Imprinting mechanisms in mammals
Wolf Reik* and Jörn Walter†

Imprinting is a genetic mechanism that determines expression or repression of genes according to their parental origin. Some imprinted genes occur in clusters in the genome. Recent work using transgenic mice shows that multiple cis-acting sequences are needed for correct imprinting. Mutation analysis in a normal chromosomal context reveals the importance of imprinting centres for regional establishment or maintenance of imprinting in a cluster. Elements that contribute to the function of imprinting centres and regional propagation of the imprints are CpG-rich differentially methylated regions (that during development retain germline imposed methylation or demethylation), direct repeat clusters, and unusual RNAs (antisense, non-translated etc.). The interaction of these cis elements with transacting factors such as methylase and chromatin factors establishes a hierarchical control system with local and regional effects.

Addresses
*Laboratory of Developmental Genetics and Imprinting, The Babraham Institute, Cambridge CB2 4AT, UK; e-mail: wolf.reik@bbsrc.ac.uk
†Max Planck Institute of Molecular Genetics, Ihnestrasse 73, D-14195 Berlin, Germany; e-mail: walter@mpimg-berlin-dahlem.mpg.de

Abbreviations

AS	Angelman syndrome
DMRs	regions of differential methylation
ES	embryonic stem
IC	imprinting centre
IME	imprinting maintenance element
NRC	nuclease-resistant chromatin
PWS	Prader–Willi syndrome
YAC	yeast artificial chromosome

Introduction
Parental, genomic, or gametic imprinting is a genetic mechanism that results in the expression or repression of imprinted genes depending on their parental inheritance [1–12]. The phylogenetic distribution of imprinting appears to be limited to seed plants, some insect species, and to mammals. Hence, in some of the classic genetic model organisms such as Drosophila, Caenorhabditis elegans, and recently Zebrafish (Danio rerio) [13] no major imprinting effects, as judged by phenotype, have been observed. Importantly, however, the absence of imprinting in these organisms does not mean the absence of the mechanistic machinery needed to achieve imprinting. A recent elegant study in Drosophila [14] describes the imprinting of a variegating gene which was achieved by bringing this gene together with different modifier alleles in the germline. Although the variegating gene and the modifier were segregating in offspring, the pattern of variegation

induced in the germline (by the modifier) was inherited. Therefore some of the genetic components necessary for the establishment and maintenance of imprinting effects are apparently evolutionarily conserved and are being used for other purposes of heritable gene activation or silencing. In addition, this study shows that imprinting is possible in organisms which do not have DNA methylation (such as Drosophila).

Imprinting may also apparently evolve for different biological purposes and yet use similar mechanisms. In the insect mealybug species (planococcus lilacinus), paternally inherited chromosomes are heterochromatised during early development in a proportion of bugs as an apparent sex-determining device (as the embryos with one heterochromatic set of chromosomes develop into males). Recently, a nuclease-resistant chromatin (NRC) fraction has been identified in males. Importantly, the NRC is already present in sperm (but the chromosomes are not heterochromatic) suggesting that it is a candidate for the imprint. DNA sequences contained in the NRC have been isolated and some show motifs similar to those present in mouse alpha-satellites and nuclear matrix attachment regions [15].

In this review, we focus on imprinting mechanisms in mammals. The formal requirements of the imprinting mechanism are the introduction of specific imprints in parental germ cells, their maintenance (and potential further evolution) during embryonic development, and their erasure in germ cells. In addition, gene-specific imprints have to be used ('read') in order to either repress or activate transcription (there are no reliable reports to date that other mechanisms of gene regulation are affected by imprinting).

The past two years have seen important advances, in particular in the identification of cis-acting requirements for imprinting and imprinted genes in mouse and human, and a growing realisation that both local and regional imprinting elements interact. Thus imprinting is seen increasingly as a regional chromosomal phenomenon, with both short and medium range (up to Megabase) effects. Cis-acting imprinting elements interact with developmentally controlled transacting factors, and in the next couple of years we expect that some of these will be identified. We summarise the key findings and publications and at the end distil the most general current model of imprinting mechanisms.

Ontogeny of allelic methylation
In mammals, DNA methylation is a key component of the imprinting mechanism. Clear evidence for the importance of DNA methylation comes from the observation that

imprinting of several imprinted genes such as *H19*, *Igf2*, *Igf2r* and *Snrpn* is disrupted in methyltransferase-deficient mice [16,17•]. Although its precise role in initiation and maintenance of imprints is not clear yet (see below), much information is now available on where localised allele-specific methylation patches occur in imprinted genes. All imprinted genes studied to date are marked by regions of differential methylation (DMRs). Whereas most of these genes are methylated on the non-expressed allele [17•,18–32], some such as *Igf2r*, *Igf2*, and *Snrpn* do also contain methylation on the expressed allele [17•,32–40]. Differential methylation often occurs either in or near CpG-rich regions (sometimes CpG islands), which contain or are adjacent to blocks (up to 2 kb) of different types of short direct repeats [41]. The differential methylation can encompass the repeat block or occur adjacent to it.

A recent interesting finding is that DMRs in some instances overlap with the transcribed region of un-usual RNAs (non-coding and antisense) [18–22,27,37,42•]. These RNAs are also frequently transcribed through the tandem repeat block. For some imprinted genes, DMRs fulfil the proposed requirements of a sex-specific imprinting mark such that differential methylation is already established in germ cells and seems to be inherited throughout development. These DMRs include region 2 (but not region 1) of *Igf2r* (methylated in the egg) [33,43,44], the upstream region of *H19* (methylated in sperm) [45,46•,47•], and both DMRs of *Snrpn* (DMR1 methylated in egg, DMR2 in sperm) [17•]. Other regions are differentially methylated in germ cells but this is lost soon after fertilisation, only to be re-established later in development (*Igf2* DMR2; J Oswald, unpublished data). Yet others do not seem to be differentially methylated in gametes but become so early in development (*Igf2* DMR1) [43,44]. Some conflicting evidence exists for *U2afbp-rs1* [48,49] and possibly also for *Xist* [50,51]. Hence, although DMRs appear to be hallmarks of all imprinted genes, their different ontogeny indicates that they may fulfil quite different functions in the control of imprinting.

Interesting insights have been gained recently by two studies on the ontogeny of the *H19* DMR [46•,47•]. These studies were carried out by bisulphite sequencing of genomic DNA which yields more information than single site methylation PCR. Upstream of *H19*, there was found an extensive region which is fully methylated in sperm but not in eggs. Following fertilization, demethylation of the sperm copy and *de novo* methylation of the egg copy (equilibration) was observed except in a core region of 2 kb in length which is located upstream of a direct repeat block. This region retained paternal methylation and maternal lack of methylation at all stages of development. Interestingly, it coincides with a silencer element that was identified in transgenic studies in *Drosophila* (see below [52•]). Following differentiation in the embryo, regions of methylation and demethylation expand again on the paternal and maternal copies of the *H19* gene,

respectively. These studies highlight the importance of regional and developmental studies of DMRs (by bisulphite sequencing) in order to define core regions. Adjacent regions that may show germline differences can undergo dynamic changes during preimplantation development. Parental differences in these regions are equilibrated, despite the fact that the core methylation imprints are maintained and that differential expression will occur [44,46•,47•,53].

Another important insight is that, during preimplantation development, DMR core regions presumably need to be protected both from demethylation — which occurs on many sequences during preimplantation development, presumably both by passive and active processes — and *de novo* methylation, such that maintenance of germline-derived methylation patterns occurs. Once methylation has been lost from an imprinted DMR (e.g. in MTase-deficient mice) it cannot be regained by reintroduction of the MTase somatically [54•], whereas methylation at nonimprinted sequences is restored. Obviously, germline passage is required to establish the methylation in DMRs [54•], suggesting that either an unknown germline specific *de novo* methyltransferase is responsible for methylation of DMRs and/or that other factors protect DMRs from *de novo* methylation in the embryo but that these protective factors are absent during (later stage) germ cell development (presumably in both sexes; see below). Protective factors (against *de novo* methylation) have indeed been demonstrated genetically in the embryo for the imprinted *RSVIgmyc* transgene [55] and have been surmised for the imprinted *U2afbp-rs1* gene in the male germline [56•].

