GENOMIC IMPRINTING: PARENTAL INFLUENCE ON THE GENOME

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Genomic imprinting affects several dozen mammalian genes and results in the expression of those genes from only one of the two parental chromosomes. This is brought about by epigenetic instructions — imprints — that are laid down in the parental germ cells. Imprinting is a particularly important genetic mechanism in mammals, and is thought to influence the transfer of nutrients to the fetus and the newborn from the mother. Consistent with this view is the fact that imprinted genes tend to affect growth in the womb and behaviour after birth. Aberrant imprinting disturbs development and is the cause of various disease syndromes. The study of imprinting also provides new insights into epigenetic gene modification during development.

EUTHERIANS
Mammals that give birth to live offspring (viviparous) and possess an allantoic placenta.

Genomic imprinting in mammals was discovered in the early 1980s as a result of two types of mouse experiment. Nuclear transplantation was used to make embryos that had only one of the two sets of parental chromosomes (uniparental embryos) and other sophisticated genetic techniques were used to make embryos that inherited specific chromosomes from one parent only (uniparental disomy). In both cases, the surprising finding was that mammalian genes could function differently depending on whether they came from the mother or the father[1–6]. The early 1990s then saw the discovery of the first imprinted genes, which were indeed expressed differently on maternal and paternal chromosomes[7–9], and the realization that imprinting had a substantial effect on human genetic disease[10,11]. It was also found that DNA methylation was a key molecular mechanism of imprinting; methylation marks the imprinted genes differently in egg and sperm, and inheritance of these epigenetic marks leads to differential gene expression[12–17].

Substantial progress has been made in our understanding of imprinting in the past few years: important phenotypic effects of imprinted genes have been discovered, particularly in the control of fetal growth and behaviour after birth; a number of *cis*-acting sequences are being defined that are important for the control of imprinted gene expression; the evolutionary understanding of imprinting and its likely biological purposes is increasing[18,19]; and the study of imprinting is providing general insights into the importance of epigenetic mechanisms in development.

Here we review these recent developments. We begin with a brief summary of imprinted genes, then look at what is known about establishment and maintenance of imprints, and the important role of the germ line. We review the various 'reading mechanisms' that convert the imprint into differential gene expression. We discuss the evolution of imprinting, and its main phenotypic effects, in healthy and diseased states. Finally, we consider the effect of imprinting on important general issues in epigenetics, such as cloning and genome reprogramming.

Imprinted genes

Using several approaches (BOX1), around 45 imprinted genes have so far been identified in the mouse (see the Harwell imprinting web site for up-to-date statistics on imprinted gene numbers and characteristics). Some of these genes have been tested in other mammals and for many (but not all), the imprinting status is conserved in humans, in some other EUTHERIAN mammals and in a marsupial[20–22] (but only a few genes have been tested). What are the genetic and epigenetic features that characterize imprinted genes?

