TIMELINE

Genomic imprinting: the emergence of an epigenetic paradigm

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Abstract | The emerging awareness of the contribution of epigenetic processes to genome function in health and disease is underpinned by decades of research in model systems. In particular, many principles of the epigenetic control of genome function have been uncovered by studies of genomic imprinting. The phenomenon of genomic imprinting, which results in some genes being expressed in a parental-origin-specific manner, is essential for normal mammalian growth and development and exemplifies the regulatory influences of DNA methylation, chromatin structure and non-coding RNA. Setting seminal discoveries in this field alongside recent progress and remaining questions shows how the study of imprinting continues to enhance our understanding of the epigenetic control of genome function in other contexts.

Genomic imprinting is a remarkable epigenetically regulated process that causes genes to be expressed in a parental-origin-specific manner rather than from both chromosome homologues. Thus some imprinted genes are expressed from the paternally inherited allele, and other genes are expressed from the maternally inherited allele. Parental-origin effects in plants, insects and mammals were noted around 40 years ago. However, it was the subsequent elegant embryological and genetic manipulations in the mouse that placed imprinting on the map and led to its recognition as an important paradigm of epigenetic inheritance. This, coupled with detailed genome mapping studies on human patients with disorders exhibiting parental-origin effects in their patterns of inheritance, provided the molecular basis for the identification of the first endogenous imprinted genes and the genomic features and epigenetic mechanisms responsible for their mono-allelic expression. These early studies of genomic imprinting established essential roles for differential DNA methylation, allele-specific histone modifications, large non-coding RNAs and dynamic developmental changes in the epigenetic programme. These factors that influence imprinted domains now have well-established roles in many other contexts, such as in stem cell programming, cancer epigenetics and *cis*-acting mechanisms of gene regulation. As such, genomic imprinting was one of the first, and remains one of the most informative, paradigms for understanding the consequences of interactions between the genome and the epigenome.

A historical perspective of some of these discoveries is presented here (with key events shown in the TIMELINE), illustrating how embryological studies, combined with classical and molecular genetic studies, have provided a framework for the more sophisticated epigenetic and genomic approaches that are applied today. This Timeline article includes consideration of the wider implications of the chromosomal secrets revealed through imprinting research and their impact on our understanding of epigenetic inheritance. The extent and functional implications of genomic imprinting are considered, its regulatory mechanisms reviewed and its contribution to health and disease discussed. Finally, some of the unresolved issues and wider questions that are emerging are suggested, thus providing a glimpse of the challenges and exciting prospects facing the future of this field.

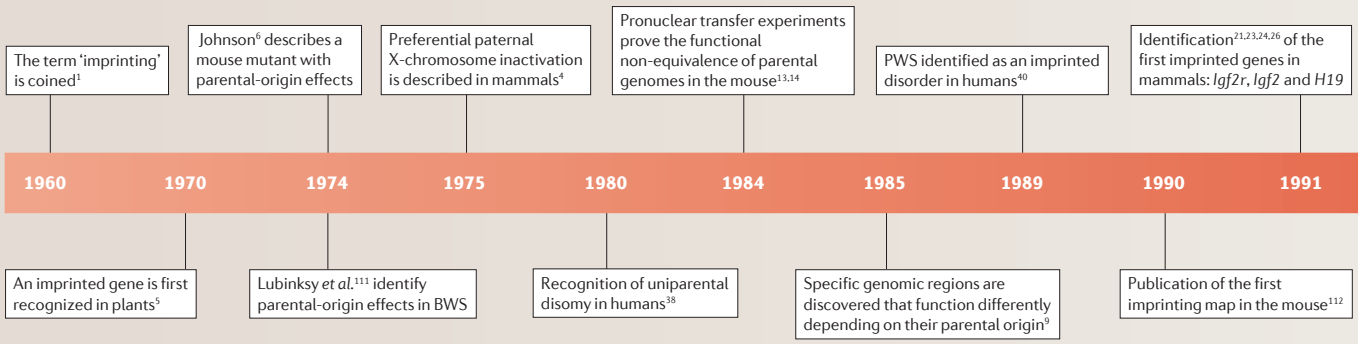
Evidence of genomic imprinting

Early observations, particularly in insects and plants, indicated that the appearance of a particular visible trait in offspring could differ depending on whether it was transmitted from the mother or the father. In some of the early studies, imprinting effects were observed cytogenetically and, as such, were seen to affect whole chromosomes. However, genetic experiments suggested that parental-origin effects could also act at the level of the gene.

Whole chromosome effects. Historically, there have been several examples of parental-origin-specific 'marking' of whole chromosomes documented in the literature. Indeed, the term 'imprinting' was first coined by the cytogeneticist Helen Crouse[1] in 1960 to describe the programmed elimination of one or two paternally derived X chromosomes in sciarid flies. Sciarid zygotes inherit two paternally derived and one maternally derived X chromosome. In female embryos, a single paternally inherited X chromosome is eliminated, but in males both paternally inherited X chromosomes are selectively lost from somatic nuclei. Crouse recognized that the imprint identifying the X chromosome as maternal or paternal in origin was determined by the sex of the germ line through which it was inherited[1]. Parental-origin effects were also described in the sex determination mechanism of coccid insects[2]. These insects lack sex chromosomes and in males the paternally derived chromosome set becomes heterochromatic, inactive and is not transmitted to offspring. Epigenetic differences between the maternally and paternally inherited coccid chromosome sets have been described but the underlying processes that establish the parent-specific imprint are not understood.

Sex chromosome dosage compensation is a well-established whole chromosome model in which parental-origin effects are studied. In mammals, females with two X chromosomes achieve parity with males in their X-linked gene dosage through epigenetic inactivation of one X chromosome. In marsupials, X-chromosome inactivation is imprinted, with the paternally inherited X chromosome being inactive in somatic

Timeline | **Key events in imprinting research**



BWS, Beckwith–Wiedemann syndrome; CTCF, CCCTC-binding factor; ICR, imprinting control region; *Igf2*, insulin-like growth factor 2; *Igf2r*, IGF2 receptor; piRNA, PIWI-interacting RNA; PWS, Prader–Willi syndrome; RNA-seq, high-throughput RNA sequencing.

