*elegans* X chromosome is dosage compensated on a gene by gene basis, as the *Drosophila* X appears to be, or by a long-range chromosomal phenomenon like X inactivation in mammals (reviewed in *1*, *47*). If the mechanism is more similar to X inactivation, then it could spread long distances to neighboring chromatin in X:A translocations. If it operates on a gene by gene basis, then X-linked genes moved to autosomes should retain dosage compensation. Data to support either of these models are scarce, in large part because of the transformation system in *C. elegans*, in which microinjected DNA is usually maintained in extrachromosomal arrays rather than within the genome in single copy. Therefore, individual X-linked genes have not been assayed for dosage compensation when inserted into autosomes (*28*). However, in an unpublished study by Hsu and Meyer (cited in *28*), an autosomal gene, *unc-54*, was dosage compensated when inserted onto the X chromosome, so in this example any hypothetical cis-acting elements must have been able to function over distances of several kilobases. In addition, in one genetic study, mutations in several autosomal loci were not fully complemented by the autosomal portion of a translocation between the X and chromosome V, suggesting that the X signals might act over long distances to repress juxtaposed chromatin (*48*). The ability to stain for DPY-27 protein on translocation chromosomes should begin to allow the resolution of this very fundamental question.

### Things to Come . . .

In this review, we have highlighted aspects of X chromosome dosage compensation in two model organisms, fruitflies and nematodes. A third system that is of extreme interest to the chromosome field is the X-inactivation mechanism used by female mammals (*47*). A potentially fundamental breakthrough in that field may be forthcoming, as researchers determine whether inactivation of a whole chromosome relies, at least in part, on the expression of a nonprotein-coding RNA molecule, termed Xist, from the inactive X chromosome. We await with excitement the elucidation of this third, distinctive mechanism of X chromosome dosage compensation.

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# Gametic Imprinting in Mammals


Denise P. Barlow



Embryonic development in mammals is distinct from that in other vertebrates because it depends on a small number of imprinted genes that are specifically expressed from either the maternal or paternal genome. Why mammals are uniquely dependent on sexual reproduction and how this dependency is dictated at a molecular level are questions that have been intensively investigated during the past 2 years. Gene inactivation experiments have confirmed predictions that imprinted genes regulate embryonic and placental growth and that DNA methylation is part of the imprinting mechanism. Despite these considerable achievements, the reason why imprinted hemizygosity is used as a mechanism to regulate the intrauterine growth of mammalian embryos remains elusive.


In mammals some genetic traits show parental dependency and are only expressed when inherited from one parent. Two types of parental dependency are currently known. The first has a trivial cause and is due to an unequal distribution of genetic information between male and female gametes. Examples of this type include traits encoded by mitochondrial genes, Y chromosome–linked genes, and maternal-effect genes. The second type, known as gametic or genomic imprinting, is more of an enigma whose role in mammalian development and disease is not yet fully appreciated. Gametic imprinting describes those parental-dependent traits in which both the male and female allele are present but function unequally in the embryo.

Genes whose expression is restricted to either the maternal or paternal allele constitute the best known example of gametic imprinting. Sixteen such genes have been described in mice and humans, 5 of which are maternally expressed and 11 paternally expressed (Table 1). However, other traits such as trinucleotide repeat amplification, host-defense methylation responses, asynchrony of sister chromatid behavior, and meiotic recombination also exhibit parental dependency (*1*). Whether these latter traits arise from gametic imprinting is not yet clear; therefore, they will not be considered further in this brief review.


The author is at the Institute of Molecular Pathology, Dr. Bohr-Gasse 7, Vienna, Austria.

## Why Are Genes Imprinted?

The existence of any parental-dependent trait in mammals is a puzzle, because the advantage offered by a biparental contribution is clear from the recessive nature of most mutations. What then is the function of the hemizygosity induced by gametic imprinting? Earlier work based on experimentally derived androgenotes (diploid embryos with two paternal genomes) and gynogenotes (diploid embryos with two maternal genomes), and on mice that were diploid but had inherited both copies of individual chromosomes from one parent, showed that imprinted genes are essential in development and may play a growth regulatory role in the embryo and neonate (*2*). Interestingly, placental development appeared to depend on paternally expressed genes, and embryonic growth on maternally expressed genes, suggesting a link between imprinting and the control of intrauterine embryonic growth. This presumed link has been strengthened by recent analyses of mice harboring inactivated copies of imprinted genes. Of five imprinted genes studied, four caused defects in embryonic or placental growth [*IGF2*, *IGF2R/MPR300*, *H19*, and *MASH2*], and one was lethal before the onset of maximal embryonic growth at midgestation (*WT1*) (Table 1). (Human gene symbols are used throughout text and in Table 1.) However, not all of these genes showed the division of labor suggested by the analysis of androgenotes and gynogenotes. *MASH2*, for example, is a maternally expressed gene essential for formation of the placenta (*3*). Although it is too soon to conclude that all imprinted genes have a similar function, the significance of these results is emphasized by the fact that of the 263 gene "knockouts" analyzed in mice, 4% affect embryonic growth, 11% are lethal before midgestation, and 85% have no growth defect (*4*).

