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Curr Opin Genet Dev. Author manuscript; available in PMC 2010 April 28.

Published in final edited form as:
Curr Opin Genet Dev. 2010 April ; 20(2): 164–170. doi:10.1016/j.gde.2010.01.009.

Genomic imprinting—an epigenetic gene-regulatory model

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Abstract

Epigenetic mechanisms (Box 1) are considered to play major gene-regulatory roles in development, differentiation and disease. However, the relative importance of epigenetics in defining the mammalian transcriptome in normal and disease states is unknown. The mammalian genome contains only a few model systems where epigenetic gene regulation has been shown to play a major role in transcriptional control. These model systems are important not only to investigate the biological function of known epigenetic modifications but also to identify new and unexpected epigenetic mechanisms in the mammalian genome. Here we review recent progress in understanding how epigenetic mechanisms control imprinted gene expression.

Introduction

The last few years have seen a tremendous breakthrough in high-throughput sequencing technologies that allow histone and DNA modifications, transcription factors, and RNA polymerases to be exactly localized throughout the genome relative to expressed or silent genes [1-3]. These studies provide important information about the chromatin state of expressed or silent genes, but give no insight into whether the chromatin state is the cause or effect of changes in gene expression. An alternative approach is to apply these mapping techniques to genes that are known to be subject to specific epigenetic regulation, to identify chromatin and transcription features of these genes and then to test the relevance of these features for gene expression. In this way a large number of epigenetic gene-regulatory models (such as yeast mating-type switching, transgene position effect variegation, transposon silencing, and centromere silencing) have been proposed as models to probe how the chromatin state controls expressed and silent genes [4]. In mammals, systems showing mono-allelic expression in diploid cells such as X-chromosome inactivation and genomic imprinting show very clear evidence of epigenetic regulation that restricts expression to one parental allele in diploid cells. Here we overview recent progress in using genomic imprinting (Box 2) as a model of epigenetic gene regulation in mammals, which indicates that epigenetic mechanisms do not always follow the most straightforward route to regulate gene expression.

It has recently been appreciated that multiple mechanistic steps lead to the stable inheritance of epigenetic phenotypes through cell division [5••]. This new operational definition of epigenetics proposes three logical steps: first an ‘epigenator signal’ from outside the cell is required to trigger an intracellular pathway, second an ‘epigenetic initiator’ responds to this pathway in a locus-specific manner, and third, ‘epigenetic maintainers’ change the

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Conflict of interests
The authors declare there is no conflict of interest.

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chromatin state at the locus recognized by the initiator and maintain this state throughout subsequent cell divisions. While little is yet known of epigenator signals, epigenetic initiators include DNA-binding proteins and non-coding RNAs, while the epigenetic maintainers include the well-known processes of DNA methylation, histone modifications, histone variants, or nucleosome remodelling [5••]. With this operational definition of epigenetics in mind, we overview here recent progress in understanding how genomic imprinting induces parental-specific gene expression.

The key role of the imprint control element (ICE) in genomic imprinting

In mammals, genomic imprinting acts as a block to parthenogenesis (Box 1) and mouse embryos carrying two maternal genomes normally die at mid-gestation [6]. However, bi-maternal mice can be generated from one normal haploid maternal genome and one immature haploid maternal genome genetically manipulated to delete two ICEs (Box 1), which normally gain a paternal methylation imprint during spermatogenesis [7••]. In these bi-maternal mice one haploid chromosome set obtained from mature oocytes carries normal maternal imprints and shows typical maternal-specific imprinted expression (Figure 1). The other genetically manipulated haploid chromosome set was transferred from an immature oocyte and so lacks maternal imprints. The combination of a lack of maternal imprints plus a lack of two normally paternally methylated ICEs not only fully substitutes for a paternally inherited chromosome set, but also shows that no other paternally methylated ICE regulates essential genes during development (note that Figure 1 only shows chromosome 7, the other deleted paternally methylated ICE in these bi-maternal mice lies on chromosome 12). While this appears to be a complicated story there is a simple explanation that highlights the two key features of the ICE: (i) On one parental chromosome the unmethylated ICE is a *cis*-acting repressor. Thus, deletion of the unmethylated ICE will relieve gene repression. In Figure 1, deletion of the normally paternally methylated ICE relieves *Igf2* repression on the maternal chromosome. (ii) On the other parental chromosome a gametic DNA methylation imprint acquired during male or female gametogenesis represses ICE function. In Figure 1 expression of *Cdkn1c* from a maternal chromosome requires maternal ICE methylation. Note that deletion of the methylated ICE will not change gene expression.

