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REVIEW ARTICLE
Mechanisms of Genomic Imprinting

Karl Pfeifer

Laboratory of Mammalian Genes and Development, NICHD/NIH, Bethesda, MD

Imprinted genes represent a curious defiance of normal Mendelian genetics. Mammals inherit two complete sets of chromosomes, one from the mother and one from the father, and most autosomal genes will be expressed from both the maternal and the paternal alleles. Imprinted genes, however, are expressed from only one chromosome, in a parent-of-origin–dependent manner. Because silent and active promoters are present in a single nucleus, the differences in activity cannot be explained by transcription-factor abundance. Thus, transcription of imprinted genes represents a clear situation in which epigenetic mechanisms restrict gene expression and, therefore, offers a model for understanding the role of DNA modifications and chromatin structure in maintaining appropriate patterns of expression. Furthermore, because of their parent-of-origin–restricted expression, phenotypes determined by imprinted genes are susceptible not only to genetic alterations in the genes but also to disruptions in the epigenetic programs controlling regulation. Imprinted genes are often associated with human diseases, including disorders affecting cell growth, development, and behavior.

Introduction

The nonequivalence of maternally and paternally contributed genomes was first identified in elegant nuclear-transfer studies (McGrath and Solter 1984; Surani et al. 1984). Subsequently, uniparental disomies (UPDs), in which either single chromosomes or parts thereof are inherited solely through the maternal or the paternal germlines, have been studied extensively in mice, to identify regions of the genome that carry imprinted genes (Cattanach 1986). For example, paternal UPD of the distal end of mouse chromosome 7 results in early embryonic lethality, a phenotype that can be explained by either loss of a maternal-specific transcript or the double dose of a paternal-specific transcript in these animals (Ferguson-Smith et al. 1991). Likewise, human geneticists have identified, by association of uniparental inheritance of these regions with specific diseases, chromosomal regions likely to carry imprinted genes; for example, paternal UPD of human 11p15.5 (syntenic with mouse distal 7) is associated with Beckwith-Wiedemann syndrome (BWS [MIM 130650]) (Henry et al. 1991; Weksberg et al. 1993*b*). Other diseases clearly associated with imprinted genes include Prader-Willi (PWS [MIM 176270]), Angelman (AS [MIM 105830]), and Russell-Silver syndromes (MIM 180860) and Albright hereditary osteodystrophy (MIM 103580). Imprinted genes contribute to language development and social affiliation (Skuse et al. 1997) and probably to other complex behavioral phenotypes, including alcohol preference, schizophrenia, and bipolar affective disorder, in humans (Nicholls 2000). Finally, disruption in the monoallelic expression of imprinted genes may be the most common mutation associated with cancer (Feinberg 2000). An excellent compilation and description of studies demonstrating parent-of-origin effects has been recently published (Morison and Reeve 1998).

Several approaches have been used to isolate imprinted genes. These include positional cloning and candidate-gene testing to find genes responsible for the UPD phenotypes in mice and humans (e.g., see Barlow et al. 1991; Lee et al. 1997), as well as genomewide scans that depend on the differential expression or epigenetic modification of maternal and paternal alleles of imprinted genes (e.g., see Hatada et al. 1993; Kaneko-Ishino et al. 1995; Piras et al. 2000). It has become clear that imprinted genes are not randomly distributed throughout the genome but, rather, are often concentrated in discrete clusters. Thus, the identification of one imprinted gene has often led to the rapid determination that nearby genes are also imprinted. Finally, allele-specific expression of several genes has been discovered serendipitously during analysis of their loss-of-function phenotypes in mouse studies. The paternal-specific expression of *IGF2* (MIM 147470), the first endogenous gene to be identified as imprinted, was discovered in this way (DeChiara et al. 1991). The frequency with

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Address for correspondence and reprints: Dr. Karl Pfeifer, Laboratory of Mammalian Genes and Development, NICHD/NIH, Bethesda, MD 20892. E-mail: kpfeifer@helix.nih.gov

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which imprinted genes have been fortuitously identified in knockout studies suggests that ~0.1%–1% of all mammalian genes are imprinted (Barlow 1995). To date, ~3 dozen imprinted genes have been identified in mice and humans.

