., Zwart, R., and Barlow, D.P. 2002. The non-coding *Air* RNA is required for silencing autosomal imprinted genes. *Nature* **415**: 810–813.
- Sleutels, F., Tjon, G., Ludwig, T., and Barlow, D.P. 2003. Imprinted silencing of *Slc22a2* and *Slc22a3* does not need transcriptional overlap between *Igf2r* and *Air*. *EMBO J.* **22**: 3696–3704.
- Spahn, L. and Barlow, D.P. 2003. An ICE pattern crystallizes. *Nat. Genet.* **35**: 11–12.
- Umlauf, D., Goto, Y., Cao, R., Cerqueira, F., Wagschal, A., Zhang, Y., and Feil, R. 2004. Imprinting along the *Kcnq1* domain on mouse chromosome 7 involves repressive histone methylation and recruitment of Polycomb group complexes. *Nat. Genet.* **36**: 1296–1300.
- Verona, R.I., Mann, M.R., and Bartolomei, M.S. 2003. Genomic imprinting: Intricacies of epigenetic regulation in clusters. *Annu. Rev. Cell Dev. Biol.* **19**: 237–259.
- Wolffe, A.P. 2000. Transcriptional control: Imprinting insulation. *Curr. Biol.* **10**: R463–R465.
- Zemel, S., Bartolomei, M.S., and Tilghman, S.M. 1992. Physical linkage of two mammalian imprinted genes, *H19* and insulin-like growth factor 2. *Nat. Genet.* **2**: 61–65.
- Zwart, R., Sleutels, F., Wutz, A., Schinkel, A.H., and Barlow, D.P. 2001. Bidirectional action of the *Igf2r* imprint control element on upstream and downstream imprinted genes. *Genes & Dev.* **15**: 2361–2366.

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