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Regulation of imprinted expression by macro non-coding RNAs

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Abstract

In mammals, imprinted genes are clustered and at least one gene in each imprinted cluster is a long i.e., macro non-coding (nc) RNA. Most genes in a cluster show concordant parental-specific expression but the ncRNA is the odd one out, and is expressed from the opposite parental chromosome. While reciprocal expression between imprinted macro non-coding RNAs and flanking mRNA genes is indicative of a functional role, only two of three tested macro ncRNAs have been shown to induce imprinted gene expression. The two known functional imprinted macro non-coding RNAs are both RNAPII transcripts with unusual transcriptional properties that may be functionally relevant and their analysis may shed light on the function of non-coding RNAs that have been shown to comprise the majority of the mammalian transcriptome.

Keywords

ncRNA; non-coding RNA; macro ncRNA; genomic imprinting; imprinted clusters

Genomic Imprinting—A Model of Epigenetic Gene Regulation

Mammalian reproduction strictly requires a mother and a father who each donate a haploid chromosome set to the embryo (Fig. 1). Classical genetics shows that most mutations are recessive and indicates that for most genes, both the maternal and paternal gene copy is equally expressed in all cells. However, a small percentage of mammalian genes (estimated at less than 1% of all genes) show parental-specific expression, such that a diploid cell will contain some genes expressed from the maternal chromosome and repressed on the paternal chromosome and vice versa. This arises from an epigenetic mechanism known as genomic imprinting.1 The existence of imprinted genes controlling essential developmental functions explains the mammalian embryo's strict requirement for both parents.

Genes showing parental-specific expression were hypothesized to exist in the mammalian genome from a series of landmark experiments conducted in the 70's and 80's that indicated that the maternal and paternal genomes do not make an equal contribution to embryonic development. These experiments included the failure of embryos to develop by parthenogenesis in the absence of fertilization, the lethal phenotype of embryos inheriting two copies of one parental chromosome in the absence of the other parental copy (known as chromosomal uniparental disomy), and the success of oocyte nuclear transfer experiments to generate viable embryos containing one maternal and one paternal pronucleus and their inability to produce viable embryos containing two maternal or paternal pronuclei.2,3 This hypothesis was proven in the 90's with the discovery of three imprinted genes: (1) the

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maternally-expressed *Igf2r* gene,4 (2) the paternally-expressed *Igf2* gene5,6 and (3), the maternally-expressed *H19* non-coding RNA.7 Today 94 genes showing imprinted expression are described on the Harwell Mouse-Imprinting-Data site http://www.har.mrc.ac.uk/research/genomic_imprinting/,8 and imprinted expression of most but not all these genes, is conserved in humans (see www.otago.ac.nz/IGC).9

Since both the expressed and repressed parental alleles of an imprinted gene are contained in the same nucleus, the mechanism controlling imprinted expression must be cis-acting i.e., restricted to one chromosome (see cover illustration). Furthermore, as imprinted expression is observed in diploid cells of inbred mice that contain genetically-identical parental chromosomes, it cannot arise from differences in the primary DNA sequence instead, it must arise from an epigenetic mechanism i.e., a modification of DNA or chromatin proteins that alters the ability of DNA to respond to external signals.10 Epigenetic modifications have the potential to regulate many nuclear functions such as nuclear packaging of long DNA molecules, the dynamic organization of chromosome structure throughout the cell cycle, the control of DNA repair and recombination, the control of transposons that comprise almost 50% of the mouse and human genome and last but not least, the regulation of gene expression. Since epigenetics has the potential to control so many nuclear functions, the identification of genes that show clear epigenetic regulation offers an important model system to determine exactly how epigenetics influence gene expression. Although many epigenetic gene regulatory systems have been described most depend on comparing cells from different developmental stages or tissues. Genomic imprinting has the advantage in that the expressed and repressed parental allele are contained in the same nucleus and repressive epigenetic modifications can be identified by comparing two parental alleles in the same environment of transacting factors.

Imprinted Genes are Clustered

In the mouse imprinted genes are found on 11 of the 19 autosomal chromosomes. Additionally, most are grouped into small clusters containing from 2–12 imprinted genes that spread over 80–3,700 kilobase pairs.11 Clustering of imprinted genes suggested two features of the epigenetic mechanism underlying imprinted expression. Firstly, that it is not genespecific but instead, affects a chromosomal domain containing multiple genes. And, secondly, since the mechanism is not gene-specific it may depend on a cis-acting imprint control element lying outside the affected gene. Both of these suggestions have now been confirmed. We know now that a single Imprint Control Element or ICE, which is subject to parental-specific epigenetic control, regulates imprinted expression of all genes in one imprinted cluster. Two interesting findings regarding imprinted gene clusters are that at least one gene in each imprinted cluster is a macro non-coding (nc) RNA, and, that while most genes in a cluster show concordant parental-specific expression, the ncRNA is the odd one out and is expressed from the other parental chromosome.12,13 Thus, within one imprinted cluster, imprinted mRNAs and imprinted ncRNAs show reciprocal-parental-specific expression. Figure 2 shows the arrangement of mRNAs and ncRNA in two imprinted clusters, the *Igf2r* cluster that spans 400 kb on Chr.17 and the *Kcnq1* imprinted cluster that spans 1,000 kb on Chr.7. Both clusters show maternal-specific expression of multiple mRNA genes and paternal-specific expression of a single macro ncRNA, respectively *Airn* and *Kcnq1ot1,* whose promoter lies in an antisense orientation in an intron of one of the repressed mRNA genes. Both *Airn* (108 kb) and *Kcnq1ot1* (91.5 kb) are considered ‘macro’ ncRNAs. *Airn* overlaps the *Igf2r* promoter while *Kcnq1ot1* spans intron 10–11 and does not overlap the *Kcnq1* promoter. Note that in both clusters more genes show imprinted expression in the placenta than in the embryo. The reciprocal expression of imprinted mRNAs genes and imprinted ncRNA genes indicates the potential of a functional relationship between ncRNA expression and repression in cis of mRNA genes.