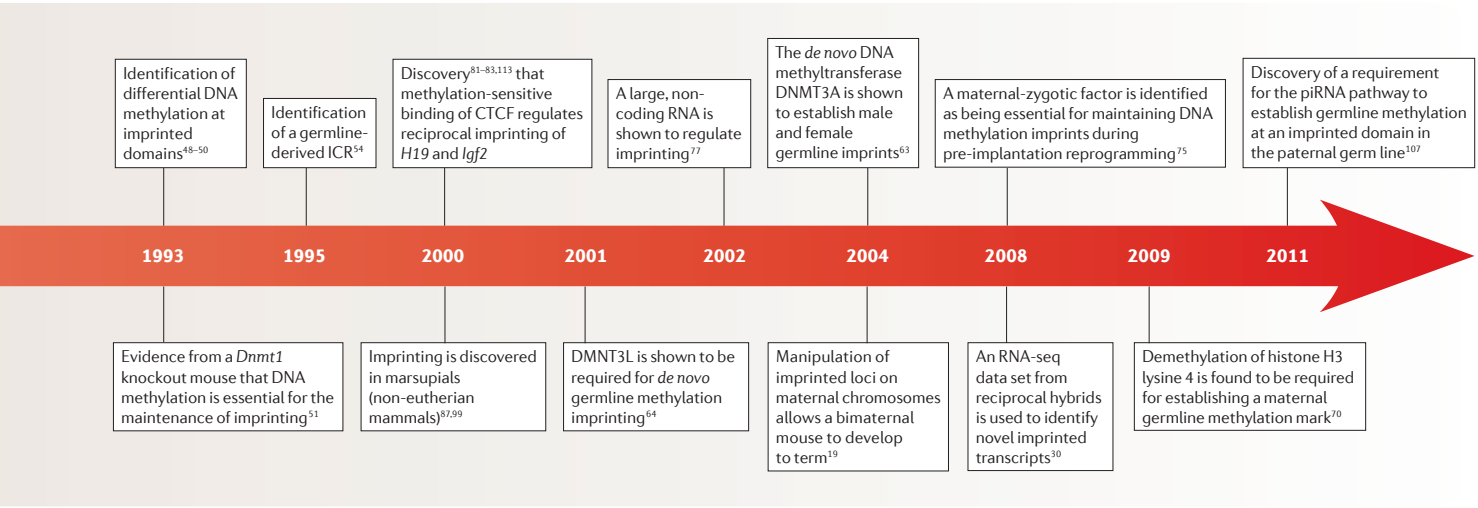
cells[3]. Interestingly, in mice, X-chromosome inactivation is similarly imprinted, but specifically during pre-implantation stages and in extra-embryonic lineages including the placenta[4]. In mice, random inactivation initiates in all embryonic components around the time of implantation. The molecular mechanism that distinguishes the paternal from the maternal X chromosome and establishes imprinted X-chromosome inactivation is currently unknown. In particular, it will be interesting to learn the extent to which the underlying epigenetic features conferring parental-origin-specific identity in phenomena involving whole chromosomes overlap with those regulating the imprinted expression and repression of individual genes.

Early evidence that specific genes are imprinted. In 1970, one of the first convincing pieces of experimental evidence for the imprinting of specific genes was described in plants. Through rigorous experiments on the inheritance of maize kernel coloration, Kermicle[5] recognized that *R* alleles carried by the female gametophyte may be functionally different from those carried by sperm. With remarkable foresight, Kermicle stated that the effect he observed was a "paragenetic rather than a conventional genetic phenomenon" and concluded that the effect was influencing the expression, not the genetic make-up, of the *R* allele. The term 'epigenetic' has now superseded Kermicle's original 'paragenetic' description. The recognition that the 'imprint' is not dependent on the DNA sequence, but rather the parental germline environment through which the gene passes, now defines the process of imprinting.

Genetic studies in the mouse also conducted in the 1970s suggested evidence for imprinting on autosomes. In 1974, Johnson[6] described a deletion at the *Tme* locus that showed *in utero* lethality when maternally inherited but not when transmitted through the male germ line. Mapping of the phenotype for this and other *Tme* deletions indicated that the parental-origin effect localized to a 0.8–1.1 Mb region of chromosome 17. This provided the basis for the identification of the first endogenous imprinted gene (see below).

Around the same time, Cattanach, Beechey and Searle were working with mice harbouring either Robertsonian translocations or reciprocal translocations, and they were able to manipulate the parental origin of particular chromosome regions. Their research extended the earlier work of Snell[7], who had first observed a genetic outcome now coined 'non-complementation lethality' in translocation intercross mice. Translocation heterozygotes can give rise to unbalanced gametes — eggs and sperm that are duplicated or deficient for chromosomal regions that were involved in the translocation. So, when such translocation heterozygotes are intercrossed, the fusion of complementary unbalanced gametes (for example, an egg with a maternally duplicated region fertilized by a sperm with deficiency for the same region) would be expected to result in fully viable, balanced diploid zygotes with so-called uniparental disomy of a whole chromosome, or uniparental duplication/deficiency for a particular chromosomal region. However, they noted that in some of these balanced cases, normal complementation did not occur and the uniparental duplication or deficiency embryos had abnormalities in their behaviour, growth and/or viability. Although the effects of egg cytoplasm or the uterine environment were not excluded[8], these results suggested differential expression from the two parental chromosome homologues. Over subsequent years, Cattanach and colleagues[9,10] used this approach to screen the whole mouse genome for defective outcomes that were caused by altering the dosage of parental chromosomes. Those studies identified around 13 subchromosomal regions for which there is a requirement for both a maternally and paternally inherited chromosome region for normal development (see also the MouseBook imprinting catalogue). Most murine imprinted genes identified to date map to these regions. Uniparental disomy conceptuses and their wild-type littermates continue to be used to understand imprinting phenotypes and for molecular analyses in which expression and epigenetic features on the maternally versus the paternally inherited chromosomes need to be distinguished.

Embryological investigations of imprinting. Perhaps the defining experiments that proved the functional non-equivalence of mammalian parental genomes were the elegant pronuclear transplantation analyses performed by the Solter and Surani laboratories[11–14] and published in the early 1980s. Newly fertilized mouse eggs were manipulated by removing and replacing the paternal pronucleus with a second maternal one to generate a diploid, genetically bimaternal (also known as gynogenetic or parthenogenetic) conceptus. Alternatively, the maternal pronucleus was replaced with a second paternal one to generate a diploid, genetically bipaternal (androgenetic)



conceptus. The failure of embryos without the two different parental genomes to proceed past mid-gestation suggested that the mammalian genome possessed genes that were somehow 'marked' differently on the two parental genomes[11–14]. This was further emphasized in studies proving that the failure of uniparental conceptuses was not due to oocyte cytoplasmic defects[15,16]. Although not the first use of the term, 'imprinting' was specifically applied in this context to refer to the phenomenon describing the non-genetic difference that distinguishes the two parental genomes, resulting in their functional non-equivalence.

Parthenogenetic conceptuses developed tissues predominantly of embryonic origin, with a failure in the development of the extra-embryonic lineages, whereas androgenetic conceptuses developed predominantly extra-embryonic lineages and lacked, or had very underdeveloped, embryonic components. This suggested that the two parental genomes provided reciprocal functions, at least during the first half of gestation, and that the absence or overexpression of imprinted genes exclusively expressed from either the maternal or paternal genome caused the developmental failure. Subsequent studies that mixed androgenetic or parthenogenetic cells with normal cells to form chimaeras provided evidence of further roles for imprinted genes in the development of particular embryonic lineages, such as mesodermal derivatives and the brain[17,18]. More recent experiments, in which the dosage of imprinted genes was genetically manipulated, have confirmed that perturbed imprinting is the only barrier to successful parthenogenetic development in mice[19,20].