The observation that imprinted genes influence embryonic growth in mammals, combined with the apparent absence of imprinted genes in oviparous vertebrates, offers support for the suggestion that the role of gametic imprinting lies in the control of intrauterine embryonic growth. This presumed role is further strengthened by the observation that gametic imprinting has also been observed in the endosperm of a few angiosperm plants, a tissue suggested to be analogous to the mammalian placenta (*5*). Although the experimental evidence supports such a role for gametic imprinting, the explanation of why imprinted genes fulfill this particular function awaits the examination of a larger number of imprinted genes. From the evidence so far, it is not possible to distinguish between simplistic explanations, such as a role in maintenance of sexual reproduction, and the more exotic explanations, exemplified by the parental conflict hypothesis (*5*) and the trophoblastic disease protection hypothesis (*6*).

**Table 1.** Mammalian genes that show parental-specific expression in certain developmental stages and tissues. P, paternal-specific; M, maternal-specific; R, random; nd, not done; *WT1*, Wilms tumor suppressor (*20*); *INS*, insulin [two mouse loci, one human locus (*22*)]; *IGF2*, insulinlike growth factor type 2 (*21*); *H19*, non–open reading frame (ORF) RNA (*21*); *p57KIP2*, cyclin-dependent kinase inhibitor (*31*); *MASH2*, helix-loop-helix transcription factor (*3*); *SNRPN*, small nuclear riboprotein particle SmN (*21*); *ZNF127*, zinc finger protein (*21*); *PAR1* and *PAR5*, anonymous transcripts in the Prader-Willi consensus region (*27*); *IPW*, non-ORF RNA (*28*); *IGF2R/MPR300*, insulinlike growth factor type 2 receptor (also known as the mannose 6-phosphate 300-kD receptor) (*21*); *MAS*, cell surface receptor, putative oncogene (*29*); *XIST*, X chromosome–inactive specific transcript (*21*); *PEG1/MEST*, mesoderm-specific transcript (*30*); *SP2*, also known as *U2af1rs1*, a protein related to U2 small nuclear ribonucleoprotein auxiliary factor (*21*).

| Gene | Expressed allele | Chromosome | |
|---|---|---|---|
| | | Mouse | Human |
| *WT1* | M | 2 | 11p |
| *INS* | P | 7 | 11p |
| *IGF2* | P | 7 | 11p |
| *H19* | M | 7 | 11p |
| *p57KIP2* | M | 7 | 11p |
| *MASH2* | M | 7 | nd |
| *SNRPN* | P | 7 | 15q |
| *ZNF127* | P | 7 | 15q |
| *PAR1* | P | nd | 15q |
| *PAR5* | P | nd | 15q |
| *IPW* | P | nd | 15q |
| *IGF2R/MPR300* | M | 17 | 6q |
| *MAS* | P | 17 | nd |
| *XIST* | P/R | X | X |
| *PEG1/MEST* | P | 6 | nd |
| *SP2* | P | 11 | nd |

## How Are Genes Imprinted?

*Imprint acquisition*. Gametic imprinting requires that parental alleles be distinguished in every cell of the early embryo. The distinguishing mark, or imprint, that almost certainly arises during gametogenesis or before pronuclear fusion in the zygote (Fig. 1) must have three properties: (i) It must be present in one gamete and be sufficiently stable in mitosis to be propagated to every cell of the embryo. (ii) It must remain restricted to one parental chromosome in diploid cells. (iii) It must be reset in the germ line after the sex of the embryo is determined. Thus, the imprinting process is predicted to have an epigenetic component (the "imprint") that marks one parental chromosome, and a genetic component (the DNA sequence or "imprinting box") that is modified by the imprint.