The ICE is a Methyl-Sensitive cis-Acting Element Required for ncRNA Expression

Early searches for epigenetic modifications of imprinted genes concentrated on DNA methylation with the goal of identifying Differentially Methylated Regions or 'DMRs' specific to one parental chromosome. Since it was assumed that epigenetic imprints used to identify parental alleles would need to be established in the gametes before fertilization, DMRs were tested to see if they were present in one of the gametes (gDMRs) and could be the cause of imprinted expression, or, if they arose in somatic cells (sDMRs) as a consequence of imprinted expression. Only gDMRs represent candidate ICEs that need to be experimentally verified. Interestingly while all imprinted clusters studied so far contain a gDMR inherited from either the maternal or paternal gamete, sDMRs only rarely modify the repressed allele of an imprinted gene.14-17 gDMRs showed two additional features in that they are physically linked to or overlap, the ncRNA promoter and gDMR DNA methylation correlates with ncRNA repression. Notably, the parental chromosome with the unmethylated gDMR expresses the ncRNA but represses the multiple mRNA genes in the cluster (white star Fig. 2).

A key experiment towards understanding the genomic imprinting mechanism was provided by the analysis of mice lacking the maintenance DNA methyltransferase DNMT1 that is needed to copy preexisting DNA methylation patterns onto newly replicated sister chromatids. Mouse embryos lacking the *Dnmt1* gene die after gastrulation between 7.5 and 8.5 days post conception (dpc) and show a general loss of imprinted expression.18 Only a few imprinted genes were tested in the original publication and they showed a surprising pattern: the *H19* ncRNA gained biallelic expression, while the *Igf2* and the *Igf2r* mRNA genes were repressed on both parental alleles. This polar difference in the response of imprinted mRNAs and imprinted ncRNAs to the absence of DNA methylation has been supported by later studies showing that the *Airn* and *Kcnq1ot1* ncRNAs are both activated in the absence of DNA methylation, while the flanking mRNA genes in these two clusters are repressed in the absence of DNA methylation.19,20 This observation that imprinted mRNA genes become repressed when imprinted ncRNA genes are activated, further strengthens the suggestion of a functional relationship between ncRNA expression and repression in cis of mRNA genes.

Model Systems to Study the Function of Imprinted ncRNAs

Imprinted gene expression has mainly been studied in vivo in embryonic tissue (Fig. 1) using mouse strains that contain genetic differences, such as single nucleotide polymorphisms (SNPs) or chromosomal deletions of one parental chromosome, to distinguish the two parental alleles. Post-implantation embryonic tissue from 12.5 dpc or later can be easily dissected to provide sufficient tissues for analysis (Fig. 1). The embryo also generates extra-embryonic tissues in the form of the placenta and umbilical cord that allow a direct connection between the fetal and maternal blood circulation, and, the membranes that surround the embryo that are used to nourish and protect the embryo before the placenta becomes fully functional. Two types of experiments have been used to test the function of imprinted ncRNAs. In the first type of experiment, ICE deletions were introduced by homologous recombination in embryonic stem (ES) cells that were used to generate male and female mice carrying the ICE deletion on one parental allele. Offspring that inherited the ICE deletion from either their mother or father were then analyzed. In this way the unmethylated ICE in the *Igf2r, Kcnq1 and Gnas* clusters (that overlaps the *Airn, Kcnq1ot1* and *Nespas* ncRNA promoters) as well as the unmethylated ICE in the *Igf2* and *Dlk1* clusters (that lie respectively 2.2 kb and 10 kb upstream of the *H19* and *Gtl2* ncRNA promoter), have been shown to be necessary for expression of the ncRNA.21-23 Notably,

while inheritance of the ICE deletion led to loss of macro ncRNA expression, it induced de-repression of all mRNAs in each cluster. The ICE deletion experiment strengthen but do not prove the hypothesis that macro ncRNAs repress mRNA genes in cis.

The second type of experiment specifically tested the role of the ncRNA while preserving the ICE sequence and its parental-specific DNA methylation status. To date only the *H19, Airn* and *Kcnq1ot1* macro ncRNAs have been tested in this way and only the latter two play a functional role. The function of the *H19* ncRNA was tested by a 3 kb deletion of the entire gene, from −235 bp relative to the promoter up to +320 bp relative to the polyA site, in ES cells that were used to generate mice heterozygous for the *H19* deletion.24 Offspring inheriting the *H19* gene deletion from their mother showed a loss of normal maternal-specific *H19* expression, but paternal-specific DNA methylation of the ICE that lies 5 kb upstream to the promoter, was maintained. Despite the loss of maternal *H19, Igf2* maintained imprinted expression in liver, but showed a small amount of de-repression in skeletal muscle. Imprinted expression of *Ins2* was not tested in this study. This experiment shows that the *H19* ncRNA plays no role in regulating imprinted *Igf2* expression in endoderm; the possibility of some effect on imprinted *Igf2* expression in mesoderm has not yet been investigated. Imprinted *Igf2* expression in endodermal tissue was later shown to be regulated by a methylation-sensitive insulator contained in the ICE, that regulates the ability of enhancers lying downstream to *H19* to physically interact with the upstream *H19* and *Igf2* promoters. On the unmethylated maternal allele, the CTCF protein binds to the insulator in the ICE and restricts the action of enhancers to the *H19* promoter. On the methylated paternal allele, CTCF cannot bind to the insulator and the enhancers preferentially interact with the *Igf2* promoter.25,26 The *Igf2* ICE is now one of the best studied examples of a mammalian insulator and is being used to unravel how insulators function.27

The *Airn* and *Kcnq1* macro ncRNAs are both very long transcripts that have an antisense overlap with one of the imprinted mRNA genes in their clusters (Fig. 2). As a result of this overlap it was not possible to delete these ncRNAs and their function was tested in a different manner—by insertion of a polyadenylation cassette a few kilobase pairs downstream of the ncRNA promoter.28-30 The introduced polyA cassette truncated the ncRNAs to 1.5–3% of the length of the wild type ncRNA but had no affect on the ICE that contains the ncRNA promoter (Fig. 2). In both these clusters offspring inheriting the truncation from their fathers produced a truncated macro ncRNA transcript and re-expressed all mRNAs from the paternal chromosome in both embryonic and placental tissue. Thus these experiments clearly show that the *Airn* and *Kcnq1ot1* macro ncRNAs function as repressors of multiple mRNA genes in cis.