It will be important to carry out detailed bisulphite analysis in other imprinted genes – in addition, all developmental stages — especially during preimplantation development — should be analysed. It cannot necessarily be assumed, for example, that a germline methylation difference is inherited stably if it is observed in egg and sperm and blastocysts. Indeed, some peculiar observations have been made: for instance, a particular HpaII site in *Igf2r* region 2 seems to be maternally methylated in 2 and 8 cell stage preimplantation embryos but unmethylated at the 4 cell stage [44]. Whether these are quirks that arise from studying single sites in a region that undergoes dynamic reorganisation, or true gaps in the methylation signal, remains undetermined.

Imprinting control elements

There are two principal ways of testing the functional significance of DMRs and other potential control regions in imprinted genes. The first involves transgenes of various sizes (from which sequences can then be deleted) and the second involves deleting sequences from their normal chromosomal context using homologous recombination in embryonic stem (ES) cells (or naturally occurring mutations in human patients). It should be borne in mind

that these two different approaches can yield different outcomes and these should be considered carefully: for example, deleting a sequence may abolish imprinting but introducing the same sequence ectopically as a transgene may not confer imprinting (see below).

Imprinting in transgenic models

The first evidence for *cis*-acting control elements came from studies on the imprinted *RSVIgmyc* transgene. Deletion analyses of this imprinted transgene pinpointed a region in the Sα region (*IgA* switch recombination sequences) which contains a cluster of direct repeats [55]. When this element is deleted, the transgene becomes methylated on paternal transmission and imprinting is lost. This could suggest that the direct repeat region is necessary to protect the paternal allele from germline-specific (in this case egg-specific) methylation; however, the situation is more complex since substitution of another sequence in the construct (RSV LTR) with an unrelated sequence leads to methylation of the paternal allele. Interestingly, the properly imprinted transgene can be made to lose imprinting (in this case by post-zygotic methylation of the paternal allele). This loss of imprinting depends on the genetic background of mice used for crosses, and the genetic analysis indicates the involvement of factor(s) that normally protect the demethylated paternal allele from *de novo* methylation (as suggested in the previous section) [55].

Analysis has been made of gene-specific imprinting control elements of endogenous imprinted genes with *H19*, *Igf2*, *Igf2r*, *U2afbprs1*, and *Snrpn* transgenes. *Igf2* and *U2afbprs1* transgenes do not show consistent imprinting, despite the presence of DMRs in the transgenes [56•,57]. This may suggest that either these signals are not properly read in a different chromosomal environment or that the transgenes do not possess the complete set of imprinting control elements, i.e. imprinting centers (ICs) and imprinting maintenance elements (IMEs), required for establishment or maintenance of proper imprinting (see below).

For *H19* and *Igf2r* transgenes, however, the rule seems to be the longer the transgene the better the imprinting. Whereas transgenic lines containing short *H19* transgenes show variable imprinting [58•,59•], a large *H19* single-copy transgene (based on a yeast artificial chromosome [YAC] construct) is consistently and properly imprinted [60•]. Short transgenes containing various sequences around region 2 in *Igf2r* are not imprinted but a YAC transgene containing the whole locus is [42•]. Despite the variable imprinting of short *H19* transgenes, however, deletion analyses emphasise the importance of some of the local control elements required for imprinting. The region upstream of *H19*—containing the core DMR and direct repeat region—is needed for initiation of methylation in the paternal germline but additional regions are also important for initiation/maintenance. These include the 5′

part of the *H19* transcription unit and the 3′ flanking region including the enhancers [58•,59•]. That the upstream region is important for the control of imprinting is also suggested from the observation in an *H19* knockout. When substituting the *H19* promoter and transcription unit with a PGK-promoter/NEO-reporter gene construct, the reporter gene shows correct imprinted expression [61•].

In a YAC transgene (130 kb) that includes the *H19* and *Igf2* genes (except DMR0 and 1 of *Igf2*), *H19* imprinting and expression is faithful in single-copy transgenics [60•]. In multicopy trangenics, however, the transcription levels per *H19* copy are progressively reduced, suggesting that multiple copies of the transgene are competing for limited availability of activating factors. Although *Igf2* repression on maternal transmission is observed in 2 out of 3 of the low copy number transgenic lines, this is no longer the case in the multicopy transgenics (i.e. the maternal copy of *Igf2* is expressed). *Igf2* derepression may be a direct consequence of the partial downregulation of some *H19* copies on the same chromosome; activation of silent *Igf2* would be expected from the 'enhancer competition' model whereby the *Igf2* and *H19* promoters compete for a common set of enhancers [60•]. The lack of proper *Igf2* repression—there is also one example in the low copy number lines—could also be a consequence of the absence of some important regulatory elements in the *Igf2* gene, such as the DMR0/DMR1 sequences upstream of *Igf2* (see next section).

Igf2r imprinting has also been investigated in YAC transgenes [42•]. These are 300 kb in length and the majority (but not all) show proper imprinting of *Igf2r* with maternal expression. These YACs were then used to investigate the role of the differentially methylated region 2 (see previous section) in *Igf2r* imprinting. A 4 kb deletion abolished imprinting and led to expression on both paternal and maternal transmission. Intriguingly, an antisense imprinted RNA was discovered (paternally expressed) the promoter or enhancer of which is contained in the 4 kb deletion. Hence, the antisense RNA is absent on the deleted YAC and, as a result, the otherwise repressed *Igf2r* promoter is activated. The antisense RNA is fairly long and this may occlude the *Igf2r* promoter. Other models of how this antisense transcript could interfere with the *Igf2r* message include local heterochromatisation (as with *Xist*), transcription factor competition, or the direct promotion of local methylation of region 1 ([4,62]; OW Smrzka, W Lerchner, DP Barlow, E Braidotti, personal communication). As pointed out above, shorter transgenes containing region 2 do not maintain egg-specific methylation (whether they attract it in the first place is not known). Importantly, whereas deletion of region 2 on a paternal YAC leads to demethylation of region 1, mutation of region 1 (on a maternal YAC) also leads to demethylation of region 2, indicating that differential methylation in both DMRs is not controlled independently (for an attempted explanation, see below).

As an apparent exception to the general rule, short DNA sequences from DMR1 and 2 of *Snrpn* have been microinjected into eggs in either unmethylated or (*in vitro*) methylated form and been shown to stably inherit their respective state of methylation to the blastocyst stage [17•]. Whether these methylation imprints would be stable to later developmental stages and switch on germline transmission appropriately is not known.

A more detailed picture of local and regional control mechanisms of imprinted genes might also emerge from the analysis of transgenic lines carrying human imprinted genes. It will be of particular interest to see whether a completely different organisation — as is the case with the human region upstream of *H19*—still confers proper imprinting in the heterologous host.

Imprinting mutations

The previous analysis suggests that combinations of local elements can confer imprinting on transgenes, albeit often imperfectly. Imprinting mutations, on the other hand, of such local elements in the normal chromosome context either abolish or alter imprinting in a region ranging from tens of kilobases to megabases. A closer look at some of these situations suggests that imprinting elements may interact in *cis* along a chromosomal region to establish and maintain proper imprinting. The clustering of imprinted genes may, therefore, reflect a general property of the imprinting mechanism.

The first imprinting mutations were described in the Prader–Willi and Angelman syndromes (PWS and AS, respectively [63–65]). A proportion of both PWS and AS patients have normal chromosome inheritance but the chromosomes appear to have undergone a regional epigenetic switch such that in PWS, several imprinted sequences over a region of 2 Mb are methylated and repressed on the mutant paternal chromosome (as well as on the normal maternal chromosome). In equivalent AS patients, by contrast, the mutant maternal chromosome is demethylated and expressed in the same sequences, as is the normal paternal one. In PWS, the phenotype may arise from repression of one or more of these genes, whereas in AS the phenotype may arise from repression of the maternally expressed *UBE3A* gene (this has not so far been shown to be repressed in AS-imprinting mutations) [66–68].