Although certain DNA binding proteins such as the *Drosophila* Polycomb proteins could fulfill the requirements of the imprint (*7*), DNA methylation is so far the best candidate. In mammals DNA methylation is restricted to cytosine, usually in a CpG dinucleotide ($^{m}$CpG). After replication DNA will contain hemimethylated CpG dinucleotides that are the unique substrate for DNA methyltransferase (DNMTase) (*8*). DNMTase action is thus restricted to previously methylated sequences and acts to stably propagate chromosome-specific methylation patterns to daughter cells. Furthermore, in keeping with the properties required of the imprint, genomic methylation patterns are removed and reset during gametogenesis and in the preimplantation embryo (*9*). Thus, methylation has all of the hallmarks required of the imprint.

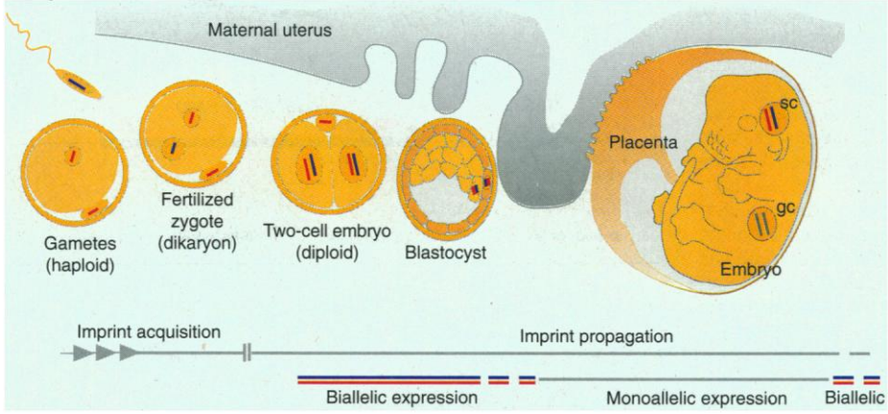


**Fig. 1.** Gametic imprinting is a multistep process. The key stages of gametic imprinting are shown. Imprint acquisition occurs when the genome is haploid, in the gametes or the dikaryon zygote, but may be modified later in development. In the later embryo, the imprint is stably propagated through mitosis and remains restricted to one parental chromosome. Monoallelic expression of several imprinted genes occurs late in the preimplantation embryo, and can be lost in some tissues of the later embryo or adult. Two types of diploid cell are depicted in the embryo: sc, somatic cell with imprinted chromosomes; and gc, germ cell with nonimprinted chromosomes. The oocyte polar body is illustrated up to the two-cell stage. Paternal expression is indicated by the blue line and maternal expression by the red line.

All imprinted genes so far examined contain DNA sequences methylated in a parental-specific manner (*8*). In some cases this methylation mark is acquired late in embryonic development. However, three imprinted genes (*IGF2R/MPR300*, *H19*, and *XIST*) and one imprinted transgene (RSVIgmyc, a complex mouse genomic fragment with an immunoglobulin–c-*myc* fusion) (*10*) have been shown to inherit a methylation imprint from one gamete. In these four cases the methylation imprint resists preimplantation demethylation, is maintained in somatic tissues, and is reset in the germ line. Interestingly, the DNA sequences carrying these gametic imprints preferentially retain methyl groups (*11*) when DNMTase activity is limiting. The significance of gametic methylation imprints has not yet been directly tested by genetic manipulation. However, a role for these imprints is supported by the finding that *H19* transgenes, containing the imprinted sequence, show the expected monoallelic expression pattern when integrated at independent loci (*12*).

The *IGF2R/MPR300* gene, the *XIST* gene, and the RSVIgmyc transgene are methylated maternally, whereas the *H19* gene is paternally methylated (*10*). DNA sequences carrying imprints from the same parent but not those imprinted by the other parent might be expected to share some common features. But, so far, no direct sequence homology has been found among the three maternally imprinted genes. The DNA sequences carrying both maternal and paternal gametic methylation imprints do, however, have two features in common. First, they resemble CpG islands, containing CG-rich regions of 200 to 1500 base pairs (bp) with a balanced CG:GC ratio (*13*). These imprinted sequences differ from standard CpG islands because they are methylated, albeit only on one parental allele. Because spontaneous deamination of $^{m}$C yields a T residue that is not repaired by the cell, methylation is expected to lead to loss of a CpG island (*13*). This may be prevented because the methylation imprint is removed early in the germ line and only reset in mature gametes (*9*); thus, imprinted islands are methylated for a relatively short period in the mammalian life span. The second feature shared by imprinted DNA sequences is that they contain, or are closely associated with, a region rich in direct repeats (Fig. 2). These repeats range in size from 25 to 120 bp, are unique to the respective imprinted regions, but have no obvious homology to each other or to highly repetitive mammalian sequences. The direct repeats may be an important feature of gametic imprinting, as they have been found in all imprinted genes analyzed to date and are also evolutionarily conserved (*14*).