This review will focus on recent experiments investigating the molecular basis for monoallelic expression of genes within two imprinted gene clusters: human 11p15.5/mouse distal 7 (associated with BWS and with Wilms tumor [MIM 194070]) and human 15q11-q13/central mouse chromosome 7 (associated with PWS and AS). Taken together, these studies indicate that mechanisms for parent-of-origin–specific gene expression are likely to vary from gene to gene, since the cell exploits cis-acting sequences and transcription factors already involved in determining the cell type–specific patterns of expression. Thus, dissection of imprinting pathways contributes to a general understanding of mechanisms for controlling the expression of nonimprinted genes in the mammalian cell.

Allele-Specific Expression: A Developmental Process

Imprinting can be considered a multistep developmental process. First, the chromosome must be marked as to its parental origin. Presumably, this occurs either during gametogenesis or in the zygote, prior to fusion of the two gametes, while the maternal and paternal chromosomes are still physically separate. Second, a parent-of-origin mark must be stably maintained as the cells divide and differentiate. The imprint or mark might remain identical to the original mark on the gametic chromosomes or may be a secondary derivative of that mark. Third, a parent-of-origin mark must be recognized by the transcriptional machinery, so as to result in monoallelic expression. Finally, and specific to germ cells, the mark must be erased and reset. A failure at any of these steps would result in a loss of imprinting (LOI) mutation. As described below, there is evidence for mutations in each of these steps in animal and human disease models.

Establishing the Mark: Imprinting and DNA Methylation

CpG methylation has received great attention as an excellent candidate for the genomic-imprinting mark, on the basis of two very useful properties of the DNA methyltransferase 1 (DNMT1) enzyme. First, DNMT1 has been demonstrated to associate with DNA-replication forks. Second, it shows a strong substrate preference for hemimethylated DNA. Given the semiconservative replication of DNA, these two features of the enzyme indicate that DNA, once methylated, will tend to stay methylated, thus providing a mechanism for the stable maintenance of an imprint during cell division and differentiation (Bestor and Verdine 1994).

Using CpG-sensitive restriction enzymes or bisulfite sequencing, researchers in many labs have identified parent-of-origin–specific differences in CpG methylation in almost all imprinted genes that have been examined. However, only for three genes—*H19* (MIM 103280), *IGF2R* (MIM 147280), and *Snrpn* (MIM 182279)—does this methylation fit the strictest criteria of a genomic imprint—that is, the differences are present in gametes and are maintained throughout development (Stoger et al. 1993; Tremblay et al. 1995; Shemer et al. 1997). Furthermore, the functional significance of the methylated sequences for each of these genes is supported by mutational analyses that demonstrate the essential role of these elements in imprinted expression of linked genes (Wutz et al. 1997; Thorvaldsen et al. 1998; Yang et al. 1998).

A role for methylation is further supported by the demonstration that mice deficient in *Dnmt1*-gene function show a loss of imprinting at almost all loci tested (Li et al. 1993; Shemer et al. 1997; Caspary et al. 1998). The exact phenotype is gene specific, consistent with the association of methylation with both silent and expressed loci; for example, the *H19* gene, which is normally methylated on the silent paternal allele, becomes biallelically expressed after loss of *Dnmt1* function. In contrast, methylation of *Igf2R* and *Igf2* is normally on the active allele, and *Dnmt1*-deficient mice fail to express these genes from either chromosome. These experiments do not distinguish between a role for *Dnmt1* function in establishing the gametic imprint, maintaining the mark in somatic cells, or altering gene expression in response to the real gametic imprint.