It is noteworthy that in humans, parthenogenetic bimaternal ovarian teratomas and androgenetic bipaternal conceptuses (complete hydatidiform moles) have related phenotypes to those in the mouse.

Identification of imprinted genes

Identification of the first endogenous imprinted genes. The first three endogenous imprinted genes in the mouse were identified in 1991. The first to be published was the gene responsible for the parental-origin effect that Johnson had described at the *Tme* locus on mouse chromosome 17. By assessing the expression of positionally cloned candidate genes falling within the minimal deletion region, Barlow and colleagues[21] showed that the gene encoding the insulin-like growth factor 2 receptor (*Igf2r*) was expressed solely from the maternally inherited chromosome; the paternally inherited copy was repressed. Subsequent studies[22] indicated that *Igf2r* is one of a cluster of imprinted genes on the proximal region of mouse chromosome 17 (FIG. 1Aa). IGF2R functions both as a mannose-6-phosphate receptor and also as a receptor for insulin-like growth factor 2 (IGF2).

The *Igf2* gene was shown in two studies to be imprinted too. Mice carrying a targeted deletion of *Igf2* on the distal portion of chromosome 7 showed a growth deficiency phenotype on paternal transmission but not on maternal transmission. Expression analyis showed that *Igf2* was transcribed from the paternally inherited chromosome and repressed on the maternally inherited one[23] — imprinting that is reciprocal to that of *Igf2r*. In a second study, *Igf2* was found to be repressed in embryos with maternal uniparental duplication and paternal deficiency of distal chromosome 7, compared to normal littermates, also indicating repression of *Igf2* on the maternally inherited chromosome and expression from the paternally inherited allele[24]. Located approximately 90 kb downstream from the murine *Igf2* gene lies the *H19* gene, a non-coding RNA of unknown function that contains a conserved mammalian microRNA, miR-675 (REF. 25). Using hybrid embryos in which expression from the two parental alleles could be distinguished by strain-specific polymorphisms, Tilghman and colleagues[26] found that *H19* was expressed from the maternally inherited chromosome. Hence two adjacent genes, *Igf2* and *H19*, show reciprocal patterns of imprinted expression (FIG. 1Ba). Interestingly, *H19* and *Igf2* are generally co-expressed, sharing mesodermal and endodermal enhancers. Therefore, the study of the organization and regulation of this imprinted domain provided an excellent paradigm for the mechanistic studies to decipher imprinting control, outlined below.

Ongoing identification of imprinted genes. It is noteworthy that the four studies describing the first three endogenous imprinted genes in mouse used four different methods to determine their imprinting. These four approaches — targeted deletion, allele-specific activity in hybrids, positional cloning and the use of uniparental duplication/deficiency conceptuses from translocation intercrosses — illustrate the range of different methodologies that allow expression from maternal or paternal chromosomes to be distinguished. Subsequently, many more imprinted genes have been identified. To date approximately 100 imprinted genes have been validated as being imprinted in