*Reading the imprint*. Monoallelic expression of imprinted genes is not always coincident with the onset of expression and can also vary in development, differentiation, and disease (Fig. 1). The embryonic genome is activated at the two-cell stage coincident with degradation of maternally stored messenger RNA (*15*). Some imprinted genes such as *XIST* and *H19* (*10*) show monoallelic expression in early preimplantation embryonic stages. However, analyses of preimplantation embryos derived from androgenotes and gynogenotes and of embryonic stem (ES) cells (which resemble blastocyst-stage embryos) show equal parental expression of several imprinted genes. The *IGF2R/MPR300* gene, for example, is expressed in preimplantation androgenetic embryos, and from both parental alleles, in ES cells and in all preimplantation stages up to late blastocyst (*16*). The separation in time between acquisition of the gametic imprint and onset of monoallelic expression, which can occur in blastocysts containing several hundred cells, suggests that the imprint itself does not have to be a primary inactivation event and that the imprinting mechanism may involve additional trans-acting molecules that read the imprint. This suggestion is supported by two examples that have been molecularly characterized. In the first, a methylation imprint appears to directly repress the mouse *H19* gene on the paternal chromosome, and maternal expression of *H19* appears to inhibit *IGF2* and *INS1* expression in cis (*17*), possibly by enhancer competition (Fig. 2). In the second example, biallelic expression of the human *IGF2* gene occurs in neonatal liver through the recruitment of an upstream promoter that appears to be insensitive to the imprint (*18*).

*Maintenance of monoallelic expression*. The extent of monoallelic expression also varies in development and differentiation and differs between humans and mice. The mouse *IGF2R/MPR300* gene is maternally expressed in every tissue in all tested mouse strains. In contrast, monoallelic expression of the human gene appears to be a polymorphic trait, occurring in all tested tissues but only in a minority of the human population (*19*). A similar polymorphism is shown by the human *WT1* gene in placental tissue (*20*). Biallelic expression of the mouse and human *IGF2* gene occurs in some neural tissues, and in the human, also in adult liver (*18, 21*). Finally, the mouse *INS1* and *INS2* genes only show monoallelic expression in the extraembryonic membranes but not in the pancreas (*22*). In addition to the variation in monoallelic expression that occurs in development and differentiation, *IGF2* has been shown to switch to biallelic expression in tumors and other human diseases (*23*), and mice with an inactive maternal *IGF2R/MPR300* allele can in some circumstances switch on their paternal allele (*16*).

One key experiment has presented strong evidence that monoallelic expression of several imprinted genes depends on DNA methylation. Mice deficient for DNMTase die in the early postimplantation period but advance sufficiently so that gene expression can be analyzed. In the absence of DNMTase, monoallelic expression of four imprinted genes (*IGF2*, *IGF2R/MPR300*, *H19*, and *XIST*) was lost in postimplantation embryos (*11*). Surprisingly, the loss of monoallelic expression occurred, as predict-