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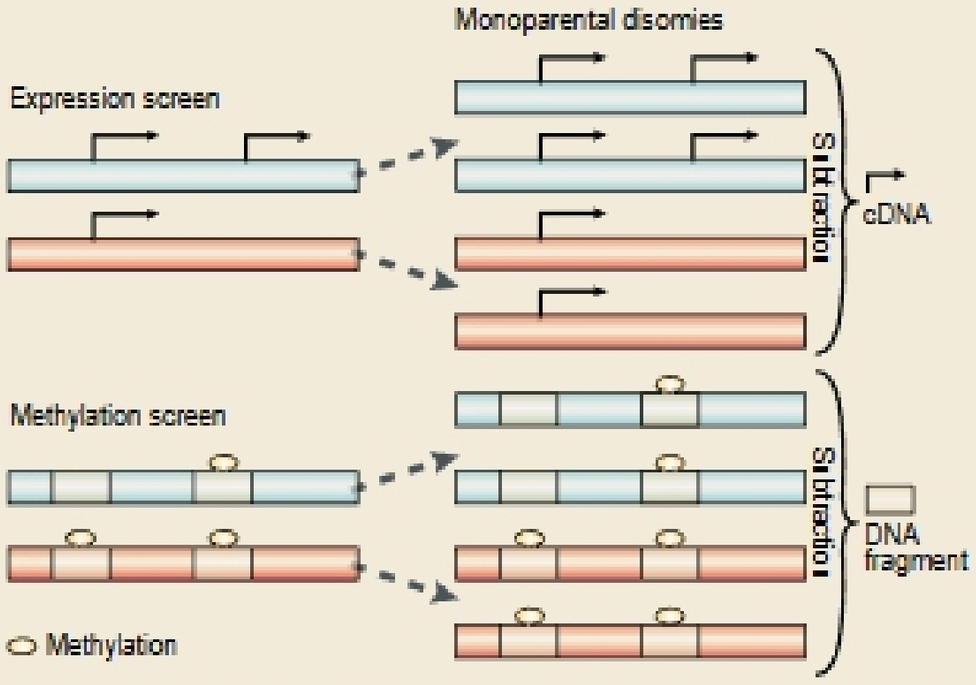
Box 1 | Finding imprinted genes



Imprinted genes have been identified in various ways: by chance (usually knockouts that then showed parent-specific expression); based on position (next to other imprinted genes or in a chromosome region associated with an imprinting phenotype); or by using two types of systematic screen. In both screens, embryos are used that have a duplication of one of the parental chromosomes or genomes, together with embryos that have the opposite parental chromosome duplicated. This results in gene expression or methylation in one type of embryo but not the other if the gene is imprinted. The first screen is based on subtraction of cDNAs between such uniparental embryos[115]. The second is based on methylation differences. One approach using restriction landmark genome scanning (by two-dimensional electrophoresis of DNA) has estimated that there are roughly 100 imprinted genes in the mouse genome[116]. Another methylation screen uses representational difference analysis[117] (RDA). The estimate of 100 imprinted genes in the genome is likely to be an underestimate but, in any event, imprinted genes constitute a minority of all the genes in the genome.

One remarkable and characteristic feature of imprinted genes is that they are rarely found on their own: around 80% are physically linked in clusters with other imprinted genes (FIG. 1). The clustered organization of imprinted genes is thought to reflect coordinated regulation of the genes in a chromosomal domain. By analogy to X-chromosome inactivation in which an X-inactivation centre controls the inactivation of the entire chromosome, imprinting centres or imprinting control elements (ICs) have been discovered in some clusters. These ICs are needed for the regional control of imprinting or imprinted expression.

No common features are recognizable when comparing the protein sequences encoded by imprinted genes, although there are functional relationships between some proteins with roles in fetal growth and development. Furthermore, two general features of the DNA sequence environment of imprinted genes have been noted. First, they are unusually rich in CPG ISLANDS[23]: around 88% of mouse imprinted genes have CpG islands, compared with the average figure of 47%. Second, clustered, direct repeats are common near to or within the CpG islands. The repeats might or might not belong to one of the known repeat families and they have been proposed to be involved in conferring or maintaining differential methylation[24]. Neither the repeats nor the CpG islands are unique to imprinted genes, so these features cannot be used in a systematic search for new imprinted genes.

CPG ISLAND
DNA region of >500 bp that has a high CpG density and is usually unmethylated. CpG islands are found upstream of many mammalian genes.

The great majority of imprinted genes examined so far show differences in DNA methylation between the parental alleles (FIG. 2), but the differentially methylated regions (DMRs) can have different properties. For example, the differential DNA methylation in some DMRs is introduced in parental germ cells and maintained in all developmental stages and tissues[25–28]. Others show considerable changes in methylation during development and acquire tissue-specific methylation patterns[29], which can be associated with tissue-specific imprinted expression. Some DMRs are methylated in the inactive gene copy, whereas others are methylated in the active one. Imprinted genes can also differ with respect to bulk chromatin structure, as well as with respect to more specific modifications, such as histone acetylation[30–36] (R. Feil and R. Gregory, personal communication).