Monoallelic expression of at least one gene, *Mash2,* proceeds even in the absence of *Dnmt1* activity (Caspary et al. 1998; Tanaka et al. 1999). This gene is part of the human 11p15.5/mouse distal 7 cluster (fig. 1*a*), where monoallelic expression of the *H19, Igf2, Kvlqt1,* and *P57Kip2* genes is dependent on methylation (Li et al. 1993; Caspary et al. 1998). Perhaps these results mean only that CpG sequences crucial for imprinting of *Mash2* are less sensitive to the loss of *Dnmt1* function, given compensation by other methyltransferases still present in the mouse. However, organisms that lack methyltransferase enzyme activity can still maintain stable states of gene activation and repression, including parent-of-origin–specific effects (e.g., see Dalgaard and Klar 1999; Wolffe and Matzke 1999). Thus, DNA methylation is not necessarily crucial in genomic imprinting.

Molecular bases by which methylation can alter transcription patterns are becoming increasingly clear (Ng and Bird 1999). Methylation can block expression directly, by interfering with the binding of transcriptional

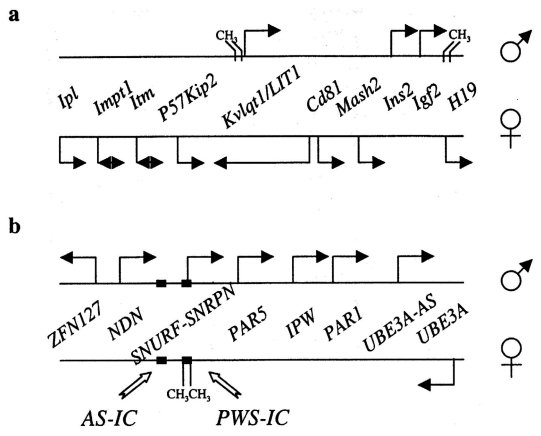

Figure 1 Clusters of imprinted genes on mouse distal 7 (syntenic with human 11p15.5) (*a*) and human 15q11-q13 (syntenic with mouse central 7) (*b*). Transcripts are depicted by arrows, with the direction indicated if known. Regions showing parent-of-origin–dependent DNA methylation are shown as CH_3. The thickened segments of the lines represent the *AS-IC* and *PWS-IC* elements, as indicated. These sites are required in *cis* for normal imprinting on human 15q11-13.

activator complexes, or it may act indirectly, by recruiting the factors that induce repressive chromatin structures. Methylation is also associated with active alleles, at several loci. In this case, the methylation is expected to interfere with recruitment of a transcriptional repressor. The exact role for methylation in monoallelic expression both in setting the genomic imprint and in altering the patterns of gene expression is best characterized for the mouse *H19/Igf2* locus.

Multiple Roles for Methylation: Imprinting at the Mouse *H19/Igf2* Locus

Understanding the molecular basis for the paternal specific expression of *Igf2* is of long-standing interest in the field of imprinting. Mouse *Igf2* was the first endogenous gene whose parent-of-origin–specific expression was recognized. In addition, overexpression of *IGF2* has been a favored mechanism for the etiology of both BWS and Wilms tumor. Overexpression of *Igf2* can occur through paternal UPD, through rearrangements on the maternal chromosome, or through LOI mutations, in which expression of the normally silent maternal *Igf2* allele is noted (Ping et al. 1989; Weksberg et al. 1993*a;* Elliot and Maher 1994; Joyce et al. 1997).

Igf2 is part of a cluster of imprinted genes whose organization is well conserved in mice and humans (fig. 1*a*). *Igf2* and its neighbor, the maternal-specific *H19* gene, are coregulated. They share enhancers, at least for expression in several endodermal tissues (Leighton et al. 1995*b*) and in skeletal muscle (Kaffer et al. 2000). These enhancers all lie 3′ of the *H19* gene and, thus, downstream of the *Igf2* and the *H19* promoters (fig. 2*a*).