Developmental Regulation of Imprinted Expression

Imprinted expression of mRNA genes does not arise immediately after fertilization but is first seen, in the *Kcnq1* imprinted cluster in the preimplantation embryo at 4.5 dpc and in the *Igf2* cluster after embryonic implantation at 6.5 dpc.31,32 In the *Igf2r* cluster, the *Igf2r* mRNA has been shown to be bi-allelically expressed in the preimplantation embryo and then to acquire imprinted maternal-specific expression during the time of implantation.32,33 These findings identify the implantation stage as an important period to establish imprinted expression. Embryos at this developmental stage are small and difficult to dissect free of maternal tissue, however, differentiating embryonic stem (ES) cells have recently been shown to provide a robust in vitro system for studying the gain of imprinted expression in the *Igf2r* cluster.34 Using ES cells carrying a distinguishing SNP on the maternal allele, *Igf2r* was shown to be biallelically expressed in undifferentiated ES cells that mimic the preimplantation embryo while the *Airn* ncRNA is not expressed at this stage. The onset of *Igf2r* imprinted expression coincides with the onset of *Airn* ncRNA expression as ES cells

are induced to differentiate by the addition of retinoic acid. These experiments show that differentiating ES cells provide an in vitro model of the gain of imprinted expression that occurs in the implanting embryo (Fig. 3). The surprising observation from these experiments was that during ES cell differentiation, low-level expression from the paternal *Igf2r* promoter remained constant, while expression from the maternal *Igf2r* promoter increased ten-fold. Notably, this low-level of paternal *Igf2r* expression persisted despite the acquisition of an sDMR (grey circle Fig. 3) and repressive heterochromatin on the *Igf2r* promoter.34,35 This revealed a novel mechanism by which *Airn* induces imprinted expression, i.e., by creating an expression bias between the two parental alleles most likely by regulating gene enhancers, instead of inducing gene silencing. It is too early to know if this observation is applicable to other imprinted clusters, but it has the potential to explain the rarity of sDMRs on repressed mRNAs promoters in imprinted clusters. The developmental regulation of *Igf2r* imprinted expression that correlates with *Airn* expression highlights the functional role of macro ncRNAs and also shows that imprinted expression can be regulated despite the universal presence of the ICE DNA methylation imprint. This study also shows that imprinted expression of *Igf2r* can be controlled by developmental regulation of the *Airn* ncRNA. The same phenomenon i.e., regulated expression of *Airn,* also explains biallelic *Igf2r* expression that is seen in embryonic and adult neurons.36

Macro ncRNAs may Function Differently in Placenta and Embryo

A common observation seen in well-studied imprinted clusters is that the macro ncRNAs represses a larger number of genes in the placenta compared to the embryo. In the *Igf2r* cluster three mRNA genes are repressed on the paternal allele in placenta, while only one is repressed in the embryo. In the *Kcnq1* cluster eleven mRNA genes are repressed in placenta, compared to four genes repressed in the embryo (Fig. 2). Notably, in these clusters imprinted expression in both placenta and embryo is controlled by the macro ncRNAs *Airn* and *Kcnq1ot1* (Fig. 2). Because the placenta is unavoidably contaminated by maternal tissues (Fig. 1), some care is needed to interpret experiments showing imprinted expression and epigenetic modifications in this tissue. Despite this caveat, there is increasing evidence that the *Airn* and *Kcnq1ot1* macro ncRNAs act differently in placenta and embryo to induce imprinted gene expression. A technique called RNA TRAP (tagging and recovery of associated proteins) has recently shown that the *Airn* ncRNA physically associates with the paternal *Slc22a3* promoter, in a manner that correlates with the imprinted expression of this gene in early but not late placenta.37 The histone methyltransferase G9A that induces H3K9me2 modification of H3 histone tails, a mark typical of repressed chromatin, was also shown to be necessary for imprinted *Slc22a3* expression in early placenta. The authors propose a model whereby *Airn* specifically interacts with the paternal *Slc22a3* promoter that lies 275 kb upstream and then recruits the G9A histone methylase that induces repressive chromatin and gene repression. However, *Igf2r* imprinted expression in the placenta was not regulated by G9A indicating an independent mechanism for *Airn* to repress this gene. G9A has also been shown to be necessary for placental-specific but not for embryonic imprinted expression in the *Kcnq1* cluster.38 The technique of RNA/DNA Fluorescence In Situ Hybridization (FISH) was recently used to show that the *Kcnq1ot1* macro ncRNA forms a discrete nuclear compartment exclusively in pre-implantation trophectoderm cells that only contribute to the placenta. This compartment is depleted for RNAPII and contains the *Kcnq1* cluster in a 3D contracted chromatin state.39 This publication also used pre-implantation mouse embryos deficient for Polycomb repressor complex (PRC) proteins (that induce the repressive histone modifications H3K27me3 and H2A ubiquitination) to show that both EZH2 (from the PRC2 complex) and RNF2 (from the PRC1 complex) are necessary for imprinted gene expression in trophectoderm cells. These studies not only highlight the importance of higher order chromatin structures and repressive histone modifications for

placental-specific imprinted expression, but also indicate that the macro ncRNA itself targets repressive modifications specifically in placenta (see model in Fig. 4).

While repressive histone modifications appear to play a role in placental-specific imprinted expression, there is no evidence yet of a functional role in regulating imprinted expression in embryo and adult tissues. Indeed, the analysis of repressive H3K27me3 modifications in differentiated ES cells and in 13.5dpc embryonic fibroblasts, shows that H3K27me3 is lost as imprinted *Igf2r* expression is gained.34,35 Furthermore, analysis of multiple repressive histone modifications (H3K9me3 and H4K20me3) as well as the heterochromatin protein HP1, shows that the *Igf2r* cluster in embryonic fibroblasts and all other imprinted clusters in ES cells, are marked only by focal modifications.35,40 This indicates that repressive chromatin marks do not spread in imprinted clusters in embryonic cells. Based primarily on short half-life of the *Airn* ncRNA20 and the above finding that repression of the paternal *Igf2r* promoter does not involve spreading of repressive chromatin in embryonic cells, we have proposed a model whereby *Airn* could repress multiple genes in cis through the act of its transcription, independently of the macro ncRNA product itself.20,41 In this model that depends on *Airn* transcriptional overlap with the *Igf2r* promoter and with a putative placental-specific enhancer, the act of *Airn* transcription is proposed to interfere with the ability of RNAPII to initiate high *Igf2r* transcription and block the interaction of enhancer-binding proteins needed to activate the upstream *Slc22a2* and *Slc22a3* genes (Fig. 4). Further work is needed to test this model and to determine if is generally applicable to other imprinted clusters.