Two other epigenetic features have been discovered that might reflect the larger-scale organization of imprinted genes into clusters or domains. First, it has been shown that the DNA in imprinted regions replicates asynchronously in the S phase of the cell cycle; for most imprinted regions, the paternal copy replicates earlier than the maternal one[37,38]. Because maternally and paternally expressed genes are interspersed in some regions, this is not likely to be a gene-specific property and its molecular basis is not understood. Second, different frequencies of meiotic recombination are found in or near to imprinted clusters, with an elevated recombination rate during male meiosis[39,40]. How these regional epigenetic features are linked with methylation and chromatin structure is not known.

The precise nature of the primary imprint and its fate during development is still a mystery, but it is likely that all the above epigenetic modifications are relevant to imprinting. However, at present there is no direct evidence that histone or other chromatin modifications have roles in imprinting that are independent of DNA methylation. Indeed, the importance of DNA methylation, at least in the maintenance of imprints, has been clearly established genetically[17]. For the most part, we therefore equate 'imprints' with 'methylation imprints' or 'differential methylation' to simplify the discussion. Imprinted expression is then a result of the 'reading' of the imprint in somatic tissues.

The life cycle of imprints

Genomic imprints change in characteristic ways during the life cycle of the organism (FIG. 3). Imprints are 'established' during the development of germ cells into sperm or eggs. After fertilization, they are 'maintained' as chromosomes duplicate and segregate in the developing organism. In the germ cells of the new organism, imprints are 'erased' at an early stage. This is followed by establishment again at a later stage of germ-cell development, thus completing the imprinting cycle. In somatic cells, imprints are maintained and are modified during development. For example,