Likewise, the reciprocal imprinting of the *Igf2/H19* gene pair is also mechanistically linked. LOI mutations associated with Wilms tumor generally show biallelic expression of *IGF2,* coupled with loss of expression of *H19* from the maternal chromosome (Steenman et al. 1994; Reik et al. 1995; Catchpoole et al. 1997). In mice, a deletion of the *H19* gene and 10 kb of upstream sequence that leaves the shared enhancers intact causes biallelic expression of *Igf2* (Leighton et al. 1995*a;* fig. 2*b*). Together, these results suggest that silencing of paternal *Igf2* might ultimately be dependent on the activity of the *H19* gene, and, thus, attention has been focused on mechanisms of imprinting at that locus.

Genetic analysis of imprinting of the *H19* gene itself has been greatly facilitated by the finding that relatively small transgenes carrying the *H19* gene, its promoter, its enhancer elements, and several kilobases of upstream sequences can mimic the maternal-specific expression of the endogenous locus (Bartolomei et al. 1993). Deletion analyses of these transgenes demonstrated that promoter and enhancer sequences required for tissue-specific and temporally appropriate expression of *H19* are not sufficient for making the transgene maternal-chromosome specific. Rather, sequences encompassing at least approximately 2 to 4/7 kb upstream of the *H19* promoter are necessary to mark the *H19* transgene as paternal in origin (Pfeifer et al. 1996; Elson and Bartolomei 1997; Ainscough et al. 1998; Kaffer et al. 2000) and to induce maternal-specific expression of the transgene.

Experiments to define differences in the paternal and maternal chromosomes underline the importance of these upstream sequences. These sequences are methylated in sperm but not in oocytes, and the paternal chromosome remains differentially hypermethylated throughout embryogenesis (Tremblay et al. 1995). Thus, this region, *H19DMR* (differentially methylated region), appears to carry a methylation imprint. During development, the hypermethylation on the paternal allele expands from *H19DMR* to include the *H19* promoter and the gene body (fig. 2*a*). More recently, several labs have independently identified nuclease-hypersensitive sites in *H19DMR* that are specific to the maternal chromosome (Hark and Tilghman 1998; Szabo et al. 1998; Khosla et al. 1999).

The maternal *H19* promoter also becomes nuclease hypersensitive, consistent with the binding of transcription factors and activation of expression of the maternal *H19* allele. (Bartolomei et al. 1993; Ferguson-Smith et al. 1993). Strikingly, no allelic differences in nuclease hypersensitivity at the *Igf2* promoter have been reported, which suggests that both the maternal and paternal *Igf2* promoters might be equally ready for acti-