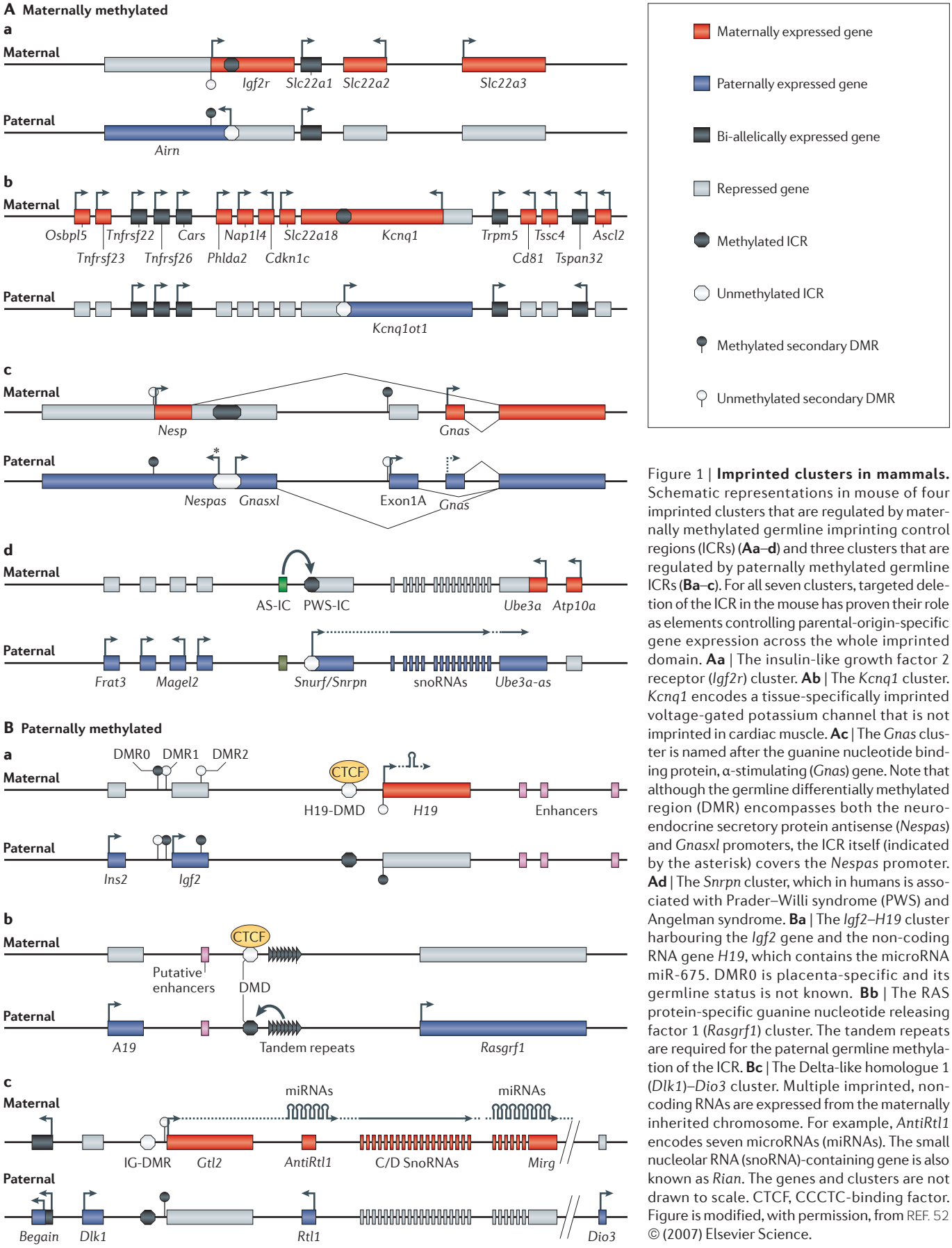


Figure 1 | **Imprinted clusters in mammals.** Schematic representations in mouse of four imprinted clusters that are regulated by maternally methylated germline imprinting control regions (ICRs) (**Aa**–**d**) and three clusters that are regulated by paternally methylated germline ICRs (**Ba**–**c**). For all seven clusters, targeted deletion of the ICR in the mouse has proven their role as elements controlling parental-origin-specific gene expression across the whole imprinted domain. **Aa** | The insulin-like growth factor 2 receptor (*Igf2r*) cluster. **Ab** | The *Kcnq1* cluster. *Kcnq1* encodes a tissue-specifically imprinted voltage-gated potassium channel that is not imprinted in cardiac muscle. **Ac** | The *Gnas* cluster is named after the guanine nucleotide binding protein, α-stimulating (*Gnas*) gene. Note that although the germline differentially methylated region (DMR) encompasses both the neuroendocrine secretory protein antisense (*Nespas*) and *Gnasxl* promoters, the ICR itself (indicated by the asterisk) covers the *Nespas* promoter. **Ad** | The *Snrpn* cluster, which in humans is associated with Prader–Willi syndrome (PWS) and Angelman syndrome. **Ba** | The *Igf2*–*H19* cluster harbouring the *Igf2* gene and the non-coding RNA gene *H19*, which contains the microRNA miR-675. DMR0 is placenta-specific and its germline status is not known. **Bb** | The RAS protein-specific guanine nucleotide releasing factor 1 (*Rasgrf1*) cluster. The tandem repeats are required for the paternal germline methylation of the ICR. **Bc** | The Delta-like homologue 1 (*Dlk1*)–*Dio3* cluster. Multiple imprinted, non-coding RNAs are expressed from the maternally inherited chromosome. For example, *AntiRtl1* encodes seven microRNAs (miRNAs). The small nucleolar RNA (snoRNA)-containing gene is also known as *Rian*. The genes and clusters are not drawn to scale. CTCF, CCCTC-binding factor. Figure is modified, with permission, from REF. 52 © (2007) Elsevier Science.

the mouse (see the Catalogue of Imprinting Effects and the Medical Research Council Harwell Genomic Imprinting homepage). Many, though not all, of these imprinted genes are located in clusters (FIG. 1).

In vivo allele-specific expression analyses — in normal hybrids, in genetic models of defective imprinting or in knockout mice — have proven that some imprinted genes show tissue-specific imprinting or cell-type-specific absence of imprinting. For example, *Igf2* is bi-allelically expressed specifically in the choroid plexus and leptomeninges of the brain[23]. Another paternally expressed imprinted gene, Delta-like homologue 1 (*Dlk1*), shows selective absence of imprinting in the postnatal neurogenic niche[27] (see below). By contrast, several other imprinted genes are imprinted in one or few tissues, with the placenta being a predominant site of tissue-specific imprinting[28,29].

Most recently, the emergence of next-generation sequencing technology and the genome-wide identification of strain-specific polymorphisms have allowed sequencing of the transcriptomes of tissues from reciprocal hybrid mice, with the potential to identify more elusive tissue-specific imprinted genes. This approach has confirmed the previously identified imprinted genes and has resulted in the identification of a few additional genes[30–32]. However, many putative imprinted genes identified by allele-specific genome-wide transcriptome approaches are yet to have their imprinting status validated.

Approaches to predict imprinting based on sequence characteristics or epigenetic features remain challenging, and to date only a minority of novel predicted imprinted genes have been validated experimentally[33,34]. Interestingly, a particular class of retrotransposon-derived imprinted genes with X-chromosome homology has been successfully recognized using this approach[35]. Nonetheless, sequencing whole transcriptomes and determining the parental origin of the transcripts by mapping reads back to sequenced heterozygous genomes (such as those of reciprocal hybrid mouse strains) currently holds the most promise for investigating the extent and cell-type-specificity of imprinting.

Insights from human disease. With a few exceptions[36,37], imprinting is found to be conserved between mice and humans, and valuable insights into the identity and organization of imprinted loci have come from the study of human disorders that show parental-origin effects in their patterns of inheritance (BOX 1). These disorders are usually caused by uniparental disomy or deletions. Indeed, uniparental disomy as a concept was described in humans by Engel[38] in 1980 through the recognition of meiotic errors that gave rise to gametes with the loss (nullisomy) or addition (disomy) of certain chromosomes. More recently an increased incidence of imprinted disorders has been associated with assisted reproductive technologies[39].

Of the many notable contributions, the analyses of patients with Beckwith–Wiedemann syndrome (BWS) — an overgrowth disorder that is also associated with an increased incidence of childhood tumours — and the neurological disorders Prader–Willi syndrome (PWS) and Angelman syndrome[40–45] provided important early advances in identifying and understanding the regulation of imprinted genes. PWS and Angelman syndrome are two distinct syndromes that map to the same domain on human chromosome 15, but that differ in the parental origin of the underlying range of genetic and epigenetic defects[46]. Studies of patients and clinical samples led to improved genomic resolution of clusters of human imprinted genes, and analyses of the consequences of microdeletions enabled the mapping of regional imprinting control regions (ICRs), which are specific loci that are required for the imprinting of all genes within a cluster (FIG. 1).

Hence, although the mouse as a model organism has been pivotal in determining the epigenetic mechanisms regulating imprinting, the contribution of human genetic studies to our understanding of imprinting control cannot be underestimated. In addition to the generation of new knowledge, these studies have generated diagnostic tools for the clinic and clarified the validity of the mouse models for more detailed analyses.

Epigenetic mechanisms of imprinting

In differentially marking the two parental chromosomes, the process of genomic imprinting has four key mechanistic principles. First, of course, it must be able to influence transcription. Second, it must be heritable in somatic lineages such that the memory of parental origin is faithfully propagated into daughter cells during cell division. Third, it is likely to be initiated on the paternally and maternally inherited chromosomes at a time when they are not in the same nucleus; that is, during gametogenesis or immediately after fertilization. Finally, the imprint must be erased in the germ line such that appropriate parental-origin-specific identity can be established in the gametes for the next generation.

What is the mark? DNA methylation is the only epigenetic modification known to fulfil all four of these properties. In mammalian cells, DNA methylation modifies predominantly CpG dinucleotides and is associated with a transcriptionally repressed state[47]. Not long after the identification of the first imprinted genes, it was shown that the two parental chromosomes at imprinted loci were differentially marked by DNA methylation[48–50]. Such regions are known as differentially methylated regions (DMRs). Not only did this finding of parental-origin-specific DNA methylation provide a starting point for determining the developmental dynamics of the epigenetic programme at these genes, but it also resulted in the evolution of an epigenetic paradigm for studying *cis*-acting mechanisms of gene regulation. Such mechanisms can act over short or long distances and can reveal links between the epigenetic state, the chromatin structure and genome function. Key experiments from Li, Beard and Jaenisch[51] in which the maintenance DNA methyltransferase DNMT1 was deleted in mice, proved the requirement for DNA methylation in genomic imprinting.