Box 1 Glossary

Epigenetics	A heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence [5••].
Cis/Trans effect	'*in cis*' refers to an action on the same chromosome, '*in trans*' refers to an action on other chromosomes.
Genomic imprinting	An epigenetic mechanism leading to parental-specific expression normally affecting small clusters of genes (Box 2).
ICE	Imprint control element is a short DNA element whose epigenetic state controls imprinted expression of all genes in one imprinted cluster (Box 2).
Imprint	An epigenetic mark on the ICE which distinguishes the parental alleles of an imprinted gene cluster in the gametes. So far only DNA methylation has been shown to act as the imprint and the imprinted chromosome is here

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	defined as the allele that gains ICE DNA methylation in one parental gamete (Box 2).
Macro (long) ncRNA	A new class of non-coding (nc) RNAs with regulatory potential defined as >200 bp whose function does not depend on processing into short/micro/pi RNAs [43-45,33].
Non-coding (nc) RNA	RNAs not translated into a protein but since they could 'code' for a function the alternative terms, non-protein-coding (npc) or untranslated (ut) are more accurate [50].
Parthenogenesis	In mammals, reproduction without fertilization in female oocytes requiring doubling of the oocyte haploid chromosome set.
Retrogene	An expressed retrotransposed gene copy that retains its protein-coding capacity.

The unmethylated ice is a *cis*-acting repressor

To date there are three known ways in which the unmethylated ICE acts as an 'epigenetic initiator' to repress genes *in cis*. Moreover, variations in imprinted expression arise because these initiator mechanisms can show tissue- and developmental-specific regulation. In the first identified mechanism, an analysis of the *Igf2* imprinted cluster on mouse chromosome 7 (Figure 2a) shows the unmethylated ICE acts as an insulator that binds the zinc finger transcription factor CTCF to block the access of *Igf2* to enhancers that lie downstream of the *H19* ncRNA [8]. Recently, COHESIN, a protein complex essential for sister chromatid cohesion, was shown to bind the same sites in mammalian genomes as CTCF [9••,10••]. Both CTCF and COHESIN have now been shown to be necessary to induce specific chromatin loops necessary for *Igf2* silencing on the maternal chromosome [11,12••]. In the second identified mechanism, an analysis of two imprinted clusters (*Igf2r* and *Kcnq1*) shows the unmethylated ICE contains an active promoter for a non-coding RNA that represses multiple genes *in cis* (Figure 2b). In the *Igf2r* imprinted cluster the 108 kb long *Airn* ncRNA represses three genes spread over 300 kb *in cis*, while in the *Kcnq1* imprinted cluster the 90 kb long *Kcnq1ot1* ncRNA represses *in cis*, 11 genes spread over 800 kb [13,14]. The *Airn* ncRNA when shortened to 3% is unable to repress genes in its imprinted cluster in all tested tissues. However, in the *Kcnq1* cluster while most genes are not repressed by a shortened *Kcnq1ot1* ncRNA, the *Cdkn1c* gene remains repressed in some, but not all tissues [15•]. This indicates that in some tissues, either a short form of the *Kcnq1ot1* ncRNA is sufficient for silencing *Cdkn1c*, or, that two distinct mechanisms operate in this cluster. In the third example (Figure 2c), analysis of the *H13* imprinted cluster shows the unmethylated ICE contains an active promoter for the *Mcts2* retrogene [16••]. Expression of *Mcts2* or the unmethylated ICE itself causes *H13* to use internal polyadenylation sites and the resulting short transcripts lack enzyme activity. Although superficially similar to the situation in the *Igf2r* and *Kcnq1* imprinted cluster, the transposed *Mcts2* retrogene retains an open-reading-frame and protein-coding capacity.

Box 2 Genomic imprinting background

While most genes in mammalian diploid somatic cells are expressed from both parental chromosomes (i.e., show bi-allelic expression), imprinted genes show parental-specific mono-allelic expression [6,51,52•]. In contrast to X-chromosome inactivation, the sex of the offspring is not important, instead, imprinted expression is dictated by the parental