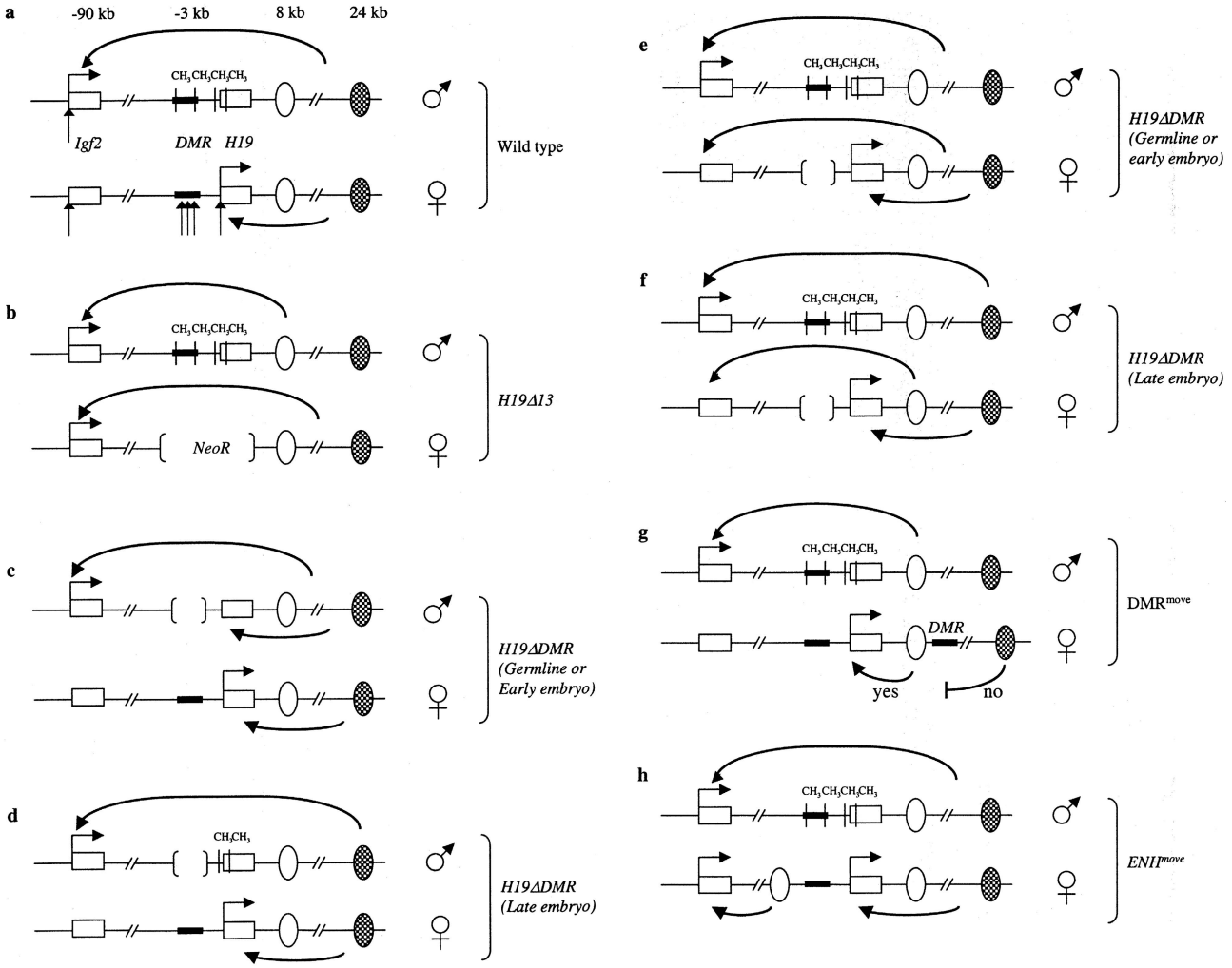

Figure 2 Effect that mutations in the *H19DMR* region have on expression of *H19* and *Igf2*. *a,* Wild-type expression. Most wild-type cells express only the maternal *H19* allele and only the paternal *Igf2* allele. Expression of both genes is driven by shared enhancer elements. Endodermal enhancers (*unblackened ovals*) and skeletal muscle enhancers (*blackened ovals*) are ~8 and ~24 kb upstream of the *H19* promoter, which is ~90 kb upstream of the *Igf2* promoter. Paternal chromosome–specific CpG methylation is noted in upstream sequences called "*H19DMR*" (*thickened segments of lines*). Differential methylation of this region is observed in sperm and is maintained during the global methylation changes observed during early embryogenesis. After implantation, the hypermethylation (CH_3) spreads to include the *H19* promoter and gene body. The maternal chromosome shows hypersensitivity to nuclease digestion (*vertical arrows*) in the *DMR* region and at the *H19* promoter, whereas the *Igf2* promoter appears to be equally sensitive to digestion on both chromosomes. *b,* Mechanistically linked imprinting of *H19* and *Igf2*. The *H19D13* allele replaces the *H19* gene, including its promoter and *H19DMR,* with the *NeoR* gene. The shared endodermal and mesodermal enhancers are unaffected by this mutation, which, on maternal inheritance, results in biallelic expression of *Igf2*. *c, H19DMR,* necessary to silence paternal expression of *H19*. *H19DDMR* removes sequences that are differentially methylated on the paternal chromosome. When inherited through the paternal germline or removed from the paternal chromosome early during embryogenesis, *H19* expression becomes biallelic. *d,* Silencing of the paternal *H19* allele, mediated by epigenetic changes driven by *H19DMR* but not directly dependent on DMR function. When DMR sequences are removed from the paternal chromosome only late during embryogenesis, *H19* expression remains monoallelic. *e, H19DMR,* necessary to silence expression of *Igf2* from the maternal allele. When inherited through the maternal germline or removed from the maternal chromosome early during embryogenesis, *Igf2* expression becomes biallelic. *f,* Silencing of the maternal *Igf2* allele, directly dependent on the action of *H19DMR*. Even when the DMR is removed from the maternal chromosome only late during embryogenesis, *Igf2* expression becomes biallelic. *g, H19DMR* and transcriptional insulator function. In DMR^{move}, *H19DMR* was inserted between the *H19/Igf2* endodermal