Where and when are imprints established? The identified DMRs fall into two categories: those that acquire their DMR status after fertilization (somatic or secondary DMRs) and those that become differentially methylated in the germ line (germline DMRs). Somatic DMRs are sometimes tissue-specific and they depend on the presence of a germline DMR[52]. Mapping microdeletions in patients, and the targeted deletion of the germline DMRs in mice, showed that these DMRs are the crucial ICRs that are essential for mono-allelic expression within an imprinted cluster[53–58]. Germline DMRs with methylation that is acquired during oogenesis are found at promoters of protein-coding genes or non-coding RNA genes, whereas those with methylation acquired in the paternal germ line are found in intergenic regions[52] (compare FIGS 1A and 1B). The evolutionary implications of this difference between the positions of the maternally and paternally methylated DMRs have been discussed elsewhere[59].

In both male and female primordial germ cells, epigenetic marks start to become erased once the migrating cells enter the

genital ridges at around day 11.5 of mouse gestation[60]. This erasure includes loss of methylation at the ICRs. Subsequently, the developing germ cells acquire new epigenetic states and this reprogramming includes the establishment of sex-specific DNA methylation marks at ICRs. In males, these new methylation imprints begin to be acquired during late fetal development[61]. By contrast, in females the process starts postnatally during the growing oocyte phase in the early neonatal period[62].

The *de novo* methyltransferase DNMT3A is required for the establishment of DMRs in the germ line[63]. Its regulatory factor, DNMT3L, is also required for the establishment of germline imprints[64]. DNMT3L interacts with DNMT3A and influences the structure of the enzyme and its ability to bind and methylate DNA[65]. DNMT3L is also essential for the repression of retrotransposons in the male germ line: male germ cells lacking this factor undergo meiotic catastrophe and pachytene arrest owing to the inappropriate activation of LTR retrotransposons and non-LTR retrotransposons in spermatogonia and spermatocytes[66]. This link between the silencing of 'parasitic' elements in the germ line and the epigenetic machinery causing imprinting was considered as early as 1993 (REF. 67) and may yet hold the elusive answer to arguably the most important outstanding question in the imprinting field: how and why does the epigenetic machinery recognize and differentially mark particular regions in the male and female germ lines? Is it related to a host defence system? Or does the answer lie in the properties of the underlying DNA sequence[65], or in germline-specific transcriptional activity across the DMR region[68]? Does the answer lie in the presence or absence of other types of modification such as histone methylation[69,70], does it depend on other currently unknown mechanisms or perhaps some combination (or all) of the above?

On fertilization, the egg and sperm transmit parental-origin-specific differential methylation to the new conceptus and dramatic epigenetic reprogramming events occur that are associated with the acquisition of totipotency and pluripotency[71]. During this time the paternally inherited genome undergoes active DNA demethylation, perhaps through a mechanism involving a hydroxymethylcytosine intermediate[72,73], with a replication-dependent passive demethylation of the maternally inherited genome. Coincident with these events, chromatin remodelling and dynamic changes in histone modifications occur[74]. To act as an epigenetic memory that is inherited from the germ line and is stable throughout development, methylation imprints must be resistant to such reprogramming events. So how are imprints maintained in an environment that is undergoing such extensive epigenetic change? Recent evidence suggests that the resistance to reprogramming is conferred through the targeting of the epigenetic machinery to ICRs. Factors important for this process have been identified, including the KRAB zinc finger protein ZFP57 (REF. 75) and developmental pluripotency-associated protein 3 (DPPA3; also known as PGC7 or Stella)[76].

The influence of ICRs

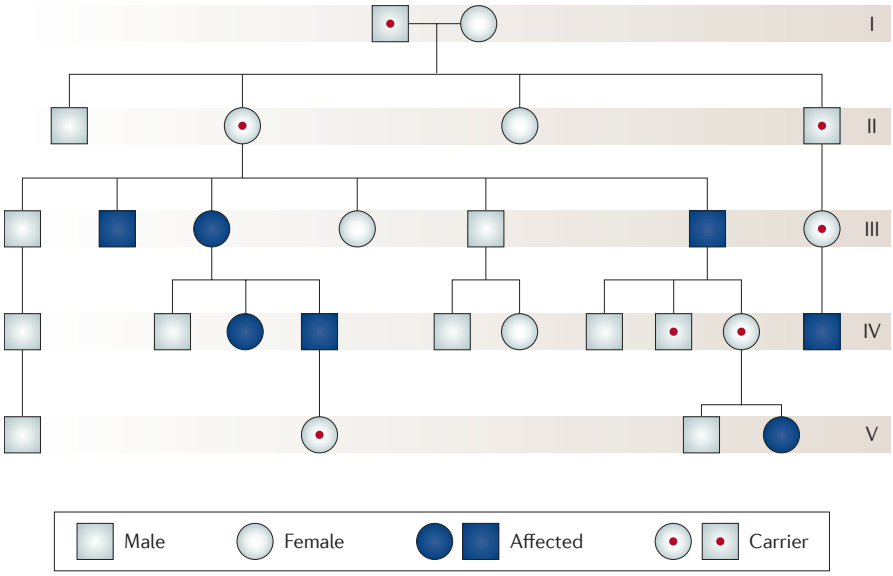
Maternal methylation. Several imprinted clusters of genes are regulated by maternally methylated ICRs at promoters that repress large non-coding or multi-functional transcripts, the activity of which on the paternal chromosome is required for the repression of protein-coding genes in *cis* on that

Box 1 | **Imprinting and inheritance**

In 1974, Lubinsky and colleagues[111] reported parental-origin effects in familial Beckwith–Wiedemann Syndrome (BWS). In their *Lancet* paper they noted, "affected offspring of either sex born only to female but not to male carriers" in the pedigree. This turned out to be a classic example of inheritance of an imprinted disorder. The figure shows a hypothetical pedigree of familial inheritance of an imprinted disorder through five generations, illustrating the parental-origin-specific mode of inheritance of the disease. Here, the mutation is in a maternally expressed imprinted gene and, therefore, when father transmits the mutation, offspring are unaffected because the paternally inherited allele is normally repressed. Half of his offspring will be carriers (as in generation II). The female offspring will transmit the mutation in the active, maternally inherited allele to 50% of their children, who will be affected, as is clearly evident in generation III.

One of the explanations for the BWS pedigree that Lubinsky *et al.* suggested was that the BWS gene acted "through factors mediated by the ovum but not by the sperm". We now know that a subset of BWS cases can be caused by maternally inherited mutations in the cyclin-dependent kinase inhibitor 1C (*CDKN1C*) gene, a maternally expressed imprinted cell cycle regulator. Imprinting of *CDKN1C* is regulated by methylation that is established in the female germ line (FIG. 1Ab), so BWS can be caused either by mutation in *CDKN1C* itself, or by the absence of methylation on the maternally inherited imprinting control region (an epimutation). Other mechanisms that cause BWS include paternal uniparental disomy for chromosome 11.