Imprinted Macro ncRNA Biology

We are in the early stages of understanding how the *Airn* and *Kcnq1ot1* macro ncRNAs act to repress genes in cis. In particular, it is of interest to know if these two macro ncRNAs possess special properties relevant for their function that could also be typical of other macro ncRNAs. Both *Airn* and *Kcnq1ot1* are RNAPII transcripts but are atypical RNPII transcripts because they are unusually large, mostly unspliced and not exported to the cytoplasm.20,42 Both are described as ‘macro’ ncRNAs as *Airn* is 108 kb and *Kcnq1ot1* is 91.5 kb (Fig. 5). A small percentage (<1%) of *Airn* transcripts are variably spliced to ncRNAs of approximately 1 kb and exported to the cytoplasm. Spliced *Airn* transcripts are 9 fold more stable than the unspliced transcript but are predicted to be non-functional. *Kcnq1ot1* appears to lack any spliced variants (Fig. 5). In contrast, the non-functional *H19* ncRNA is a fully spliced ncRNA that is exported to the cytoplasm43 however, it is atypical in that it has a low intron/exon ratio (Fig. 5).

While mammalian genes are often larger than 50 kb—e.g., the DMD gene is the largest known mouse gene that is 2,256 kb long and spliced to a 13.8 kb transcript containing 79 exons; mature spliced mRNA transcripts are rarely larger than 10–15 kb. Thus mammalian mRNA genes normally have a high intron to exon ratio. Single exon mammalian mRNA genes do exist, however, they are normally short transcripts in the order of 1 kb that are exported to the cytoplasm. A consequence of the large size and unspliced nature of *Airn* and *Kcnq1ot1* is that they contain transposons, which comprise approximately 50% of the mouse genome and are equally distributed in genes and intergenic regions.44 Thus an additional atypical feature is that imprinted macro ncRNAs contain transposons, which are only rarely found in mRNAs (Fig. 5). Since transposons are usually subject to DNA methylation and silenced in a differentiated cell genome,45 mature RNAs containing transposon sequences are rare and open the possibility that they contribute to the repressor function of imprinted macro ncRNAs.

Experiments to identify functional regions within *Airn* and *Kcnq1ot1* are in progress and we currently lack a clear picture of key regions within these ncRNAs. However, it is possible that the absence of splicing is related to macro ncRNA function. Exon-junction-complexes that mark spliced junctions may be used to target the nuclear export machinery and absence of splicing in macro ncRNAs may prevent nuclear export. The control of mRNA splicing is still a topic of active investigation. However, it is generally thought that RNAPII transcripts are co-transcriptionally processed to spliced products that are exported to the cytoplasm, because the elongating form of RNAPII recruits both splicing and polyadenylation proteins.46 Recently, the polyadenylation complex was shown in yeast to recruit nuclear export factors.47 Since RNAPII is recruited to the promoter, this may indicate a unique property of macro ncRNA promoters is to recruit a form of RNAPII that is unable to subsequently recruit splicing and polyadenylation factors. This has been directly tested for the *Airn* promoter by experiments that deleted the endogenous promoter and replaced it with the *Pgk1* promoter that normally transcribes a 16.6 kb gene spliced to a 1.8 kb transcript containing 11 exons.48 Surprisingly, the resultant *Pgk-Airn* transcript retained all properties of the endogenous *Airn* ncRNA. *Pgk-Airn* transcripts were 108 kb long and only produced a low level of spliced variants, and importantly, they retained the ability to repress *Igf2r* in cis. Thus the lack of *Airn* splicing appears to be regulated independently of its promoter sequence and RNAPII recruitment. This result contrasts with a study in yeast that swapped two mRNA promoters and showed that splicing regulation was promoter driven.49 Further work will be needed to identify functional regions within the *Airn* and *Kcnq1ot1* macro ncRNAs.

Conclusions and Future Directions

Genomic imprinting provides a remarkable model of epigenetic gene regulation in mammals. The model is remarkable because it has already allowed the identification of epigenetic cis-regulatory factors that had been predicted, such as insulators, as well as macro ncRNAs that were not anticipated. The *Airn* and *Kcnq1ot1* imprinted ncRNAs are now an important part of future strategies to understand how macro ncRNAs are processed and how they exert their cis-regulatory functions. The mammalian genome contains 25 known imprinted clusters and one future important goal is to determine how many contain macro ncRNAs and if they resemble the non-functional *H19* ncRNA or the functional *Airn* and *Kcnq1ot*1 ncRNAs.

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References

1. Barlow, DP.; Bartolomei, MS. Genomic imprinting in mammals. EPIGENETICS Chapter 19. David Allis, C.; Jenuwein, Thomas; Reinberg, Danny, editors. Cold Spring Harbor Laboratory Press; New York: 2007.
2. Solter D. Imprinting today: end of the beginning or beginning of the end? Cytogenet Genome Res. 2006; 113:12–6. [PubMed: 16575157]
3. Cattanach BM, Beechey CV, Peters J. Interactions between imprinting effects: summary and review. Cytogenet Genome Res. 2006; 113:17–23. [PubMed: 16575158]