The PWS- and AS-imprinting mutations have been mapped to the *SNRPN* exon 1 region, and the upstream transcripts (BD transcripts) of *SNRPN* (which are also paternally expressed but at a very low level and probably not translated), respectively [69•]. It has been proposed that these regions constitute an IC involved in imprint switching in the germline (see below) which has a bipartite structure [69•]. The imprintor (BD RNA transcript) acts on the switch-initiation site — *SNRPN* exon 1; a CpG-rich region with a direct repeat structure — and this switch
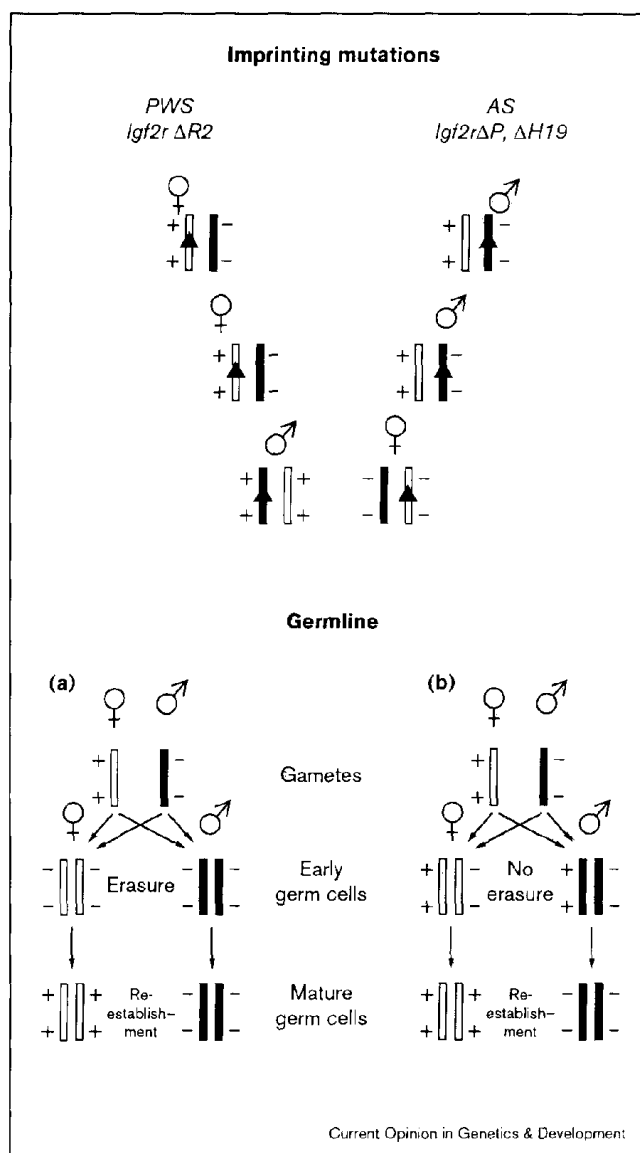
initiation site (SIS) becomes methylated (presumably in the female germline). In the male germline, the SIS initiates demethylation of the IC. Methylation (in the case of the maternal chromosome) or demethylation (in the paternal chromosome) then spreads along the region, either already in the germlines, or postzygotically. We note that the untranslated RNA (BD transcript) is associated with methylation of the CpG-rich region with the direct repeats in *cis*. The imprintor RNA transcript should therefore be expressed in the female germline to initiate the paternal→maternal switch, whereas it should not be expressed (or not active) in the paternal germline where the SIS initiates the maternal→paternal imprint switch.

Imprintor mutations can be transmitted silently on paternal chromosomes — because these are demethylated — but reveal themselves on transmission through a female (leading to AS). By contrast, SIS mutations can be transmitted silently on maternal chromosomes (because they are methylated) but lead to problems on paternal transmission (PWS; Figure 1). This has led to the suggestion that, in normal germline passage, the epigenotype (methylation and chromatin) of the same-sex chromosome (e.g. a grandpaternal chromosome in a male germline) is transmitted unaltered but the opposite sex epigenotype (e.g. a grandmaternal chromosome in a female germline) is switched [69•]. This addresses fundamental properties of how imprinting is erased and re-established in germ cells and is addressed in the next section [70,71].

Another set of imprinting mutations is the deletion of the differentially methylated region 2 in *Igf2r*, or mutation of the promoter of *Igf2r*, as described in the previous section [4,42•]. Although this was on a YAC transgene, the conclusions are probably the same for the endogenous locus. Two further interesting points emerge from this analysis. First, deletion of region 2 affects regional methylation (region 1) via an RNA (antisense), and mutation of the promoter (in region 1) affects regional methylation (region 2) possibly via an RNA (sense). The general theme that presence of an RNA near a 'switch' element (CpG-rich, direct repeats) leads to methylation of this element, whereas its absence leads to demethylation, seems to be upheld. Second, if one draws a pedigree of the transmission of *Igf2r* ∆R2 and *Igf2r* ∆P mutations, like the ones for the PWS/AS mutations, these look surprisingly similar to each other (Figure 1). Hence, *Igf2r* ∆R2 is transmitted 'silently' through females but causes a 'phenotype' when transmitted paternally, and *Igf2r* ∆P is transmitted silently through males and is activated on maternal transmission.

The first imprinting mutations made in the mouse were in the *H19* gene and its 3′ endoderm enhancers. The enhancer deletion abolishes *H19* transcription in endoderm-derived tissues (on the maternal chromosome) and *Igf2* transcription on the paternal chromosome [72]. Thus the two reciprocally imprinted genes can access

Figure 1

Imprinting mutations

PWS
Igf2r ΔR2

AS
Igf2rΔP, ΔH19

Germline

(a) ♀ ♂

Gametes

♀ ╱╲ ♂

Early
germ cells

Erasure

Mature
germ cells

Re-
establish-
ment

(b) ♀ ♂

♀ ╱╲ ♂

No
erasure

Re-
establish-
ment

Current Opinion in Genetics & Development

Imprinting mutations and role of the germline. Upper: an imprinting mutation (▲) disrupts regional setting or resetting of imprints (+/-). A particular property of imprinting mutations (PWS/AS, Igf2rΔR2, Igf2rΔP, ΔH19; see text) is that they can be transmitted through one sex germline (e.g. PWS through females) without effect on regional imprinting. However, when they are passed through the opposite sex germline (male for PWS mutation), the maternal imprint or epigenotype (+) remains on the chromosome. This is now brought together with a normal maternal chromosome with a maternal epigenotype and causes a functional disomy. The failure to establish the appropriate regional epigenotype could be caused by failure to erase the previous imprints (i.e. the maternal ones in the example) or failure to establish or maintain (in the germline or postzygotically) the paternal imprints. Open bars: maternal chromosomes; filled bars: paternal chromosomes. Lower: role of the germline. Two different models can be envisaged. (a) Imprints are first erased early in germ cell development, and then later on re-established according to the sex of the germ cells. (b) Imprints of the same sex (e.g. grandmaternal chromosome in maternal germline) are not erased, but imprints of the opposite sex (e.g. grandpaternal chromosome in maternal germline) are switched and re-established.

the same enhancer. In addition, deletion of the H19 gene and 10 kb of upstream sequences (including the core DMR and repeats) from the maternal chromosome leads to derepression of Igf2 (and Ins2) on the same chromosome, and so does (to a lesser extent) another deletion of H19 which is limited to the promoter and gene body [61•,73]. These observations have given support to the elegant 'enhancer competition' model of H19-Igf2 imprinting [9]. In its simplest form, this model states that the Igf2 and H19 promoters compete for enhancers (one of these is located 3' of H19) and that the competition is controlled by imprinting of H19, which is achieved by the upstream control element. There are a number of situations in normal development and in disease, however, where either the maternal Igf2 is not repressed (thus being expressed together with H19 on the same chromosome) or the paternal Igf2 is not expressed (with a silent H19 on the same chromosome; this situation is not as frequent as the previous one). As H19 deletion leads to an altered pattern of allelic methylation of Igf2 in DMRs 0–2 [74•], we have extended this model to suggest that interaction of the H19 locus with elements in Igf2 in cis determines allelic methylation of Igf2 and enhancer competition subsequently operates within the confines of the allelic methylation patterns of both Igf2 and H19 [75]. Fittingly, the direct repeat/DMR1 region in Igf2 is overlapped by an imprinted antisense transcript, again establishing an association between methylation of a potential imprint switch/imprint maintenance element and the presence of an unusual RNA in cis [37]. There is some preliminary evidence that deletion of this region in fibroblasts can lead to failure to maintain Igf2 imprinting [76]. In addition to elements in Igf2 and H19 there may be other elements further away in the imprinting cluster that are involved in its imprint establishment or maintenance. For example, in the human translocations in KVLQT which is further centromeric [77], can result in loss of repression of the maternal allele of IGF2 [78].