It is important to emphasize the distinction between parental-origin effects that are due to imprinting and sex-linked inheritance. In sex-linked inheritance, the trait maps to sex chromosomes and hence, when transmitted to offspring, males and females differ in their manifestation of the trait. This is fundamentally different from parental-origin effects associated with genomic imprinting, in which it is the sex of the transmitting parent that matters. Imprinted traits almost exclusively map to autosomes and importantly, when transmitted to offspring, males and females are equally affected (see the figure). Furthermore, the manifestation of the imprinted trait will be silenced and will seem to skip a generation when inherited from one carrier parent: for example, the trait is not observed when inherited from the father, but re-emerges in the offspring of his daughters (see generations III–V in the figure).



chromosome. One of the best characterized examples of this comes from studies of antisense *Igf2r* RNA (*Airn*), a non-coding RNA that regulates imprinting at the *Igf2r* cluster[77] (FIG. 1Aa). On the unmethylated, paternally inherited allele, the ICR is a promoter for the *Airn* transcript, which is expressed in an antisense direction on the paternally inherited chromosome. On the maternally inherited chromosome, where the *Airn* promoter is germline-methylated and repressed, *Igf2r* is expressed. Deletion of the unmethylated *Airn* promoter region, or premature truncation of the *Airn* RNA itself, results in bi-allelic expression of *Igf2r* though the failure to repress the paternally inherited allele[54,75,77]. It is interesting that *Airn* also represses the other imprinted genes in the cluster, including the tissue- and temporal-specific *Slc22a3* solute carrier gene located further 5′ of *Airn*. Evidence suggests that the *Airn* RNA can recruit and target repressive histone modifications to the *Slc22a3* promoter in a tissue- and stage-specific manner[78]. At least three other imprinted clusters regulated by maternal germline methylation seem to be controlled by large, multifunctional transcripts, the expression of which is required for the repression of adjacent protein-coding genes in *cis*[68,78–80] (FIG. 1Ab–d). However, the extent to which these transcripts act to occlude protein-coding transcription, or influence the recruitment of inactivating epigenetic states to repressed imprinted domains, remains to be determined.

Paternal methylation. The best characterized paternally methylated ICR is the H19-DMR. This intergenic DMR is required for normal imprinting of *H19* and the reciprocally imprinted linked gene *Igf2* (REF. 55). The element functions through interaction with the zinc-finger protein CCCTC-binding factor (CTCF), which binds DNA in a methylation-sensitive manner and preferentially targets the unmethylated maternal chromosome[81–83] (FIG. 1Ba). When bound, CTCF insulates the *Igf2* promoter from downstream enhancers that it shares with *H19* and facilitates *H19* promoter–enhancer interactions by generating loops that influence chromatin topology[84,85]. *Igf2* is therefore not activated on the maternal chromosome. By contrast, CTCF does not bind to the methylated, paternally inherited chromosome, so the enhancers are free to interact with the *Igf2* promoter, and the *H19* promoter is repressed and becomes methylated. Some other paternally methylated DMRs, such as the IG-DMR on mouse chromosome 12 (FIG. 1Bc), do not bind CTCF and hence may influence imprinting of the adjacent genes using a different, currently unknown mechanism[86]. It therefore seems that, although a consistent theme in imprinting control is the establishment of germline-derived DMRs, how they act in *cis* to confer mono-allelic expression differs from locus to locus.

Principles of imprinted regulation applied more widely. Nonetheless, the mechanisms of imprinting at the above loci seem to be conserved between mammalian species[25,86,87]. Their analysis has taught us several perhaps unexpected but important principles about the relationship between DNA methylation and gene expression more widely. Two of these principles are particularly noteworthy. First is a role for DNA methylation in repressing promoters of non-coding transcripts and the regulatory importance of the non-coding transcripts themselves. When unmethylated and expressed, the non-coding RNA prevents transcription of adjacent protein-coding genes (FIG. 1A). The genome is rich in large, non-coding transcripts, generally of unknown function, but with the potential to contribute to the repression of adjacent protein-coding genes. Second, the promoters of some repressed imprinted genes are secondary DMRs — regions that acquire their methylation after fertilization in response to a germline DMR. Secondary methylation is often acquired after the allele has become repressed[88], perhaps even as a consequence of transcriptional repression rather than having an initiating role. It is often assumed that the acquisition of promoter methylation is instructive for gene repression, but imprinting studies indicate that determining the temporal relationship between the acquisition of methylation and the repression of transcription is important for interpreting whether methylation has indeed caused repression. Other insights that have emerged from imprinting studies that can be applied more widely include contributions to understanding the relative

Glossary

Choroid plexus
A rich network of blood vessels located in the brain that is responsible for the production of cerebrospinal fluid.

Coccid insects
Scale insects of the order *Hemiptera*.

Complete hydatidiform mole
A product of conception that has two paternally derived genomes and is devoid of maternally inherited chromosomes. It develops as a rapidly growing mass, is derived from cells that would normally contribute to the placenta and lacks fetal tissue.

CCCTC-binding factor
(CTCF). A highly conserved zinc finger protein that influences chromatin organization and architecture and is implicated in diverse regulatory functions including transcriptional activation, repression and insulation.

Heterochromatic
A cytogenetic term to describe chromosomes or chromosomal regions that remain condensed and heavily stained during interphase.

Histone modifications
Reversible post-translational modifications, such as methylation and acetylation, that occur on the amino-terminal tails of core histone proteins.

Leptomeninges
Two of the three layers of membrane that protect the brain and the spinal cord; cerebrospinal fluid circulates between these two layers.

LTR retrotransposons
A long terminal repeat (LTR)-containing class of genetic element that can replicate and insert into a host genome through an RNA intermediate.

Neurogenic niche
A specific microenvironment in which neural stem cells can respond to endogenous and exogenous cues and can undergo self-renewal, proliferation and/or differentiation.

Ovarian teratomas
A tumour, derived from egg cells, which consists of cells that resemble fetal tissue-derived cells.

Pachytene
A stage of meiosis in which the chromosome homologues are closely synapsed. This is the stage when crossing-over between the homologous chromosomes occurs.

Pronucleus
The haploid nucleus from a male or female gamete.

Reciprocal translocation
The interchange of genetic material between two chromosomes that are non-homologous.

Robertsonian translocation
A chromosomal abnormality in which two acrocentric chromosomes become joined by a common centromere.

Sciarid flies
Dipteran insects of the genus *Sciara*, also known as fungus gnats.

Uniparental disomy
A cellular or organismal phenomenon in which both chromosome homologues are derived from one parent with none derived from the other parent. It can be the result of fertilization involving a disomic gamete and a gamete that is nullisomic for the homologue.

hierarchy of epigenetic changes associated with developmental programming, the influence of epigenetic states on DNA–protein interactions and the relationships between DNA and histone modifications.