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origin of the chromosome and affected genes show the same parental-specific expression in both male and female offspring. To date 131 maternally or paternally expressed imprinted genes have been identified in mice and many of these control essential functions in embryonic development (http://www.mousebook.org/catalog.php?catalog=imprinting [49••]). Notably, although imprinted gene expression can vary in development, differentiation and disease; the parental origin of the expressed allele does not. For example, the mouse *Igf2r* (*Insulin-like growth factor type 2 receptor*) gene shows bi-allelic expression in early pre-implantation embryos that changes to maternal-specific expression in early post-implantation embryos, and in post-mitotic neurons only, *Igf2r* reverts to bi-allelic expression [53,54]. The key to understanding how imprinted expression can show tissue-specific or developmental variation, was the appreciation of two features: (i) that DNA methylation imprints (Box 1) are not gene-specific but instead, repress a long-range regulatory element (known as the imprint control element or region: ICE or ICR) that represses clusters of genes *in cis* (Box 1), and (ii), that the repressor activity of the ICE is developmentally and tissue-specifically regulated [55,56]. Thus, the ICE that is universally present in all somatic cells is 'imprinted' on one chromosome by DNA methylation or 'not imprinted' on the other chromosome, and genes regulated by the non-imprinted unmethylated ICE, show imprinted expression in some tissues and bi-allelic expression in others.

Epigenetic initiators at the unmethylated ICE have been shown to control parental-specific silencing in all types of mouse tissue (references in Figure 2). However, recent studies indicate that epigenetic maintainers responding to the epigenetic initiator may differ in mouse embryonic/adult tissues and extra-embryonic tissues. The latter comprise the placenta and membranes of mouse embryos that are short-lived tissues and only present during the 21-day embryonic gestation period [17•]. For example, in placenta the *Airn* ncRNA represses three genes *Slc22a3, Slc22a2*, and *Igf2r* (Figure 2). In embryo and adult tissues, only *Igf2r* is repressed by *Airn*. In the placenta, *Airn* was recently shown to recruit G9A (KMT1C) a histone H3-K9-dimethylase to silence *Slc22a3* (*Slc22a2* could not be tested as it is not expressed before G9A null embryos die at 10.5 dpc) [18••]. *Igf2r* was not affected by the loss of G9A, indicating *Slc22a3* and *Igf2r* are silenced by different mechanisms in placenta. The *Kcnq1ot1* ncRNA was also recently shown to localize to a nuclear compartment with Polycomb group (PcG) proteins that induce a contracted state in the paternal allele necessary for silencing of some, but not all genes in extra-embryonic tissues, but not in embryonic tissues [19••].

An earlier analysis of mice deficient in the EED Polycomb protein, similarly showed loss of imprinted expression of only 4/14 tested genes in extra-embryonic tissues but no effect in embryonic tissues [20]. These data indicate that histone modifications play a partial role as epigenetic maintainers of extra-embryonic imprinted expression. In contrast, histone modifications have not yet been shown to play an epigenetic maintainer role in mouse embryonic tissues. Genome-wide maps of repressive histone H3K9me3 marks in embryonic stem (ES) cells have shown they are focally restricted to the DNA methylated ICE and do not spread throughout imprinted clusters [21••]. We have recently shown that the *Igf2r* gene is devoid of Polycomb-dependent H3K27me3 modifications when it shows imprinted expression in differentiated embryonic cells. Moreover, although this mark is present in undifferentiated embryonic stem (ES) cells, it is lost from both parental alleles when *Igf2r* gains imprinted expression in differentiated ES cells [22••,23••]. This contrasts with tissue-specific silent genes that are contained in broad domains of H3K27me3 in differentiated embryonic cells [24••]. Notably, very few genes repressed *in cis* by the unmethylated ICE, subsequently gain promoter DNA methylation. The mouse *Igf2r* promoter and *Cdkn1c* CpG island promoters are an exception and both are methylated when repressed (Figure 2).

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However, both of these genes are silenced by their respective macro-ncRNAs in the absence of genome-wide DNA methylation [25-28]. A new model for generating imprinted expression based on kinetic experiments at the *Igf2r* imprinted cluster demonstrated that the repressed allele maintained low-level basal expression, and that imprinted expression arose from an expression bias between the two parental alleles [23••]. While it remains to be tested how general this expression-bias model is, it may explain the widespread lack of repressive marks on genes repressed by the active ICE, and the frequent finding that the expression of imprinted genes becomes biallelic, but is reduced to basal levels in the absence of ICE methylation [29-31].