Again, the formal genetics of the H19 imprinting mutations (deletions) are similar to the PWS/AS situation. Transmission of H19 deletions is silent through the paternal line but reveals itself on maternal transmission (Figure 1). What the opposite mutation would be like is not clear at the moment but it could be something that interferes with the establishment of the H19 imprint (leading to repression) in the paternal germline.

Lastly and briefly in this section, we should point out that imprinting mutations can also arise without mutations at the DNA level — that is, they would be epimutations. Thus, methylation on the maternal H19 and IGF2 genes (associated with H19 repression and IGF2 activation) has been found in isolated sporadic cases of Beckwith–Wiedemann syndrome and mutations have not yet been found [79]. Similar observations have been made in AS patients and it is a possibility that these represent

imprinting defects in the sense of epimutations, rather than be explained by mutational mechanisms [80].

The germline

The germline has the crucial role of erasure and re-establishment of imprints. Two different models could be envisaged of how this might work (Figure 1). First, the epigenotype of the same sex chromosome could be maintained (grandpaternal chromosome in paternal germline) [65,69•], but the opposite sex chromosome needs to be switched (grandmaternal chromosome in paternal germline). As a result, there could be epigenetically 'old' chromosomes in the population which have been passed through only one germline for multiple generations. Alternatively, epigenetic information on both chromosomes becomes 'erased' and re-established at a later stage. All the available information on methylation and expression of imprinted genes in the germline suggests the latter view is correct. Hence, methylation in imprinted genes — as well as in nonimprinted genes — is lost at early stages of germ cell development in either sex. Several days later, *de novo* methylation occurs in DMRs [43]. In addition, in all cases tested, expression of imprinted genes in the germline is biallelic, suggesting that there are no epigenetic differences between parental chromosomes left [81,82].

Early stage germ cells of either sex, explanted into culture as embryonic germ cells, have a dominant demethylating affect when fused with lymphocytes; this includes effects on imprinted and nonimprinted genes [83•]. Thus early germ cells may have a strong demethylase activity [84•] combined with absence of factors that protect specific regions from demethylation.

Interactions in *trans*

A considerable number of molecular properties have now been associated with imprinted genes (Table 1) and some have been discussed extensively already. Others are listed in the table but no further mechanistic insights have been gained since the original publications and these are not discussed any further here. Some recent interesting observations are worth mentioning, however, because they expand our thinking of imprinting essentially as a *cis*-acting chromosome phenomenon. The first is that introduction of imprinted transgenes into the genome sometimes results in interactions with the endogenous copies. *Igf2* transgenes introduced into mice via ES cells are silenced but the endogenous genes are hyperactivated (and this is accompanied by methylation changes in both the transgene and the endogenous locus) [85•]. *U2afbp–rs1* transgenes can lead to methylation of the endogenous copy of the gene in testis and to transmission of methylated copies from these males to offspring [56•]. *Xist* transgenes interact (and presumably count) with the endogenous *Xist* locus [86–88]. These observations are unusual in mammals — transgenes are known to interact with endogenous counterparts in plants, and

more recently in *Drosophila* — but they may suggest that dosage-dependent factors (such as repressors, activators, or chromatin components) are important in the repression or activation of imprinted genes and that transgenes can titrate these factors, thus leading to altered expression of the endogenous copies. Indeed, in the maternal *H19* knockout, methylation in *Igf2* is increased in *cis* but at the same time decreased in *trans* (on the paternal chromosome) [74•]. Other observations of *trans*-allelic effects [89] may be explained by interactions between homologues in imprinted regions in late S-phase [90•] but the mechanisms need to be explored. Methylation transfer between homologues as observed in *Ascobolus immersus* is one of the candidate mechanisms [91].

Table 1

Imprinted genes.

Mechanistic features	References
Differential DNA methylation	Review [32]
Differential chromatin organisation	Review [98]
Intron size and content	[99]
Direct repeat clusters	[41]
Transcripts upregulated on growth arrest	[100]
Transcriptional silencers	[101]
Unusual RNAs	Review [4]
Differential DNA replication timing	Review [5]
Differential chromatin extension	(a)
Clustering	Review [5,7]
Pairing in late S-phase	[90•]
Differences in meiotic recombination rate	[102,103]
Silencers in *Drosophila*	[52•]
Transactivation/*trans*-effects	[56•,74•,85•,86–89]

(a) K Okumura, personal communication.

General imprinting model and conclusions

A number of common features and common players seem to emerge from the analysis of different imprinting systems (in particular PWS/AS, *Igf2r, Igf2–H19*). The most general model suggested by these findings is shown in Figure 2. The players and their roles are ICs and IMEs; the distinction between these two is sometimes difficult and they may depend on each other in *cis*. The IC and the IME contain CpG-rich regions, which are differentially methylated. These regions overlap with or are adjacent to direct repeat clusters. The DMRs in the IC are likely to be core DMRs — that is, those elements that maintain germline-specific methylation throughout development. Whether IMEs have core DMRs or not is not known at present. Interestingly, two core DMRs (*H19, SNRPN*) have been shown to act as silencers in *Drosophila*, suggesting perhaps that they can act as PRE (polycomb response element) type sequences ([52•], F Lyko, K Buiting, B Horsthemke, R Paro, personal communication). Binding of polycomb type or other factors might protect from *de novo* or demethylation. Co-operative binding of this type of chromatin factor together with further local *cis*-acting DNA signals may lead to local spreading of methylation/demethylation. The
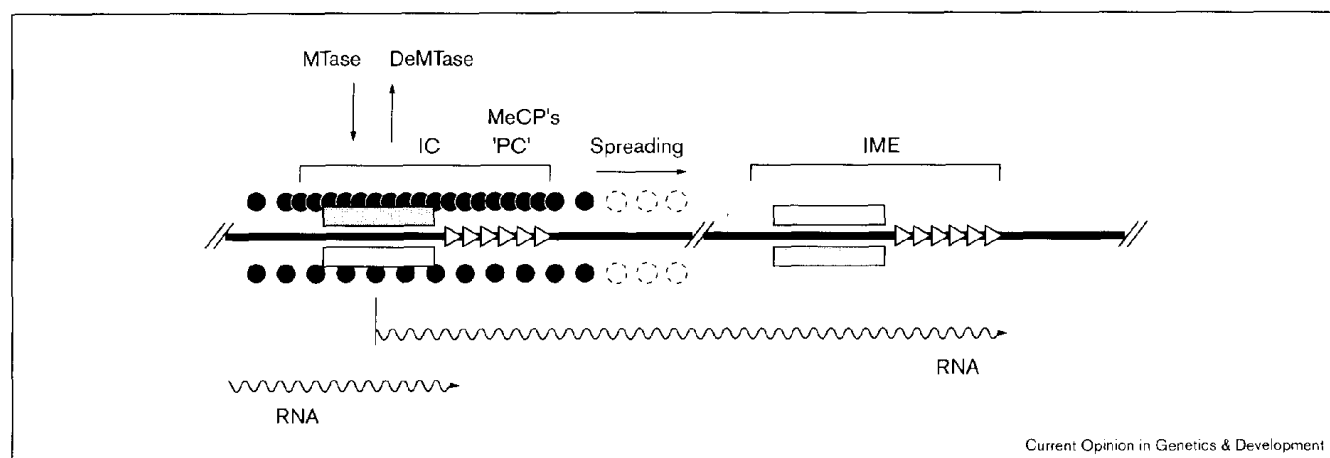
IC or its vicinity can be the starting point of RNA transcripts, which are not necessarily translatable. These transcripts might reach the next IME and probably lead to methylation (or demethylation) of the IME, perhaps through local interaction with protective factors and the direct repeats. The RNAs do not necessarily have to be long as ICs and IMEs could be brought into close physical contact via nuclear matrix/scaffold attachment binding and higher-order chromatin [92]. It is not excluded that on contact between IC and IME, epigenotypes are transferred without the involvement of RNA, for example through local chromatin factors. The IC could also be receiving information in *cis* via RNAs starting elsewhere. One model of how RNAs act in *cis* is the *Xist*-encoded RNA, the expression of which is also associated with methylation of CpG rich sequences in *cis*.