Histone modifications. Despite correlations between the presence of active and repressive histone modifications at imprinted loci and the mono-allelic behaviour of imprinted genes, there is no substantial evidence that these modifications are instructive for imprinting. Rather, such marks contribute to the hierarchy of epigenetic states that is built on DNA and chromatin in response to the germline DMR[89]. These modified states may facilitate the maintenance of regional activity or repression and indeed may be modulated developmentally, as has been shown for other non-imprinted regions of the genome[90,91]. However, it has been shown that the histone H3 lysine 4 trimethylation (H3K4me3)-modified chromatin may shield DNA from methylation, a finding based on the lower affinity of the DNMT3A–DNMT3L complex for chromatin harbouring this mark[69]. Furthermore, and in support of this, in the female germ line, demethylation of H3K4 is required in order that *de novo* DNA methylation can occur at some ICRs[70]. It therefore seems likely that histone modifications might be instructive in the germ line, thus contributing to the recruitment of the heritable germline DNA imprint. Further analysis is required to determine the extent to which modulation of histone modifications is required for germline DNA methylation.

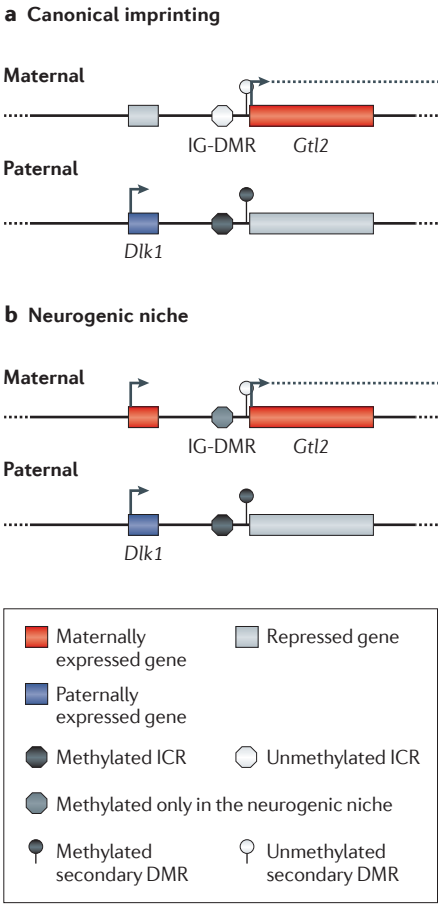


Figure 2 | **Tissue-specific imprinting effects.** Schematic representation of the Delta-like homologue 1 (*Dlk1*) imprinted locus. **a** | The state of the imprinted *Dlk1* and *Gtl2* (also known as *Meg3*) genes in most tissues (canonical imprinting). **b** | In the postnatal neurogenic niche, there is a selective absence of imprinting at the *Dlk1* gene, and this increase in overall *Dlk1* expression is required for neurogenesis[27]. Selective activation of the maternal allele of *Dlk1* is associated with increased methylation at the IG differentially methylated region (IG-DMR) imprinting control region.

Function and evolution of imprinted genes

Over the years, the phenotypic characterization of patients with imprinted disorders and mice with loss or gain of allelic activity at imprinted genes has elaborated on the early embryological and genetic studies indicating that imprinted genes function to control prenatal growth, placentation and the development of lineages such as the musculoskeletal system and the brain[92–94]. More recently, several studies have indicated that imprinted genes also play important parts in postnatal processes including adaptation to feeding, social behaviour and metabolism[95–97], processes that may be particularly responsive to environmental influences. Perhaps genomic imprinting, as an epigenetic mechanism to regulate gene dosage, might have evolved in response to extrinsic and/or intrinsic signals to allow levels of these genes to be modulated as conditions required. Consistent with this are findings that different imprinted genes show a wide range of tissue- and/or temporal-specific imprinting during development[94–98].

Dynamic control. Evidence in support of the idea that the control of gene dosage may be a modulatable process has come from the study of the paternally expressed imprinted gene, *Dlk1* in mice (FIG. 2). During embryogenesis *Dlk1* is exclusively expressed from the paternally inherited chromosome. However, in the neurogenic niche shortly after birth *Dlk1* selectively loses its imprinting, and its dosage dramatically increases. This is coincident with a requirement for both parental alleles for the maintenance of the neural stem cell pool and the subsequent generation of new neurons in the adult and throughout the remaining lifetime of the animal. This selective absence of imprinting correlates with the acquisition of methylation at the germline DMR on the maternal allele and suggests that gain or loss of imprinting may be a dynamic, epigenetically regulated mechanism that controls gene dosage in particular developmental contexts[27] (FIG. 2). The extent of such modulation, and whether this may also apply to other imprinted genes, remains to be elucidated. Rather than complicating our understanding of imprinting, these observations perhaps illustrate the importance of dynamic changes in epigenetic states as a mechanism to control gene dosage as the need arises. Further analyses of these events may contribute more generally to our understanding of the epigenetic control of dosage in normal developmental contexts, in environmentally stimulated diseases (for example, in cancer and adult-onset metabolic disease) and in mechanisms underlying the phenotypic consequences of ageing.

Evolutionary forces. Imprinting is believed to have evolved independently in plants, and is not found in lower vertebrates. Comparisons of imprinting between a wide range of eutherian and non-eutherian mammals have shown both conservation and lack of conservation of imprinting. For example, imprinting has not yet been found in prototherian mammals (the egg-laying platypus and the echidna). In metatherian mammals (the marsupials) only a subset of loci imprinted in eutherians show conservation of imprinting. Domains such as the *Igf2–H19* and *Igf2r* clusters are imprinted in marsupials[25,87,99] but others, such as the *Dlk1* and *Snrpn* imprinted clusters, are not; their organization and imprinting evolved after the divergence of eutherians and metatherians from their common ancestor[100,101].

These findings imply that different evolutionary selective pressures, acting at different times on different gene clusters, led to the evolution of dosage control by imprinting. This might reflect the different physiological roles of imprinted genes and their ability to adapt to new levels of functional complexity through changes in their gene dosage. For example, it is well-established that imprinted genes contribute to the regulation of nutritional resources to the fetus *in utero*. This occurs predominantly through the placenta, a recently evolved feto-maternal organ that mediates the supply and demand

of nutrients to the growing fetus. Even marsupial mammals have a yolk sac and/or placenta for their short gestation[102]. Genes such as *Igf2* and *Igf2r,* which are important for regulating resource acquisition through the placenta, are imprinted in both marsupials and rodents.

Evolution of imprinting at the *Igf2* and *Igf2r* loci fits with the 'parental-conflict hypothesis', one of the most popular theories explaining why imprinting evolved[103]. This theory is based on the premise that the mother can bear and raise offspring from multiple fathers and, despite the mother being equally related to all her offspring, each father is only related to a subset of these offspring. Therefore, although the mother distributes her resources to all offspring equally, the father's genes are driven to maximize resources for his own offspring at the expense of their mother and siblings, resulting in a 'transcriptional arms race' between maternally and paternally expressed genes. Paternally expressed imprinted genes such as *Igf2* therefore act to maximize growth and resource acquisition, whereas maternally expressed imprinted genes are growth suppressing and designed to minimize the burden on future pregnancies. However, the conflict hypothesis does not necessarily apply to genes that may be more important for postnatal adaptations. The imprinting of these genes could have evolved owing to the influence of different selective pressures; for example, pressures associated with the brain and behaviour[104]. One would predict that evolution of the imprinting of these genes may not follow the same temporal trajectory as those associated with parental conflict. Consistent with this is the finding that the imprinting of postnatally important genes seems to have evolved later than genes that regulate prenatal growth.