and mesodermal enhancers. After maternal inheritance of the DMR^{move} chromosome, *H19* expression in skeletal muscle, but not in the liver, was lost. *h,* Biallelic expression of *Igf2,* allowed by moving of the endodermal enhancer elements closer to *Igf2,* where they are no longer separated from the *Igf2* promoter by the DMR. "Early embryo" (*c* and *e*) indicates that deletions were generated in preimplantation embryos. "Late embryo" (*d* and *f*) describes deletions generated in differentiated muscle cells.

vation but that some other factor prevents enhancement on the maternal chromosome (Sasaki et al. 1992).

Mice inheriting a deletion of the *H19DMR* through the father show loss of imprinting at *H19*—that is, these mice are biallelic for expression of *H19* (Thorvaldsen et al. 1998; fig. 2*c*). Thus, *H19DMR* acts to silence the paternal *H19* promoter. To address the mechanism of silencing, *H19DMR* was flanked with Cre *lox* sites and was deleted in a temporally regulated manner, by use of cell type–specific Cre recombinase transgenes (Srivastava et al. 2000). It was therefore possible to generate mice that had inherited a wild-type paternal chromosome in which *H19DMR* was deleted either in early embryos or in differentiated cells (fig. 2*c* and *d*). When *H19DMR* was deleted from the paternal chromosome in differentiated cells, the paternal *H19* allele remained silent. However, when a wild-type chromosome is inherited through the sperm but *H19DMR* is deleted early during embryogenesis, repression of the paternal allele is lost. Thus, *H19DMR*'s role in monoallelic expression is temporary, and its silencing activity is not required for direct interaction with the transcriptional machinery. Bisulfite-sequencing experiments demonstrate that *H19DMR* is required during early embryogenesis, to direct methylation of the *H19* promoter. These changes are then stably maintained independent of *DMR* and probably directly repress the paternal allele. These results are consistent with earlier experiments, which showed that the repression of the paternal *H19* is actually a process that proceeds as the embryo develops (Jinno et al. 1995; Szabo and Mann 1995; Svensson et al. 1998).

In sum, genetic experiments demonstrate that *H19DMR* is necessary to maintain the *H19* imprint during early development and to convert the imprint into a signal that actually represses the transcriptional machinery. Molecular studies support the notion that *DMR* carries the actual imprint, but genetic studies to date have not been able to address this issue.