The remaining players are the *de novo* and maintenance methylation system, and the demethylase system [84•], both of which interact with (positive or negative) *trans*-acting factors. The developmental sequence of events can be evisaged thus: *de novo* methylation is established in core DMRs during the later stages of germ cell development and needs *de novo* methylase, absence of inhibitory factors, and possibly the interaction with *cis* RNA and direct repeats. Local, and regional (IME), spreading in the germline is possible. Protection from the demethylase system, or absence of this system, is necessary. During early development, core DMRs are protected probably both from *de novo* methylation and from demethylation, respectively, whereas flanking regions undergo both (equilibration). Regional spreading (via RNAs) may occur to IMEs during early or later developmental stages. Following differentiation in the embryo, local spreading is initiated again and leads to larger regions of differential methylation in imprinted genes in tissues. This may involve a number of chromatin mechanisms, including perhaps histone acetylation/deacetylation [93].

In early stage germ cells, the demethylase system is active and protective factors of DMRs are presumably absent, as are possibly *cis*-acting RNAs (although their presence may not necessarily hinder demethylation). Whether direct repeat clusters are also required for demethylation is not known. DMRs together with direct repeats and interacting RNAs therefore constitute imprint/methylation switch elements which can exist in either a methylated or unmethylated form. Mutation of ICs will lead to regional deregulation of imprinting, involving IMEs in *cis*. Mutations in IMEs may halt regional progression but may even feed back to alter IC imprints, in which case the distinction between IC and IME is difficult and perhaps meaningless. Mutations in the RNAs and disruptions between IC and IME (translocations) will also interfere with regional progression. Mutations in the *trans*-acting factors involved — as exemplified by the Dnmt1 (*Mtase*) mutations — will lead to local or regional problems with the establishment, maintenance, or erasure. Polymorphic

Figure 2

General model of imprinting control. An imprinting centre (IC) and an imprinting maintenance element (IME) are shown. The IC is composed of a core differentially methylated region (DMR; shaded box, methylated; open box, unmethylated) together with a cluster of direct repeat sequences (▷). The IME also has a DMR and repeats; this does not need to be a core DMR (see text). *Trans*-acting factors (e.g. Methyltransferase [Mtase], Demethylase [DeMtase], Methyl Cytosine binding proteins [MeCPs], Polycomb-like [PC] chromatin proteins etc.) interact with the IC and lead to methylation/demethylation and some local spreading. Differences in the chromatin structure are indicated by the density of filled circles. The IC region may coincide with the transcriptional start of an RNA (e.g. antisense) and transcription is influenced by the epigenetic state of the IC. This transcript may reach the next IME in *cis* (this may be aided by secondary chromatin structures that bring ICs and IMEs into close physical contact). The current available evidence suggests that RNA transcripts through IMEs lead to their methylation. However, if ICs and IMEs are brought into close physical contact in *cis*, transfer of epigenotypes by chromatin factors is not excluded. The IC epigenotype might also be influenced by RNA transcripts from other elements in *cis*. Direct repeats might represent local boundary elements (with a directionality) for the local spreading of epigenetic modifications.

alleles in either the *cis*-acting sequences or transacting factors in, for example, the human population may explain variability of imprinting in the population [94].

This model extends previous ones with similarities [95,96], and can also incorporate the expression competition model [4], with the exception that the present model does not predict that an imprinting mutation should only affect an oppositely imprinted gene in cis.

How the imprints are read, that is converted into gene activity/inactivity is not addressed in this model, because it involves more general mechanisms of positive and negative control (by methylation and other chromatin mechanisms) of transcriptional and post-transcriptional mechanisms [97].

Note added in proof

The work referred to as F Lyko, K Buiting, B Horsthemke, R Paro, personal communication, has now been published [104].

Acknowledgements

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* • of special interest
* •• of outstanding interest

1. Reik W, Surani MA (Eds): *Genomic Imprinting.* Oxford: Oxford University Press; 1997.

2. Ohlsson R, Hall K, Ritzen M (Eds): *Genomic Imprinting: Causes and Consequences.* Cambridge: Cambridge University Press; 1995.

3. John RM, Surani MA: **Imprinted genes and regulation of gene expression by epigenetic inheritance.** *Curr Opin Cell Biol* 1996, **8**:348-353.

4. Barlow DP: **Competition – a common motif for the imprinting mechanism?** *EMBO J* 1997, **16**:6899-6905.

5. Lalande M: **Parental imprinting and human disease.** *Annu Rev Genet* 1996, **30**:173-195.

6. Efstratiadis A: **Parental imprinting of autosomal mammalian genes.** *Curr Opin Genet Dev* 1994, **4**:265-280.

7. Horsthemke B, Dittrich B, Buiting K: **Imprinting mutations on human chromosome 15.** *Hum Mutat* 1997, **10**:329-337.

8. Reik W: **Genetic imprinting: the battle of the sexes rages on.** *Exp Phys* 1996, **81**:161-172.

9. Leighton PA, Saam JR, Ingram RS, Tilghman SM: **Genomic imprinting in mice: its function and mechanism.** *Biol Reprod* 1996, **54**:273-278.

10. Neumann B, Barlow DP: **Multiple roles for DNA methylation in gametic imprinting.** *Curr Opin Genet Dev* 1996, **6**:159-163.

11. Tycko B: **DNA methylation in genomic imprinting.** *Mut Res Rev Mut Res* 1997, **386**:131-140.

12. Turker MS, Bestor TH: **Formation of methylation patterns in the mammalian genome.** *Mut Res Rev Mut Res* 1997, **386**:119-130.

13. Corleysmith GE, Lim CTJ, Brandhorst BP: **Production of androgenetic Zebrafish (Danio rerio).** *Genetics* 1996, **142**:1265-1276.

14. Bishop CP, Jackson CM: **Genomic imprinting of chromatin in *Drosophila melanogaster*.** *Genetica* 1996, **97**:33-37.

15. Khosla S, Kantheti P, Brahmachari V, Chandra HS: **A male-specific nuclease resistant chromatin fraction in the mealybug *Planococcus lilachinus*.** *Chromosoma* 1996, **104**:386-392.

16. Li E, Beard C, Jaenisch R: **Role for DNA methylation in genomic imprinting.** *Nature* 1993, **366**:362-365.

17. Shemer R, Birger Y, Riggs AD, Razin A: **Structure of the imprinted mouse *Snrpn* gene and establishment of its parental-specific methylation pattern.** *Proc Natl Acad Sci USA* 1997, **94**:10267-10272.
•
The murine *Snrpn* gene contains two parent-specific methylated regions (DMRs 1 and 2). Methylation in these DMRs is erased in early primordial germ cells, re-established in a sex-specific way during later gametogenesis, and maintained throughout preimplantation development. *Snrpn* imprinting is disrupted in methyltransferase-deficient mice, leading to biallelic expression.

18. Ferguson SA, Sasaki H, Cattanach BM, Surani MA: **Parental-origin-specific epigenetic modification of the mouse *H19* gene.** *Nature* 1993, **362**:751-755.

19. Bartolomei MS, Webber AL, Brunkow ME, Tilghman SM: **Epigenetic mechanisms underlying the imprinting of the mouse *H19* gene.** *Genes Dev* 1993, **7**:1663-1673.

20. Zhang YH, Shields T, Crenshaw T, Hao Y, Moulton T, Tycko B: **Imprinting of human *H19* allele specific CpG methylation, loss of the active allele in Wilms tumor and potential for somatic allele switching.** *Am J Hum Genet* 1993, **53**:113-124.

21. Jinno Y, Sengoko K, Nakao M, Tamate K, Miamoto T, Matsuzaka T, Sutcliffe JS, Anan T, Takuma N, Nishiwaki K *et al.*: **Mouse/human sequence divergence in a region with a paternal-specific methylation imprint at the human *H19* locus.** *Hum Mol Genet* 1996, **5**:1155-1161.

22. Norris DP, Patel D, Kay GF, Penny GD, Brockdorff N, Sheardown SA, Rastan S: **Evidence that random and imprinted *Xist* expression is controlled by preemptive methylation.** *Cell* 1994, **77**:41-51.

23. Hatada I, Sugama T, Mukai T: **A new imprinted gene cloned by a methylation-sensitive genome scanning method.** *Nucleic Acids Res* 1993, **21**:5577-5582.

24. Smrzka OW, Fae I, Stoger R, Kurzbauer R, Fischer GF, Henn T, Weith A, Barlow DP: **Conservation of a maternal-specific methylation signal at the human *IGF2R* locus.** *Hum Mol Genet* 1995, **4**:1945-1952.