4. Barlow DP, Stoger R, Herrmann BG, Saito K, Schweifer N. The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. Nature. 1991; 349:84–7. [PubMed: 1845916]
5. DeChiara TM, Robertson EJ, Efstratiadis A. Parental imprinting of the mouse insulin-like growth factor II gene. Cell. 1991; 64:849–59. [PubMed: 1997210]
6. Ferguson-Smith AC, Cattanach BM, Barton SC, Beechey CV, Surani MA. Embryological and molecular investigations of parental imprinting on mouse chromosome 7. Nature. 1991; 351:667–70. [PubMed: 2052093]
7. Bartolomei MS, Zemel S, Tilghman SM. Parental imprinting of the mouse H19 gene. Nature. 1991; 351:153–5. [PubMed: 1709450]
8. Beechey, C.; Cattanach, BM.; Blake, A.; Peters, J. World Wide Web Site—Mouse Imprinting Data and References. MRC Harwell; Oxfordshire: 2008.
9. Morison IM, Ramsay JP, Spencer HG. A census of mammalian imprinting. Trends Genet. 2005; 21:457–65. [PubMed: 15990197]
10. Bird A. Perceptions of epigenetics. Nature. 2007; 447:396–8. [PubMed: 17522671]
11. Thorvaldsen JL, Bartolomei MS. SnapShot: imprinted gene clusters. Cell. 2007; 130:958. [PubMed: 17803916]
12. Royo H, Cavaille J. Non-coding RNAs in imprinted gene clusters. Biol Cell. 2008; 100:149–66. [PubMed: 18271756]
13. Peters J, Robson JE. Imprinted noncoding RNAs. Mamm Genome. 2008; 19:493–502. [PubMed: 18815833]
14. Sasaki H, Jones PA, Chaillet JR, Ferguson-Smith AC, Barton SC, Reik W, Surani MA. Parental imprinting: potentially active chromatin of the repressed maternal allele of the mouse insulin-like growth factor II (Igf2) gene. Genes Dev. 1992; 6:1843–56. [PubMed: 1383088]
15. Stoger R, Kubicka P, Liu CG, Kafri T, Razin A, Cedar H, Barlow DP. Maternal-specific methylation of the imprinted mouse Igf2r locus identifies the expressed locus as carrying the imprinting signal. Cell. 1993; 73:61–71. [PubMed: 8462104]
16. Takada S, Paulsen M, Tevendale M, Tsai CE, Kelsey G, Cattanach BM, Ferguson-Smith AC. Epigenetic analysis of the Dlk1-Gtl2 imprinted domain on mouse chromosome 12: implications for imprinting control from comparison with Igf2-H19. Hum Mol Genet. 2002; 11:77–86. [PubMed: 11773001]
17. Kobayashi H, Suda C, Abe T, Kohara Y, Ikemura T, Sasaki H. Bisulfite sequencing and dinucleotide content analysis of 15 imprinted mouse differentially methylated regions (DMRs): paternally methylated DMRs contain less CpGs than maternally methylated DMRs. Cytogenet Genome Res. 2006; 113:130–7. [PubMed: 16575172]
18. Li E, Beard C, Jaenisch R. Role for DNA methylation in genomic imprinting. Nature. 1993; 366:362–5. [PubMed: 8247133]
19. Green K, Lewis A, Dawson C, Dean W, Reinhart B, Chaillet JR, Reik W. A developmental window of opportunity for imprinted gene silencing mediated by DNA methylation and the Kcnq1ot1 noncoding RNA. Mamm Genome. 2007; 18:32–42. [PubMed: 17245608]
20. Seidl, CI. PhD thesis. 2006. Transcriptional characterization of the Air ncRNA and its role in regulation of imprinted expression in the Igf2r cluster in visceral yolk sac.
21. Wutz A, Smrzka OW, Schweifer N, Schellander K, Wagner EF, Barlow DP. Imprinted expression of the Igf2r gene depends on an intronic CpG island. Nature. 1997; 389:745–9. [PubMed: 9338788]
22. Thorvaldsen JL, Duran KL, Bartolomei MS. Deletion of the H19 differentially methylated domain results in loss of imprinted expression of H19 and Igf2. Genes Dev. 1998; 12:3693–702. [PubMed: 9851976]
23. Williamson CM, Turner MD, Ball ST, Nottingham WT, Glenister P, Fray M, Tymowska-Lalanne Z, Plagge A, Powles-Glover N, Kelsey G, Maconochie M, Peters J. Identification of an imprinting control region affecting the expression of all transcripts in the Gnas cluster. Nat Genet. 2006; 38:350–5. [PubMed: 16462745]
24. Schmidt JV, Levorse JM, Tilghman SM. Enhancer competition between H19 and Igf2 does not mediate their imprinting. Proc Natl Acad Sci USA. 1999; 96:9733–8. [PubMed: 10449763]