Gametically acquired DNA methylation imprints repress ICE function

The situation on the one parental chromosome carrying the methylated ICE can be reduced to the question of how an ICE gains DNA methylation in one gamete and avoids methylation in the other gamete. Since the targeting and maintenance of DNA methylation patterns in mammals is poorly understood, ICE methylation offers an important model system. To date 15 ICEs have been provisionally identified based on their gain of maternal methylation during oogenesis (12 examples) or paternal methylation during spermatogenesis (3 examples) [32]; however, only 6 of these have been genetically shown to have ICE activity [33•]. The *de novo* and maintenance DNA methyltransferase complexes that are responsible for genome-wide DNA methylation in somatic tissues [34•] are also responsible for gametic ICE methylation. In particular, the germline specific DNMT3A2 *de novo* methyltransferase isoform is necessary for ICE DNA methylation in male and female gametes, while the DNMT3L accessory protein is required specifically for maternal gametic ICE methylation, but not for paternal ICE methylation [35,36]. DNMT3L has been shown to play an important role in targeting DNA methylation to regions containing histone H3 that lacks K4 methylation [37••]. Recently, an H3K4 demethylase (KDM1B) was also shown to be necessary for establishing maternal ICE methylation in 4/7 tested ICEs, but played no role in methylation acquisition of 2/3 tested paternally methylated ICEs [38••]. Once ICE methylation imprints have been acquired in male and female gametes, maintenance of a subset of methylated ICE requires additional proteins such as the germ cell and oocyte-specific nuclear PGC7/Stella protein, and a KRAB zinc finger protein encoded by the *Zfp57* locus [39••,40••].

While these experiments have clearly identified some of the key players in the acquisition and maintenance of methylation imprints, they do not explain how methylation is targeted to ICEs that lack obvious sequence-specificity apart from a high CpG content and the presence of tandem direct repeats in some ICEs [32,41]. One possibility suggested from an analysis of the ICE in the mouse *Gnas* imprinted cluster, is that overlapping transcription from a protein-coding mRNA gene may be required for oocyte (maternal) ICE methylation [42••]. All the 12 known maternally methylated ICEs [32] are overlapped by protein-coding genes and many of these were also shown to be transcribed in oocytes [42••], indicating this could be a general epigenetic initiator mechanism leading to maternal-specific ICE methylation. These data are not contradicted by the above demonstration that H3K4 demethylation may be a prerequisite for DNA methyltransferase targeted via DNMT3L [38••], as H3K4 methylation is not distributed equally through transcribed genes, but is concentrated focally at promoters and enhancers [1].

Conclusions

The analysis of epigenetic mechanisms at imprinted gene clusters has yielded a wealth of information particularly at the level of the locus-specific epigenetic initiator, where the unexpected appears to rule. The discovery of the ICE, which is a long-range *cis*-acting

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repressor that is itself repressed by DNA methylation, not only sheds light on the domain regulation of genes, but also demonstrates that one biological role of DNA methylation in mammals is to express genes normally repressed by default. The subsequent discovery that the unmethylated ICE can repress genes *in cis* by different mechanisms including insulator formation and macro ncRNA or retrogene expression also has a wider implication for mammalian genome biology. We do not know yet if methyl-sensitive long-range regulatory elements are common features of the mammalian genome outside of imprinted clusters. Macro ncRNAs that have long been associated with imprinted gene clusters [33•] are, however, now being seen as part of the genome-wide regulatory repertoire in mammals [43-45]. Notably, macro ncRNAs have also been shown to regulate non-imprinted disease phenotypes [46••,47,48••]. It is yet too early to know if genomic imprinting provides an accurate epigenetic regulatory model of tissue-specific silencing or, whether it better reflects abnormal gene silencing occurring, for example, in aging or in tumor development. However, since the analysis of imprinted gene clusters has been unevenly concentrated on 6 of the known 26 regions containing imprinted genes [33•,49••], we can be certain they will continue to provide one of the best discovery models for epigenetic gene regulation in mammals.

Acknowledgments

We thank the whole Barlow Group for lively discussions on the themes presented here and Michael Jantsch, Florian Pauler and Quanah Hudson for reading the manuscript. The authors are supported by EU-FW6 IP ‘HEROIC’ (LSHG-CT-2005-018883), The EU-FW6 NoE ‘THE EPIGENOME’ (LSHG-Ct-2004-053433) and the FWF Modulators of RNA Fate and Function (SFB-P1718 B10).

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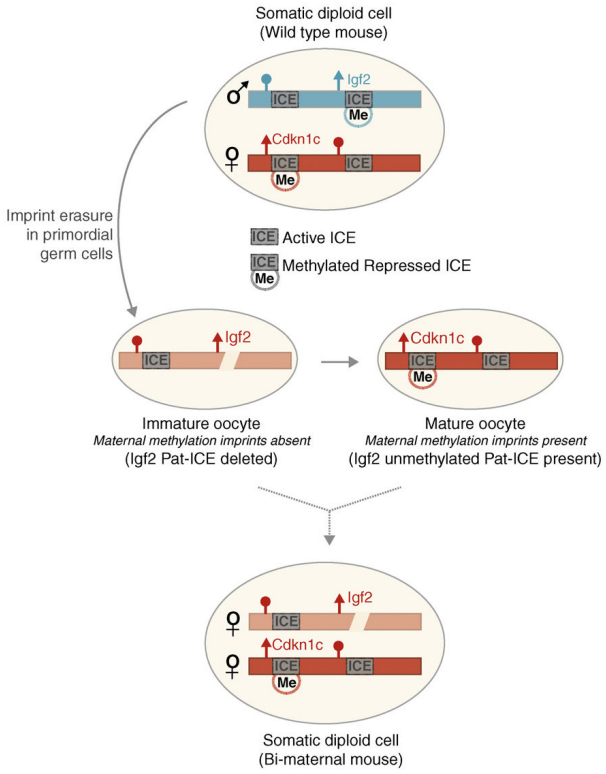