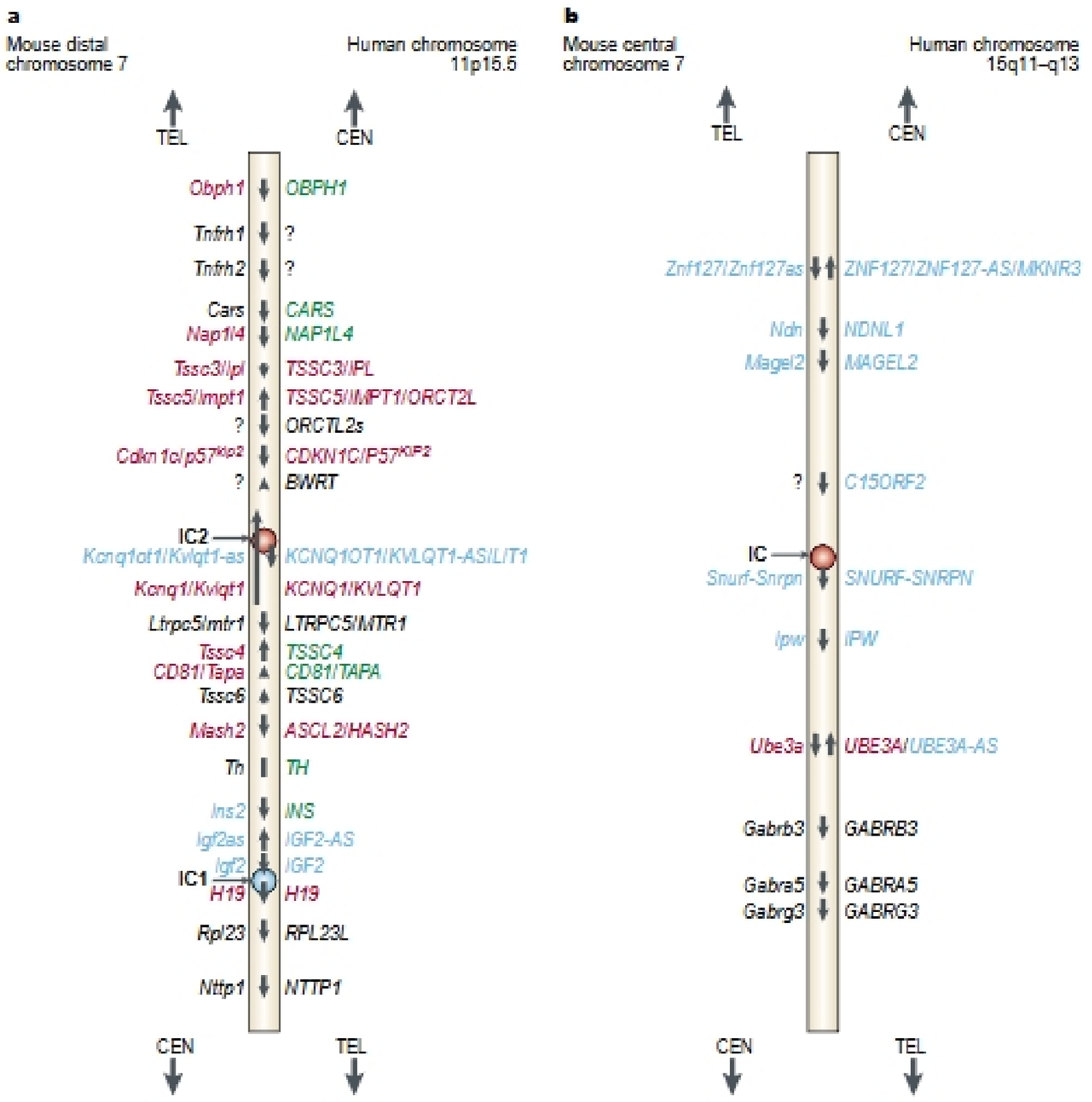


Figure 1 | **Imprinting clusters in human and mouse genomes.** Human chromosomes **a** | 11p15.5 and **b** | 15q11–q13 and orthologous clusters on **a** | mouse chromosome distal 7 and **b** | central 7. The relative location and transcriptional orientation of genes are indicated by arrows. The imprinting status is shown in red (maternally expressed), blue (paternally expressed), black (biallelic expression) and green (imprinted expression not known or not yet precisely defined). Question marks (?) indicate that the orthologues of the mouse or human genes, respectively, are not known. The drawings are not to scale. The Beckwith–Wiedemann (BWS) cluster (a) comprises about 1 Mb, and the Prader–Willi syndrome/Angelman syndrome (PWS/AS) cluster (b) roughly 2 Mb. Imprinting centres (IC) are marked by circles coloured according to the parental origin of the imprint.

methylation may spread from an IC into the promoter. The imprints are eventually read, resulting in parent-specific gene expression.

Erasure. The germ line has the role of resetting imprints such that in mature gametes they reflect the sex of that germ line. For most imprints, current evidence indicates that there might be two stages for this resetting process — the first one is erasure. This is followed later by establishment. During erasure, there is marked and apparently genome-wide demethylation in germ cells, which is completed by embryonic day 12–13 (E12–13) in both sexes[41,42] (FIG. 4). Indeed, germ cells cultured from these stages (EG cells) have a dominant demethylating activity when fused with somatic cells[43]; whether this demethylation is active or passive is not known. The evidence so far indicates that all methylation imprints probably become erased at this stage[41,42,44,45]. This is important because it implies that imprints inherited from a parent with the same sex as the developing embryo are erased and are unlikely to persist unchanged.

There is preliminary evidence that methylation imprints are still present and may be functionally intact before the erasure stage[46]. After erasure, functional evidence from nuclear transplantation experiments with both male and female germ-cell nuclei indicates that imprints have indeed been substantially altered[47,48]; expression of imprinted genes in these reconstituted embryos reflects their lack of methylation (for example, *H19* is expressed and *Igf2* is not expressed). In some instances, this has led to interest-