Future directions

Extensive epigenetic studies in model systems and model organisms have indicated that epigenetic mechanisms function to regulate chromosome architecture, the transcriptional repression of repetitive elements (such as retroviral sequences and transposons) as well as gene activity and repression during development. Correlations between the epigenetic state at imprinted loci and those at repressed, repetitive sequences have been made but the underlying DNA sequence influences, if any, are not understood. ICRs share some epigenetic properties with retroviruses, retrotransposons or other repeats. These shared properties include: the ability to recruit proteins associated with the KAP1 chromatin co-repressor complex[75,105], the specific presence of H4K20me3 (REFS 91,106), the recruitment of PIWI-interacting RNA (piRNA)-mediated silencing mechanisms[107] and targeting by DNMT3A–DNMT3L based on sequence periodicity[65,66,108].

To what extent does the underlying DNA sequence impose epigenetic control and how does this influence phenotype? Given the impressive technological advances in the sequencing of genomes and epigenomes, current coordinated international efforts to characterize normal and disease epigenomes, and to investigate genotype–epigenotype–phenotype correlations, are timely (for example, through the International Human Epigenome Consortium). Some insights have recently emerged through the consideration of the parental origin of variants in genome-wide association studies. For example, correlations have been made between diseases, including breast cancer and type 1 and type 2 diabetes, and the parental origin of variants at imprinted domains[109,110]. One of these studies has provided evidence that such variation has epigenetic consequences[110]; analyses such as these, integrating sequence variation, epigenetic mechanisms and phenotypic outcomes, will surely enrich our perspective of the wider implications of genotype–epigenotype variation in healthy, diseased and ageing populations.

One major challenge associated with describing genome-wide epigenetic marks is deciphering which marks are instructive, which marks are occurring as a consequence of a function such as a change in transcription (perhaps to facilitate the maintenance of transcription or repression) and which marks are less relevant than others. Applying genetic approaches to established developmental models, such as genomic imprinting, is likely to continue to contribute to addressing this challenge and to play a major part in deciphering the functional elements of the epigenome.

The recent appreciation of hydroxymethylation as a DNA modification that is likely to have an impact on the dynamic programming of epigenetic states[72,73], and the mechanisms associated with the formation of this modification through the hydroxylation of 5-methylcytosine, has drawn attention to the possibility that other currently unrecognized modifications may influence DNA and chromatin and might have an impact on genome function in health and disease. Perhaps these 'new' epigenetic marks will contribute to the regulation of genomic imprinting.

Identifying the function of these marks and integrating them into existing epigenetic frameworks will require the development of novel methods for identifying, sequencing and manipulating them, and for determining their place in the increasingly colourful epigenetic palette that controls our DNA, paints our chromatin and builds our chromosomes.

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Competing interests statement
The author declares no competing financial interests.

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Catalogue of Imprinting Effects: http://igc.otago.ac.nz/home.html
Epigenesys (EU Network of Excellence): http://www.epigenesys.eu
International Human Epigenome Consortium: http://www.ihec-epigenomes.org
MouseBook imprinting catalogue: http://www.mousebook.org/catalog.php?catalog=imprinting
Medical Research Council Harwell Genomic Imprinting homepage: http://www.har.mrc.ac.uk/research/genomic_imprinting
University of Cambridge Centre for Trophoblast Research: http://www.trophoblast.cam.ac.uk

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VIEWPOINT

The future of model organisms in human disease research

Timothy J. Aitman, Charles Boone, Gary A. Churchill, Michael O. Hengartner, Trudy F. C. Mackay and Derek L. Stemple

Abstract | Model organisms have played a huge part in the history of studies of human genetic disease, both in identifying disease genes and characterizing their normal and abnormal functions. But is the importance of model organisms diminishing? The direct discovery of disease genes and variants in humans has been revolutionized, first by genome-wide association studies and now by whole-genome sequencing. Not only is it now much easier to directly identify potential disease genes in humans, but the genetic architecture that is being revealed in many cases is hard to replicate in model organisms. Furthermore, disease modelling can be done with increasing effectiveness using human cells. Where does this leave non-human models of disease?

Q *Why do we still need model organisms to understand human disease?*

Timothy J. Aitman. In May 2008, *Nature Genetics* published a Focus issue on rat genetics. An article in that issue[1], co-authored and supported by over 250 rat geneticists, outlined six principles that underline the case for continuing or even strengthening efforts in animal genetics. These principles, which apply equally to other model organisms, include: the wealth of literature accumulated over 100 or more years for models such as the mouse and rat; the genome resources that, as for humans, have accelerated the pace of gene identification for a wide range of phenotypes; and the opportunities for *in vivo* phenotyping and gene targeting that have catalysed our understanding of genetic mechanisms.

However, genome-wide association studies (GWASs) in humans have recently identified hundreds of genes and gene loci for common human diseases. Furthermore, new sequencing technologies have accelerated the pace of discovery for Mendelian traits, providing insights into their molecular basis by direct study of the human diseases rather than models. Surely it is not sufficient to continue studying animal models just because it has always been this way?

One of the arguments in favour of continuing studies of model organisms is that the genetic studies of common human diseases have significant limitations. The genes and gene loci found by GWASs are mostly of small effect and explain a relatively low proportion of the overall heritability for a particular disease or trait. So establishing the mechanism through which they act has been elusive for all but a few GWAS hits. In addition, the environmental variation and outbred, heterogeneous genetic backgrounds of human studies reduce statistical power to detect gene effects, particularly *trans*-regulated effects (where sequence variation at one locus acts by influencing gene expression at a second locus that is remote from the first), and gene–environment interactions. Curiously, although gene loci identified in human GWASs explain only a small proportion of the heritability of complex traits, such as body mass and blood pressure, studies in rodents explain several times the total genetic variance for similar traits[2,3].

In the rat, integrating linkage analysis with expression profiling has proved a particularly powerful approach. The application of this approach using adipose tissue led to the identification of *Cd36* as an insulin resistance gene in rats and humans[4]. This was among the first complex trait genes to be positionally cloned in any mammal. Building on this integrative strategy led to detection of thousands of rat expression quantitative trait loci (eQTLs) in multiple tissues[5,6] and identification of rat genes for cardiac mass, cardiac failure, glomerulonephritis and hypertension[1,7], all of which show conserved function in humans. A high proportion of other complex trait genes identified in the rat have conserved phenotypes in humans, in several cases more strongly so than corresponding mouse models. Particular examples are: the polycystic