Figure 1.

Bypassing paternal imprints to generate bi-maternal mice. On mouse chromosome 7, a paternal DNA methylation imprint (Me/blue circle) represses the ICE and allows expression of *Igf2* from the paternal chromosome in normal diploid embryonic cells (arrow). *Igf2* is not expressed from a maternal chromosome that has an active unmethylated ICE (lollipop). A similar but opposite situation occurs in a neighboring imprinted cluster on this chromosome, where expression of *Cdkn1c* depends on a maternally methylated ICE (Me/red circle). Note that chromosome 7 contains only one of the two normally paternally methylated ICEs deleted to generate bi-maternal mice [7••]. DNA methylation imprints on ICEs are erased in primordial germ cells of the developing gonad and in females these imprints are reacquired during oocyte maturation. Chromosomes in immature oocytes lack maternal ICE imprints and, if they also genetically lack Pat-ICEs (oblique rectangle) that are normally modified by paternal gametic DNA methylation, then this haploid chromosome set will have a maternal origin with the imprinted expression pattern of the paternal genome [7••].

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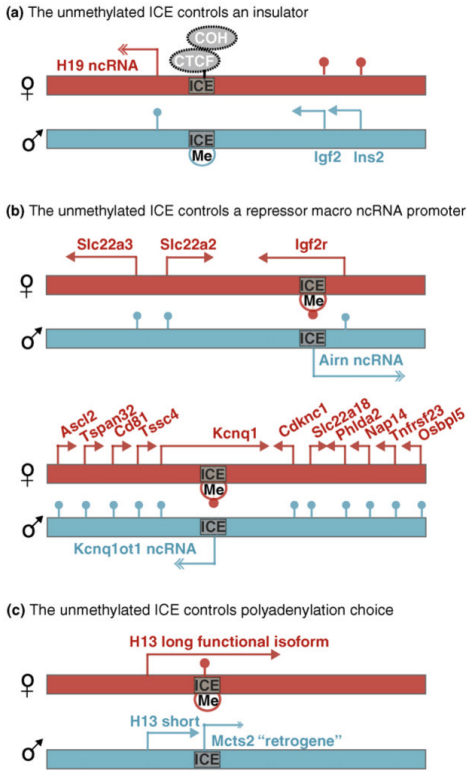

Figure 2.

The unmethylated ICE is a *cis*-acting repressor. Three examples of how the unmethylated ICE can repress mRNA genes *in cis* are known. **(a)** The unmethylated ICE in the mouse *Igf2* cluster on chromosome 7 forms an insulator on the maternal chromosome by binding CTCF and COHESIN (COH) proteins, which blocks the access of *Igf2* to enhancers located downstream to the *H19* ncRNA [8,12••]. **(b)** The unmethylated ICE in the mouse *Igf2r* imprinted gene cluster on chromosome 17 (top) and in the mouse *Kcnq1* imprinted gene cluster on chromosome 7 (bottom) contains an active promoter, respectively, for the *Airn* and *Kcnq1ot1* macro ncRNAs. Both these ncRNAs repress multiple genes *in cis* on the paternal chromosome [13,14,15•]. **(c)** The unmethylated ICE in the mouse *H13* (Minor histocompatibility antigen *H13* encoding a signal-peptide peptidase) imprinted cluster on chromosome 2 contains the active promoter for the *Mcts2* retrogene, and either the unmethylated ICE or *Mcts2* expression induces premature polyadenylation of *H13* transcripts that lack enzyme activity [16••]. The maps are not drawn to scale and show imprinted expression in the visceral yolk sac (A), for placenta (B) and in adult brain (C); genes showing bi-allelic expression are not indicated. Arrow: expressed gene, Double-headed arrow: expressed ncRNA or retrogene, lollipop: silent gene, Me/blue circle: paternal gametic methylation imprint, Me/red circle: maternal gametic methylation imprint.