25. Bell AC, Felsenfeld G. Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. Nature. 2000; 405:482–5. [PubMed: 10839546]
26. Hark AT, Schoenherr CJ, Katz DJ, Ingram RS, Levorse JM, Tilghman SM. CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. Nature. 2000; 405:486–9. [PubMed: 10839547]
27. Wallace JA, Felsenfeld G. We gather together: insulators and genome organization. Curr Opin Genet Dev. 2007; 17:400–7. [PubMed: 17913488]
28. Sleutels F, Zwart R, Barlow DP. The non-coding Air RNA is required for silencing autosomal imprinted genes. Nature. 2002; 415:810–3. [PubMed: 11845212]
29. Mancini-Dinardo D, Steele SJ, Levorse JM, Ingram RS, Tilghman SM. Elongation of the Kcnq1ot1 transcript is required for genomic imprinting of neighboring genes. Genes Dev. 2006; 20:1268–82. [PubMed: 16702402]
30. Shin JY, Fitzpatrick GV, Higgins MJ. Two distinct mechanisms of silencing by the KvDMR1 imprinting control region. EMBO J. 2008; 27:168–78. [PubMed: 18079696]
31. Lewis A, Green K, Dawson C, Redrup L, Huynh KD, Lee JT, Hemberger M, Reik W. Epigenetic dynamics of the Kcnq1 imprinted domain in the early embryo. Development. 2006; 133:4203–10. [PubMed: 17021040]
32. Szabo PE, Mann JR. Allele-specific expression and total expression levels of imprinted genes during early mouse development: implications for imprinting mechanisms. Genes Dev. 1995; 9:3097–108. [PubMed: 8543154]
33. Lerchner W, Barlow DP. 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. 1997; 61:141–9. [PubMed: 9076684]
34. Latos PA, Stricker SH, Steenpass L, Pauler FM, Huang R, Senergin BH, Regha K, Koerner MV, Warczok KE, Unger C, Barlow DP. An In Vitro ES Cell Imprinting Model Shows That Imprinted Expression Of The Igf2r Gene Arises From An Allele-Specific Expression Bias. Development. 2009; 136:1–11. [PubMed: 19036804]
35. Regha K, Sloane MA, Huang R, Pauler FM, Warczok KE, Melikant B, Radolf M, Martens JH, Schotta G, Jenuwein T, Barlow DP. Active and repressive chromatin are interspersed without spreading in an imprinted gene cluster in the mammalian genome. Mol Cell. 2007; 27:353–66. [PubMed: 17679087]
36. Yamasaki Y, Kayashima T, Soejima H, Kinoshita A, Yoshiura K, Matsumoto N, Ohta T, Urano T, Masuzaki H, Ishimaru T, Mukai T, Niikawa N, Kishino T. Neuron-specific relaxation of Igf2r imprinting is associated with neuron-specific histone modifications and lack of its antisense transcript Air. Hum Mol Genet. 2005; 14:2511–20. [PubMed: 16037066]
37. Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R, Fraser P. The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. Science. 2008; 322:1717–20. [PubMed: 18988810]
38. Wagschal A, Sutherland HG, Woodfine K, Henckel A, Chebli K, Schulz R, Oakey RJ, Bickmore WA, Feil R. G9a histone methyltransferase contributes to imprinting in the mouse placenta. Mol Cell Biol. 2008; 28:1104–13. [PubMed: 18039842]
39. Terranova R, Yokobayashi S, Stadler MB, Otte AP, van Lohuizen M, Orkin SH, Peters AH. Polycomb Group Proteins Ezh2 and Rnf2 Direct Genomic Contraction and Imprinted Repression in Early Mouse Embryos. Dev Cell. 2008 in press.
40. Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, Alvarez P, Brockman W, Kim TK, Koche RP, Lee W, Mendenhall E, O'Donovan A, Presser A, Russ C, Xie X, Meissner A, Wernig M, Jaenisch R, Nusbaum C, Lander ES, Bernstein BE. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature. 2007
41. Pauler FM, Koerner MV, Barlow DP. Silencing by imprinted noncoding RNAs: is transcription the answer? Trends Genet. 2007
42. Pandey RR, Mondal T, Mohammad F, Enroth S, Redrup L, Komorowski J, Nagano T, Mancini-Dinardo D, Kanduri C. Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. Mol Cell. 2008; 32:232–46. [PubMed: 18951091]

43. Brannan CI, Dees EC, Ingram RS, Tilghman SM. The product of the H19 gene may function as an RNA. Mol Cell Biol. 1990; 10:28–36. [PubMed: 1688465]
44. Rabinowicz PD, Palmer LE, May BP, Hemann MT, Lowe SW, McCombie WR, Martienssen RA. Genes and transposons are differentially methylated in plants, but not in mammals. Genome Res. 2003; 13:2658–64. [PubMed: 14656970]
45. Walsh CP, Bestor TH. Cytosine methylation and mammalian development. Genes Dev. 1999; 13:26–34. [PubMed: 9887097]
46. Egloff S, Murphy S. Cracking the RNA polymerase II CTD code. Trends Genet. 2008; 24:280–8. [PubMed: 18457900]
47. Johnson SA, Cubberley G, Bentley DL. Cotranscriptional Recruitment of the mRNA Export Factor Yra1 by Direct Interaction with the 3′ End Processing Factor Pcf11. Mol Cell. 2008
48. Stricker SH, Steenpass L, Pauler FM, Santoro F, Latos PA, Huang R, Koerner MV, Sloane MA, Warczok KE, Barlow DP. Silencing and transcriptional properties of the imprinted Airn ncRNA are independent of the endogenous promoter. EMBO J. 2008
49. Moldon A, Malapeira J, Gabrielli N, Gogol M, Gomez-Escoda B, Ivanova T, Seidel C, Ayte J. Promoter-driven splicing regulation in fission yeast. Nature. 2008; 455:997–1000. [PubMed: 18815595]
50. Zwart R, Sleutels F, Wutz A, Schinkel AH, Barlow DP. Bidirectional action of the Igf2r imprint control element on upstream and downstream imprinted genes. Genes Dev. 2001; 15:2361–6. [PubMed: 11562346]
51. Schoenfelder S, Smits G, Fraser P, Reik W, Paro R. Non-coding transcripts in the H19 imprinting control region mediate gene silencing in transgenic Drosophila. EMBO Rep. 2007; 8:1068–73. [PubMed: 17948025]

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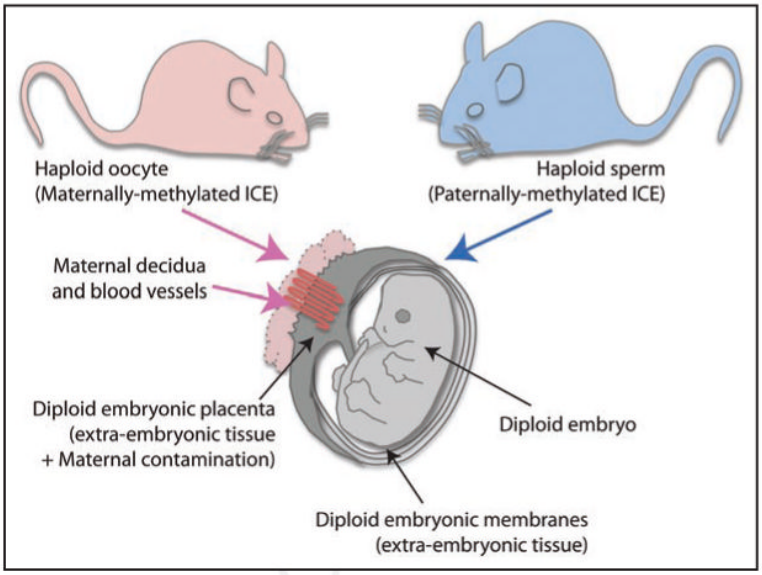

Figure 1.