25. Hayashizaki Y, Shibata H, Hirotsune S, Sugino H, Okazaki Y, Sasaki N, Hirsoe K, Imoto H, Okuizumi H, Muramatsu M *et al.*: **Identification of an imprinted *U2af* binding protein related sequence on mouse chromosome 11 using the RLGS method.** *Nat Genet* 1994, **6**:33-40.

26. Feil R, Boyano MD, Allen ND, Kelsey G: **Parental chromosome-specific chromatin conformation in the imprinted *U2af1-rs1* gene in the mouse.** *J Biol Chem* 1997, **272**:20893-20900.

27. Wevrick R, Francke U: **An imprinted mouse transcript homologous to the human imprinted in Prader-Willi syndrome (IPW) gene.** *Hum Mol Genet* 1997, **6**:325-332.

28. Lefebvre L, Viville S, Barton SC, Ishino F, Surani MA: **Genomic structure and parent of origin specific methylation of Peg1.** *Hum Molec Genet* 1997, **6**:1907-1915.

29. Kobayashi S, Kohda T, Miyoshi N, Kuroiwa Y, Aisaka K, Tsutsumi O, Kaneko IT, Ishino F: **Human PEG1/MEST, an imprinted gene on chromosome 7.** *Hum Mol Genet* 1997, **6**:781-786.

30. Riesewijk AM, Hu L, Schulz U, Tariverdian G, Hoglund P, Kere J, Ropers HH, Kalscheuer VM: **Monoallelic expression of human PEG1/MEST is paralleled by parent-specific methylation in fetuses.** *Genomics* 1997, **42**:236-244.

31. Nishita Y, Yoshida I, Sao T, Takagi N: **Genomic imprinting and chromosomal localization of the human *Mest* gene.** *Genomics* 1996, **36**:539-542.

32. Shemer R, Razin A: **Establishment of imprinted methylation patterns during development.** In *Epigenetic Mechanisms of Gene Regulation*. Edited by Russo VEA, Martienssen RA, Riggs AD. Plainview, New York, USA: Cold Spring Harbor Laboratory Press; 1996:215-229. [Cold Spring Harbor Monograph Series, 32.]

33. Stoger R, Kubicka P, Liu CG, Kafri T, Razin A, Cedar H, Barlow DP: **Maternal-specific methylation of the imprinted**

mouse *Igf2r* locus identifies the expressed locus as carrying the imprinting signal. *Cell* 1993, **73**:61-71.

34.   Riesewijk AM, Schepens MT, Welch TR, Vandenbergloonen EM, Mariman EM, Ropers HH, Kalscheuer VM: **Maternal-specific methylation of the human *IGF2R* gene is not accompanied by allele specific transcription.** *Genomics* 1996, **31**:158-166.

35.   Sasaki H, Jones PA, Chaillet JR, Ferguson SA, Barton SC, Reik W, Surani MA: **Parental imprinting: potentially active chromatin of the repressed maternal allele of the mouse insulin-like growth factor II (*Igf2*) gene.** *Genes Dev* 1992, **6**:1843-1856.

36.   Feil R, Walter J, Allen ND, Reik W: **Developmental control of allelic methylation in the imprinted mouse *Igf2* and *H19* genes.** *Development* 1994, **120**:2933-2943.

37.   Moore T, Constancia M, Zubair M, Bailleul B, Feil R, Sasaki H, Reik W: **Multiple imprinted sense and antisense transcripts, differential methylation and tandem repeats in a putative imprinting control region upstream of mouse *Igf2*.** *Proc Natl Acad Sci USA* 1997, **94**:12509-12514.

38.   Reik W, Brown KW, Slatter RE, Sartori P, Elliott M, Maher ER: **Allelic methylation of H19 and Igf2 in the Beckwith-Wiedemann syndrome.** *Hum Mol Genet* 1994, **3**:1297-1301.

39.   Issa JPJ, Vertino PM, Boehm CD, Newsham IF, Baylin SB: **Switch from monoallelic to biallelic human *IGF2* promoter methylation during aging and carcinogenesis.** *Proc Natl Acad Sci USA* 1996, **93**:11757-11762.

40.   Glenn CC, Saitoh S, Jong MT, Filbrandt MM, Surti U, Driscoll DJ, Nicholls RD: **Gene structure, DNA methylation and imprinted expression of the human *SNRPN* gene.** *Am J Hum Genet* 1996, **58**:335-346.

41.   Neumann B, Kubicka P, Barlow DP: **Characteristics of imprinted genes.** *Nat Genet* 1995, **9**:451.

42.   Wutz A, Smrzka OW, Schweifer N, Schellander K, Wagner EF,
•   Barlow DP: **Imprinted expression of the *Igf2r* gene depends on an intronic CpG island.** *Nature* 1997, **389**:745-749.
YAC transgenes containing the *Igf2r* gene reproduce the imprinted methylation and expression pattern of the endogenous *Igf2r* gene. Proper imprinting and parent specific methylation of these transgenes depend on the presence of multiple elements including the differentially methylated region 2, the promoter region of *Igf2r*, and possibly a novel antisense transcript that is initiated in region 2.

43.   Brandeis M, Kafri T, Ariel M, Chaillet JR, McCarrey J, Razin A, Cedar H: **The ontogeny of allele-specific methylation associated with imprinted genes in the mouse.** *EMBO J* 1993, **12**:3669-3677.

44.   Shemer R, Birger Y, Dean WL, Reik W, Riggs AD, Razin A: **Dynamic methylation adjustment and counting as part of imprinting mechanisms.** *Proc Natl Acad Sci USA* 1996, **93**:6371-6376.

45.   Tremblay KD, Saam JR, Ingram RS, Tilghman SM, Bartolomei MS: **A paternal-specific methylation imprint marks the alleles of the mouse *H19* gene.** *Nat Genet* 1995, **9**:407-413.

46.   Tremblay KD, Duran KL Bartolomei MS: **A 5′ 2-kilobase-pair**
•   **region of the imprinted mouse *H19* gene exhibits exclusive paternal methylation throughout development.** *Mol Cell Biol* 1997, **17**:4322-4329.
A 2 kb region upstream of the *H19* gene is shown to be methylated differentially throughout embryonic development in contrast to the 350 bp promoter-proximal region, which is largely devoid of methylation on both alleles in blastocysts. These data identify the 2 kb differentially methylated region as a key regulatory domain for imprinted *H19* expression.

47.   Olek A, Walter J: **The pre-implantation ontogeny of the *H19***
•   **methylation imprint.** *Nat Genet* 1997, **17**:275-276.
Methylation differences between paternal and maternal chromsomes in the upstream region of *H19* gene are equilibrated during early preimplantation development except for a core region of about 2 kb in which the imprint is maintained. This suggests the existence of active protective mechanisms which preserve differential methylation in imprinting core elements, whereas all other differences originating from the gametes are equilibrated.

48.   Hatada I, Kitagawa K, Yamaoka T, Wang XD, Arai Y, Hashido K, Ohishi S, Masuda J, Ogata J, Mukai T: **Allele-specific methylation and expression of an imprinted *U2af1-rs1 (SP2)* gene.** *Nucleic Acids Res* 1995, **23**:36-41.

49.   Shibata H, Ueda T, Kamiya M, Yoshiki A, Kusakabe M, Placc C, Held WA, Sunahara S, Katsuki M, Muramatsu M, Hayashizaki Y: **An oocyte-specific methylation imprint center in the mouse *U2afbp-rs/Usaf1-rs1* gene marks the establishment of allele-**

specific methylation during preimplantation development. *Genomics* 1997, **2**:171-178.

50.   Zuccotti M, Monk M: **Methylation of the mouse Xist gene in sperm and eggs correlates with imprinted Xist expression and paternal X-inactivation.** *Nat Genet* 1995, **9**:316-320.

51.   Ariel M, Robinson E, Mccarrey JR, Cedar H: **Gamete-specific methylation correlates with imprinting of the murine *Xist* gene.** *Nat Genet* 1995, **9**:312-315.

52.   Lyko F, Brenton JD, Surani MA, Paro R: **An imprinting element**
•   **from the mouse *H19* locus functions as a silencer in *Drosophila*.** *Nat Genet* 1997, **16**:171-173.
A murine *H19* transgene containing a 1.2 kb portion of the upstream sequence is shown to function in *cis* as a parent-of-origin independent silencing element in *Drosophila*. Strikingly, this *cis*-acting element is located within an upstream region necessary for *H19* imprinting in mice. The results point to evolutionary conserved mechanisms in both gene silencing in *Drosophila* and imprinting in mice.