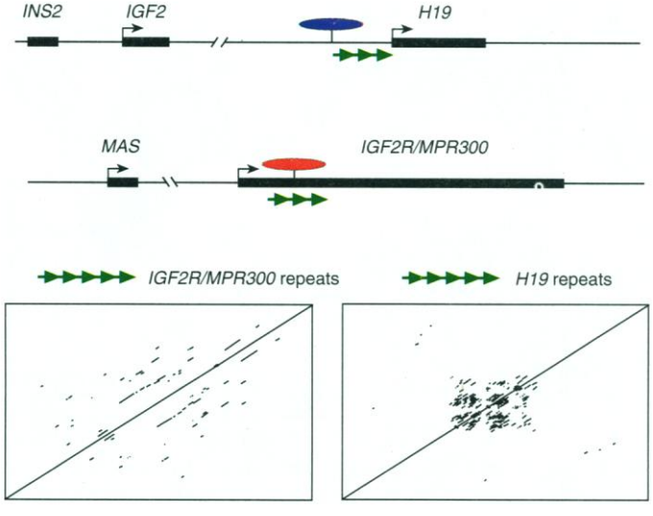


**Fig. 2.** Gametic methylation imprints and direct repeats in the *H19* and *IGF2R/MPR300* genes. The *H19* gene inherits a paternal methylation imprint (blue ellipse) proximal to a series of direct repeats (green arrows) upstream to the transcription start site. Sequences containing the *H19* imprint and repeats have been shown to affect the imprinting of the flanking *INS2* and *IGF2* genes (*17*). The *IGF2R/MPR300* gene inherits a maternal methylation imprint (red ellipse) directly covering a series of direct repeats (green arrows) that lie in the second intron (*10*). The relative position of the *MAS* gene, which is imprinted only when weakly expressed, is shown (*29*) (the significance of the *IGF2R/MPR300* intronic repeats and of the close linkage to *MAS* has not been analyzed). The boxes at the bottom show the extent of the direct repeats in the *H19* and *IGF2R/MPR300* genes in a 1500-bp region (covered by the green arrows). The boxes were generated by a sequence comparison program that aligns the sequence to itself, as described (*14*).

ed, by the gametic methylation imprints and not, as predicted, by the expected role of methylation in silencing gene expression. The silent, methylated *H19* and *XIST* alleles were activated, whereas the active, methylated *IGF2R/MPR300* allele was silenced. The active *IGF2* allele, which has no obvious gametic imprint, was also silenced. The simplest interpretation of these results is that methylation modulates the access of DNA binding proteins that repress or activate transcription. However, this may be an oversimplification, because in the case of *IGF2*, allele-specific repression most likely results from activation of the upstream *H19* gene (*17*). It remains to be seen if a similar mechanism of enhancer competition could also explain the apparent dependency of *IGF2R/MPR300* expression on methylation.

Because mammalian DNMTase has primarily a maintenance activity, it is unlikely to be involved in the de novo establishment of the gametic imprint. It remains possible that there are other methylating enzymes that carry out this role whose action is maintained by DNMTase in the postimplantation embryo. Alternatively, gamete-specific proteins may convert DNMTase into an imprinting enzyme. The recent characterization of genomic sequences bearing gametic methylation imprints may facilitate the identification of the enzyme responsible for the gametic imprint and, in turn, the identification of as yet unknown imprinted genes.

## Future Developments

The 16 imprinted genes listed in Table 1 have been arranged to highlight their clustering into chromosomal domains. Seven imprinted genes [and possibly three more (*PAR1*, *PAR5*, and *IPW*) that have not yet been mapped in the mouse] lie in two separate domains on mouse chromosome 7 (*2*). These two imprinted chromosomal domains are separated in the human onto chromosomes 11 and 15. Genetic experiments in which more than 80% of the mouse genome was analyzed identified only seven chromosomal domains that contained genes expressed in a parental-specific manner in embryonic development (*2*). Because the total number of imprinted genes in mammals is predicted (*24*) to be between 100 to 200, clusters of 15 to 30 imprinted genes would be expected. Although clustering of imprinted genes may predict aspects of the imprinting mechanism, there are two caveats that must be considered. First, the observed clustering may reflect a sampling bias arising from concentrating on regions that flank known imprinted genes (additionally, imprinted expression of flanking genes may also represent nonspecific effects on weak promoters). Second, the number of imprinted domains may have been underestimated if some imprinted genes do not have a major effect on embryonic development. Despite these caveats, it is clear that imprinted genes are clustered. This may imply that groups of genes are imprinted by a common cis-acting sequence, or that other long-range effects are involved.

An analysis of the imprinted clusters on mouse chromosome 7 and human chromosome 15q11-13 (Table 1) has confirmed that a single gene or regulatory element can regulate monoallelic expression of distant imprinted genes in cis (*17*, *25*). The identification of an unusual non–open reading frame–imprinted RNA in both of these clusters, one of which (*H19*) shares structural homology with the *XIST* gene (*14*), adds further support to the concept that imprinted genes are functionally grouped. In addition, the description of other parental-specific effects on chromosomal function, such as asynchrony of sister chromatid behavior and meiotic recombination, which span several megabase pairs of DNA and encompass nonimprinted and imprinted genes (*1*), also indicates that long-range effects are part of the imprinting mechanism. Functional grouping of imprinted genes would predict that genes would not retain imprinted behavior when transferred to other chromosomal sites. However, the *H19* gene and the RSVIgmyc transgene do retain imprinted expression at other chromosomal positions (albeit in a background-dependent manner), whereas the *IGF2* gene showed inconsistent behavior (*10*, *12*, *26*), suggesting that cis-acting or domain effects may not always be dominant. Thus, although the significance of chromosomal clustering of imprinted genes is not yet clear, it may be that gene mapping will offer a shortcut to isolating other imprinted genes.

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