Mammalian embryos require a maternal and paternal parent. Mammals are diploid and produce haploid gametes that carry either a maternal or paternal, methylated Imprint Control Element or ICE. In diploid embryonic and placental cells, the ICE maintains its parental-specific methylation imprint through the action of the maintenance DNA methyltransferase (DNMT1). A 12.5 dpc embryo is shown contained inside three membranes (the amnion and visceral and parietal yolk sacs) and attached via the umbilical cord, to the placenta. The membranes, umbilical cord and placenta are embryo-derived tissues that do not contribute to the embryo itself and thus are known as extra-embryonic tissues. The placenta is an invasive tissue that contains maternal blood vessels, maternal blood cells and maternal uterine tissue (i.e., the decidua).

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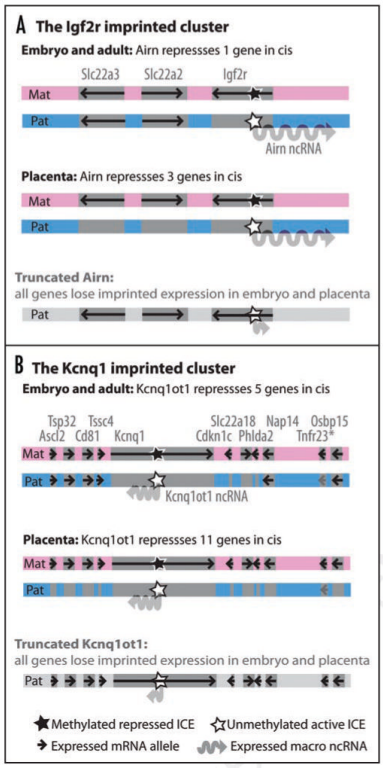

Figure 2.

The *Igf2r* and *Kcnq1* imprinted clusters. (A) The *Igf2r* imprinted cluster on Chr.17 contains three maternally-expressed imprinted mRNA genes (*Slc22a2, Slc22a3* and *Igf2r*) and the paternally-expressed *Airn* macro ncRNA. In the embryo and adult only *Igf2r* and *Airn* show imprinted expression, in the placenta the *Slc22a2* and *Slc22a3* genes also show imprinted expression. *Slc22a2* shows imprinted expression in early and late placenta but *Slc22a3* imprinted expression is lost in the late placenta.50 Black arrow: expressed gene and transcription orientation, gray wavy arrow: macro ncRNA, Mat: maternal chromosome, Pat: paternal chromosome. (B) The *Kcnq1* cluster on Chr.7 contains eleven maternally-expressed imprinted mRNA genes and the paternally-expressed *Kcnq1ot1* macro ncRNA. In the embryo and adult only 5 genes show imprinted expression, in the placenta an additional 6 genes also show imprinted expression (gray arrow indicates reduced expression). In both imprinted clusters, the maternal chromosome that expresses the mRNA genes carries the methylated ICE (black star) while the paternal chromosome contains the unmethylated ICE (white star) that overlaps with the ncRNA promoter. The function of the 108 kb *Airn* and 91.5 kb *Kcnq1ot1* ncRNAs, was tested by insertion of a polyA cassette downstream of the promoter and thereby generating a transcript truncated to 1.5–3% of wildtype length.28-30 This truncated ncRNA was expressed normally, i.e., only from the paternal chromosome and the ICE was normally methylated, i.e., only on the maternal chromosome, but resulted in loss of paternal repression of all mRNA genes in each imprinted cluster in both the embryo and placenta. Thus the ncRNA and not the ICE, represses multiple mRNA genes in cis in each cluster.

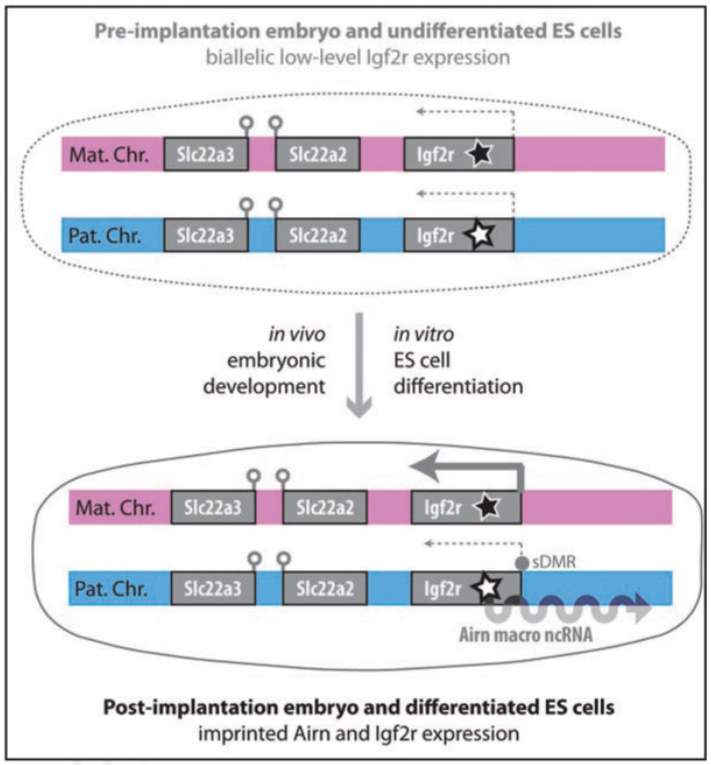

Figure 3.

Developmental regulation of *Igf2r* imprinted expression. Preimplantation embryos and undifferentiated ES cells express low levels of *Igf2r* from both parental chromosomes (dashed arrows). *Airn* is not expressed. The ICE (star) carries a maternal-specific DNA methylation imprint that was inherited from the oocyte (black star). In post-implantation embryos and differentiated ES cells, *Igf2r* is upregulated on the maternal chromosome but not on the paternal chromosome that expresses the *Airn* ncRNA from the unmethylated ICE. The paternal *Igf2r* promoter continues to be expressed at low-levels despite the acquisition of a somatic DNA methylation imprint (sDMR) in late differentiated cells. *Slc22a2* and *Slc22a3* are not expressed in embryonic or ES cells (white circles).