53.   Szabo PE, Mann JR: **Maternal and paternal genomes function independently in mouse ova in establishing expression of the imprinted genes *Snrpn* and *Igf2r*— no evidence for allelic trans-sensing and counting mechanisms.** *EMBO J* 1996, **15**:18-25.

54.   Tucker KL, Beard C, Dausman T, Jacksongrusby L, Laird PW,
•   Lei H, Li E, Jaenisch R: **Germline passage is required for establishment of methylation and expression patterns of imprinted but not of nonimprinted genes.** *Genes Dev* 1996, **10**:1008-1020.
Complementation of hypomethylated methyltransferase-deficient male ES cells with a *Dnmt* cDNA does not restore the methylation/expression patterns of imprinted genes but restores methylation in other regions. By passaging such 'rescued' ES cells through the germline in chimeric mice, however, monoallelic methylation and expression of imprinted genes is reestablished. These results indicate the existence of *de novo* DNA methylation activities (enzymes and/or protective/enhancing factors) during gametogenesis, distinct from those observed after implantation.

55.   Chaillet JR, Bader DS, Leder P: **Regulation of genomic imprinting by gametic and embryonic processes.** *Genes Dev* 1995, **9**:1177-1187.

56.   Hatada I, Nabetani A, Arai Y, Ohishi S, Suzuki M, Miyabara S,
•   Nishimune Y, Mukai T: **Aberrant methylation of an imprinted gene *U2af1-rs1(SP2)* caused by its own transgene.** *J Biol Chem* 1997, **272**:9120-9122.
The expression and methylation of the endogenous imprinted gene *U2af1-rs1* can be affected by interaction with its own transgene in the testis. This suggests *trans*-acting mechanisms during gametogenesis which protect the *U2af1-rs1* gene from methylation in the male but not in the female germline.

57.   Lee JE, Tantravahi U, Boyle AL, Efstratiadis A: **Parental imprinting of an *Igf2* transgene.** *Mol Reprod Dev* 1993, **35**:382-390.

58.   Pfeifer K, Leighton PA, Tilghman SM: **The structural *H19* gene**
•   **is required for transgene imprinting.** *Proc Natl Acad Sci USA* 1996, **93**:13876-13883.
The paper describes transgenic studies with the *H19* gene indicating that not only sequences upstream and downstream of the structural gene but also parts in the *H19* gene itself are required for both the establishment and maintenance of imprinting. Furthermore, *H19* transgenic expression does not rescue the loss of *Igf-2* imprinting in *trans* in *H19* deletion mice, implying a *cis* requirement of the *H19* gene for maintenance of *Igf2* imprinting.

59.   Elson DA, Bartolomei MS: **A 5′ differentially methylated**
•   **sequence and the 3′ flanking region are necessary for *H19* transgene imprinting.** *Mol Cell Biol* 1997, **17**:309-317.
Transgenic lines were constructed containing a 14 kb genomic fragment of the *H19* gene including 4 kb of 5′-flanking sequence and 8 kb of 3′-flanking sequence. The transgenes show imprinted expression which is most pronounced in high-copy-number transgenics. Deletion derivates of this transgenic construct reveal that elements in both the 5′ differentially methylated region and the 3′ region, which includes the endodermal *H19* enhancers, are necessary for proper imprinting and expression of the gene.

60.   Ainscough JFX, Koide T, Tada M, Barton S, Surani MA: **Imprinting**
•   **of *Igf2* and *H19* from a 130 kb YAC transgene.** *Development* 1997, **124**:3621-3632.
YAC transgenes containing both a *LacZ*-tagged *Igf2* and an unmodified *H19* gene show proper imprinting after maternal and paternal transmission, respectively. Multicopy transgenic lines, however, exhibited copy number dependent downregulation of *H19* after maternal transmission and concomitant loss of *Igf2-LacZ* repression. This indicates that the tandem arrangement of multiple transgene copies can influence the proper control of imprinted expression at individual loci.

61.   Ripoche MA, Kress C, Poirier F, Dandolo L: **Deletion of the *H19***
•   **transcription unit reveals the existence of a putative imprinting control element.** *Genes Dev* 1997, **11**:1596-1604.

Maternal transmission of a targeted deletion of the *H19* transcription unit leads to the disruption of *Igf2* imprinting and biallelic expression of this gene. As the neomycin cassette in the targetted *H19* allele is imprinted, the imprinting of the *H19* locus is independent of the *H19* gene itself.

62. Reik W, Constancia M: **Genomic imprinting: Making sense or antisense?** *Nature* 1997, **389**:689-671.

63. Reis A, Dittrich B, Greger V, Buiting K, Lalande M, Gillessen KG, Anvret M, Horsthemke B: **Imprinting mutations suggested by abnormal DNA methylation patterns in familial Angelman and Prader-Willi syndromes.** *Am J Hum Genet* 1994, **54**:741-747.

64. Sutcliffe JS, Nakao M, Christian S, Orstavik KH, Tommerup N, Ledbetter DH, Beaudet AL: **Deletions of a differentially methylated CpG island at the *SNRPN* gene define a putative imprinting control region.** *Nat Genet* 1994, **8**:52-58.

65. Buiting K, Saitoh S, Gross S, Dittrich B, Schwartz S, Nicholls RD, Horsthemke B: **Inherited microdeletions in the Angelman and Prader-Willi syndromes define an imprinting center on human-chromosome-15.** *Nat Genet* 1995, **9**:395-400.

66. Vu TH, Hoffman AR: **Imprinting of the Angelman syndrome gene, *UBE3A*, is restricted to brain.** *Nat Genet* 1997, **17**:12-13.

67. Rougeulle C, Glatt H Lalande M: **The Angelman syndrome candidate gene, *UBE3A/E6-AP*, is imprinted in brain.** *Nat Genet* 1997, **17**:4-5.

68. Albrecht U, Sutcliffe JS, Cattanach BM, Beechey CV, Armstrong D, Eichele G, Beaudet AL: **Imprinted expression of the murine Angelman syndrome gene *Ube3a* in hippocampal and Purkinje neurons.** *Nat Genet* 1997, **17**:75-78.

69. Dittrich B, Buiting K, Korn B, Rickard S, Buxton J, Saitoh S,
•   Nicholls RD, Poustka A, Winterpacht A, Zabel B, Horsthemke B: **Imprint switching on human-chromosome 15 may involve alternative transcripts of the *Snrpn* gene.** *Nat Genet* 1996, **14**:163-170.
Novel alternative *SNRPN* transcripts are described which extend into the Angelman syndrome part of the imprinting centre upstream of the previously described exon 1 of the *SNRPN* gene. These novel exons lack protein-coding potential and are expressed from the paternal chromosome only. Together with the description of intragenic deletions and a point mutation, the authors suggest that imprint switching (paternal to maternal) on human chromosome 15 may involve alternative *SNRPN* transcripts.

70. Ferguson-Smith AC: **Imprinting moves to the center.** *Nat Genet* 1996, **14**:119-121.

71. Kelsey G, Reik W: **Imprint switch mechanism indicated by mutations in Prader-Willi and Angelman syndromes.** *Bioessays* 1997, **19**:361-365.

72. Leighton PA, Saam JR, Ingram RS, Stewart CL, Tilghman SM: **An enhancer deletion affects both *H19* and *Igf2* expression.** *Genes Dev* 1995, **9**:2079-2089.

73. Leighton PA, Ingram RS, Eggenschwiler J, Efstratiadis A, Tilghman SM: **Disruption of imprinting caused by deletion of the *H19* gene region in mice.** *Nature* 1995, **375**:34-39.

74. Forne T, Oswald J, Dean W, Saam JR, Bailleul B, Dandolo L,
•   Tilghman SM, Walter J, Reik W: **Loss of the maternal *H19* gene induces changes in Igf2 methylation in both *cis* and *trans*.** *Proc Natl Acad Sci USA* 1997, **94**:10243-10248.
Allelic methylation patterns in the *Igf2* gene are influenced by deletions in the *H19* gene. After maternal transmission of such deletions, the maternal allele of *Igf2* adopts the epigenotype of the paternal allele indicating that this alteration is either directly or indirectly influenced by *H19* gene expression. Moreover, maternal *H19* deletions also influence methylation of the paternal *Igf2* allele in *trans*, suggesting the existence of *H19*-dependent mechanisms controlling allelic *Igf2* methylation in *cis* and *trans*.