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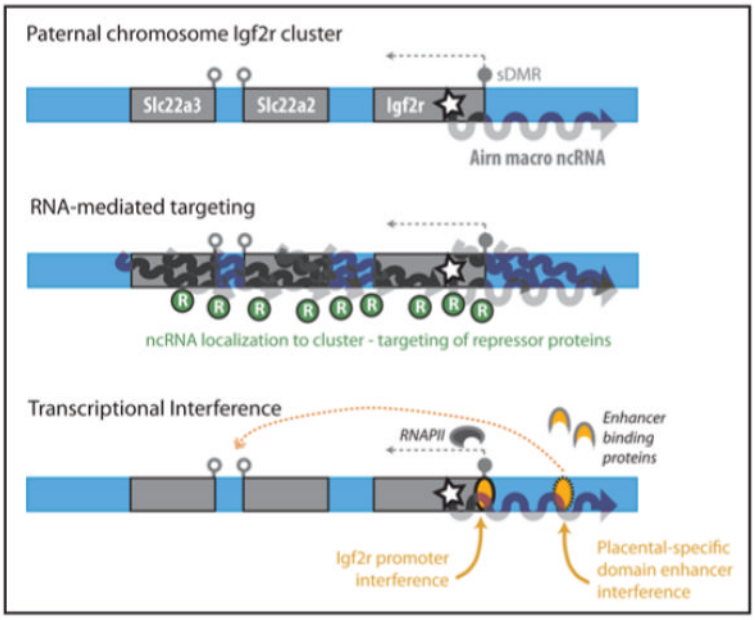

Figure 4.

Two models of repression by the *Airn* ncRNA. Top: Placental-specific expression of the paternal allele of the imprinted *Igf2r* cluster. *Slc22a3, Slc22a2* and *Igf2r* are repressed on the paternal chromosome, but only *Igf2r* has a somatic DNA methylation imprint (sDMR) that is acquired in late embryonic development (filled grey circle). The ICE that contains the *Airn* promoter is unmethylated (white star). The *Airn* ncRNA is necessary for repressing all three genes, but only has an antisense overlap with the *Igf2r* promoter. Middle: in the RNA-mediated targeting model, the *Airn* ncRNA localizes to the chromosome that expresses it, but only to the domain containing the *Slc22a3/Slc22a2/Igf2r* genes and then attracts repressor proteins (circle with R) that mediate gene repression. This model is analogous to the mechanism proposed for the *Xist* ncRNA (Leeb et al. this volume), and also allows for the possible involvement of putative cis-acting RNAi type mechanisms. Bottom: in the transcription interference model that depends only on *Airn* transcription and not on the ncRNA itself, *Airn* is proposed to repress the *Igf2r* promoter via transcription interference with RNAPII initiation because of its antisense transcription overlap. Transcription interference could also repress the *Slc22a2* and *Slc22a3* genes that lie 200 kb upstream if they were subject to cis-activation (orange dotted arrow) by a placental-specific enhancer (orange oval), that is inactivated by *Airn* transcription.

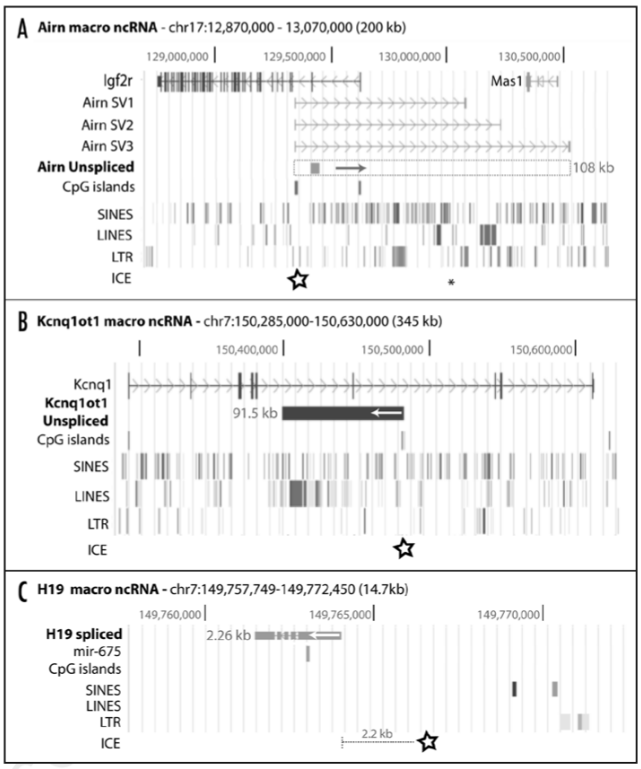

Figure 5.

Genomic organization of imprinted macro ncRNAs. The genome position (UCSC Genome Browser on Mouse July 2007 Assembly), size and orientation of the 108 kb *Airn* ncRNA (A), the 91.5 kb *Kcnq1ot1* ncRNA (B), and the 2.26 kb *H19* ncRNA (C) is shown. The *Airn* and *Kcnq1ot1* promoters are associated with CpG islands that lie in the ICE (white star), the *H19* promoter lies 5 kb downstream of the ICE that is only moderately CG rich. *Airn* transcripts are mostly unspliced and nuclear-localized, but 5% of transcripts are spliced with the indicated organization and exported to the cytoplasm.20 The *Airn* spliced variants (SV) all use the same splice donor (at +53 bp) but all use different 2nd exons. The dotted rectangle indicates the full length of the unspliced *Airn* transcript as shown by oligo tiling array hybridization.48 The small box inside the dotted rectangle indicates an annotated *Airn* EST. *Kcnq1ot1* appears to lack spliced transcripts and is localized the nucleus.42 *H19* appears to be fully spliced but shows an unusually high exon/intron ratio and is exported to the cytoplasm. A biallelically-expressed and nuclear-localized transcript of unknown function has also been identified at the *H19* ICE in embryonic liver tissue.51 Note that *Airn* and *Kcnq1ot1* have an antisense transcription overlap with one gene however, only *Airn* overlaps a promoter. The presence of interspersed repeats that are contained in the *Airn* and *Kcnq1* but not *H19* macro ncRNAs is shown underneath. *H19* also contains the mir-675 miRNA. *Airn* contains a small oocyte RNA from the *Au76* pseudogene (*).