75. Walter J, Krueger T, Engemann S, Allen ND, Kelsey G, Feil R, Forne T, Reik W: **Genomic imprinting and modifier genes in the mouse.** In *Epigenetic Mechanisms of Gene Regulation.* Edited by Russo VEA, Martienssen RA, Riggs AD. Plainview, New York, USA: Cold Spring Harbor Laboratory Press; 1996:195-213. [Cold Spring Harbor Monograph Series, 32.]

76. Hu JF, Vu TH, Hoffman AR: **Genomic deletion of an imprint maintenance element abolishes imprinting of both insulin-like growth factor II and H19.** *J Biol Chem* 1997, **272**:20715-20720.

77. Lee MP, Hu RJ, Johnson LA, Feinberg AP: **Human *KVLQT1* gene shows tissue-specific imprinting and encompasses Beckwith-Wiedemann syndrome chromosomal rearrangements.** *Nat Genet* 1997, **15**:181-185.

78. Brown KW, Villar AJ, Bickmore W, Clayton SJ, Catchpoole D, Maher ER, Reik W: **Imprinting mutation in the**

Beckwith-Wiedemann syndrome leads to biallelic *IGF2* expression through an *H19*-independent pathway. *Hum Mol Genet* 1996, **5**:2027-2032.

79. Reik W, Brown KW, Schneid H, Le BY, Bickmore W, Maher ER: **Imprinting mutations in the Beckwith-Wiedemann syndrome suggested by altered imprinting pattern in the *IGF2-H19* domain.** *Hum Mol Genet* 1995, **4**:2379-2385.

80. Burger J, Buiting K, Dittrich B, Gross S, Lich C, Sperling K, Horsthemke B, Reis A: **Different mechanisms and recurrence risks of imprinting defects in Angelman syndrome.** *Am J Hum Genet* 1997, **61**:88-93.

81. Szabo PE, Mann JR: **Biallelic expression of imprinted genes in the mouse germline: implications for erasure, establishment and mechanisms of genomic imprinting.** *Genes Dev* 1995, **9**:1857-1868.

82. Villar AJ, Eddy EM, Pedersen RA: **Developmental regulation of genomic imprinting during gametogenesis.** *Dev Biol* 1995, **172**:264-271.

83. Tada M, Tada T, Lefebvre L, Barton SC, Surani MA: **Embryonic**
•   **germ cells induce epigenetic reprogramming of somatic nucleus in hybrid cells.** *EMBO J* 1997, **16**:6510-6520.
Using cell fusions between murine embryonic germ cells and thymic lymphocytes, the authors show that the embryonic germ cells possess activities which influence the methylation/expression status of genes such that imprinted and nonimprinted genes of the somatic nucleus are reprogrammed/demethylated. The epigenetic modifications in these cell hybrids are comparable to the reprogramming events occuring during germ cell development.

84. Weiss A, Keshet I, Razin A, Cedar H: **DNA demethylation *in**
•   **vitro*— involvement of RNA.** *Cell* 1996, **86**:709-718.
DNA demethylation was studied in an *in vitro* system showing that the 5-methylcytosine nucleotide unit is transfered from DNA substrates to RNase-sensitive molecules. Experiments with extracts of various cell types show that gene-specific demethylation may be controlled by additional protein factors.

85. Sun FL, Dean WL, Kelsey G, Allen ND, Reik W: **Transactivation**
•   **of *Igf2* in a mouse model of Beckwith-Wiedemann syndrome.** *Nature* 1997, **389**:809-815.
An ectopic integration of an *Igf2* transgene into ES cells leads to transactivation of the endogenous *Igf2* gene and changes in DNA methylation. The consequent overexpression of *Igf2* in chimeric mice results in most of the symptoms characteristic of Beckwith-Wiedemann syndrome in human.

86. Lee JT, Jaenisch R: **Long-range cis effects of ectopic X-inactivation centres on a mouse autosome.** *Nature* 1997, **386**:275-279.

87. Lee JT, Strauss WM, Dausman JA, Jeanisch R: **A 450 kb transgene displays properties of the mammalian X-inactivation center.** *Cell* 1996, **86**:83-94.

88. Herzing L, Romer JT, Horn JM, Ashworth A: *Xist* **has properties of the X-chromosome inactivation centre.** *Nature* 1997, **386**:272-275.

89. Duville B, Bucchini D, Tang T, Jami J, Paldi A: **Imprinting at the mouse *Ins2* locus: evidence for *cis*- and *trans*-allelic interactions.** *Genomics* 1998, **47**:52-57.

90. LaSalle JM, Lalande M: **Homologous association of oppositely**
•   **imprinted chromosomal domains.** *Science* 1996, **272**:725-728.
During late S phase, maternal and paternal chromosomes in the Prader-Willi and Angelman syndrome region are temporally and spatially associated as shown by three-dimensional fluorescence *in situ* hybridization. Chromosomes from PWS and AS patients were deficient in such associations, indicating that normal imprinting involves mutual recognition and preferential association of maternal and paternal chromosomes.

91. Colot V, Maloisel L, Rossignol JL: **Interchromosomal transfer of epigenetic states in ascobolus-transfer of DNA methylation is mechanistically related to homologous recombination.** *Cell* 1996, **86**:855-864.

92. Banerjee S, Smallwood A: **A chromatin model of *IGF2/H19* imprinting.** *Nat Genet* 1995, **11**:237-238.

93. Svensson K, Mattson R, James TC, Wentzel P, Pilartz M, McLaughlin J, Miller S, Olsson T, Erickson UJ, Ohlsson R: **The paternal allele of the *H19* gene is silenced in a stepwise manner during early mouse development: the acetylation status of histones may be involved in the generation of variegated expression patterns.** *Development* 1998, **125**:61-69.

94. Giannoukakis N, Deal C, Paquette J, Kukuvitis A, Polychronakos C: **Polymorphic functional imprinting of the human *IGF2* gene**

among individuals in blood cells is associated with *H19* expression. *Biochem Biophys Res Comm* 1996, **220**:1014-1019.

95.   Brockdorff N: **Convergent themes in X-chromosome inactivation and autosomal imprinting.** In *Genomic Imprinting.* Edited by Reik W, Surani MA. Oxford: Oxford University Press; 1997:191-210.

96.   Feinberg AP: **Genomic imprinting as a developmental process disturbed in cancer.** In *Genomic Imprinting.* Edited by Reik W, Surani MA. Oxford: Oxford University Press; 1997:165-176.

97.   Kass SU, Pruss D, Wolffe AP: **How does DNA methylation repress transcription?** *Trends Genet* 1997, **13**:444-449.

98.   Feil R, Kelsey G: **Genomic imprinting: a chromatin connection.** *Am J Hum Genet* 1997, **61**:1213-1219.

99.   Hurst LD, McVean G, Moore T: **Imprinted genes have few and small introns.** *Nat Genet* 1996, **12**:234-237.

100.   Hayashida T, Eversole-Cire, Jones PA, Sasaki H: **Imprinted genes are upregulated by growth-arrest in embryonic fibroblasts.** *J Biochem* 1997, **112**:901-903.

101.   Ward A, Fisher R, Richardson L, Pooler JA, Squire S, Bates P, Shaposhnikov R, Hayward N, Thurston M, Graham CF: **Genomic regions regulating imprinting and insulin-like growth factor 2 promoter 3 activity in transgenics: novel enhancer and silencer elements.** *Genes Funct* 1997, **1**:25-36.

102.   Paldi A, Gyapay G, Jami J: **Imprinted chromosomal regions of the human genome display sex-specific meiotic recombination frequencies.** *Curr Biol* 1995, **5**:1030-1035.

103.   Robinson WP, Lalande M: **Sex-specific meiotic recombination in the Prader–Willi/Angelman syndrome imprinted region.** *Hum Mol Genet* 1995, **4**:801-806.

104.   Lyko F, Buiting K, Horsthemke B, Paro R: **Identification of a silencing element in the human 15q11-q13 imprinting center by using transgenic *Drosophila*.** *Proc Natl Acad Sci USA* 1998, **95**:1698-1702.