H19DMR is also required for monoallelic expression of *Igf2* (Thorvaldsen et al. 1998; fig. 2*e*). Its deletion on the maternal chromosome results in inappropriate activation of the maternal *Igf2* allele. Thus, *H19DMR* is also a silencer for *Igf2*. Strikingly, the mechanism for silencing of *Igf2* is distinct from the mechanism used to silence paternal *H19* (Srivastava et al. 2000; fig. 2*e–f*). Deletion of *DMR* in a cell type–specific manner demonstrates that its presence is continually necessary to silence the maternal *Igf2* promoter, indicating that maternal *H19DMR* and/or bound proteins interact directly with the transcriptional machinery, to block expression of maternal *Igf2*. One model to explain *H19DMR*'s action in suppressing maternal *Igf2* expression posits that it functions as a transcriptional insulator. On the maternal chromosome, the insulator prevents activation of the *Igf2* promoter by the distal enhancers. On the paternal chromosome, the methylation imprint inactivates the insulator, allowing activation of the paternal *Igf2* allele.

The hallmark of a transcriptional insulator is its ability to prevent activation of a promoter, in a strictly position-dependent manner. An insulator prevents transcription only when juxtaposed between a promoter and the enhancers on which that promoter depends for its activation. The ability of *H19DMR* sequences to act as a transcriptional insulator has been demonstrated in vivo by use of transgenes inserted at heterologous positions in the chromosome (Hark et al. 2000) and by manipulation of the *H19/Igf2* locus itself (Kaffer et al. 2000; fig. 2*g*). In the latter experiment, *DMR* was removed to a position downstream of the *H19* gene, between the endodermal and skeletal muscle enhancer elements. This construct, DMR^{move}, thus places the putative insulator between the *H19* promoter and the muscle enhancers and thereby mimics the topology of the *Igf2* locus (fig. 2*g*). With maternal inheritance, activation of *H19* in skeletal muscle but not in liver was blocked, consistent with the presence of insulator activity on the *H19DMR* insert. With paternal inheritance, the relocated *H19DMR* is methylated, and its insulator function abrogated (C. R. Kaffer and K. Pfeifer, unpublished observations).

The ability of *H19DMR* sequences to act as insulators has also been demonstrated in vitro by use of integrated (Bell and Felsenfeld 2000; Hark et al. 2000; Kaffer et al. 2000) and episomal (Kanduri et al. 2000) minigene constructs. The ability of transfected DNA constructs to mimic expression patterns and chromatin conformations of the maternal locus is good evidence that imprinting at *H19/Igf2* is solely a paternal marking of the chromosome.

The molecular basis for paternal-specific inactivation of the insulator is provided by biochemical analyses that show that binding of the CCCTC-binding factor (CTCF) protein to DMR sequences is inhibited by CpG methylation of the protein's recognition sites (Bell and Felsenfeld 2000; Hark et al. 2000). CTCF is a transcription factor that has been demonstrated elsewhere to play a role in insulator activity at the chicken β-globin locus (Bell et al. 1999). That CTCF actually plays a role in the cell is strongly supported by in vivo footprinting results that demonstrate maternal chromosome–specific CTCF (Szabo et al. 2000).

Together, these results present a conceptually simple model for imprinting at *H19/Igf2*. The maternal chromosome represents the default or unimprinted state. The *H19* promoter is active, as is a transcriptional insulator that prevents activation of the *Igf2* promoter by the shared enhancer elements. The paternal chromosome is marked or imprinted at *DMR*, and this imprint

has two functions. First, it directs further epigenetic modification of the *H19* promoter, which blocks its activity. Second, the methylation prevents binding of CTCF protein and thus prevents activation of the insulator element, thereby permitting enhancer-mediated activation of the *Igf2* promoter. Experiments that introduced chromosome 11 into mouse cells demonstrated that these mechanisms for transcriptional regulation are likely to be conserved in humans (Gabriel et al. 1998*b*).

LOI Mutations at Human *IGF2*

Biallelic expression of *IGF2* without deletion or rearrangement on either chromosome is frequently associated with BWS and with Wilms tumor. In some cases, this LOI at *IGF2* is associated with normal, maternal-specific expression of *H19* (Brown et al. 1996; Joyce et al. 1997). In other cases, LOI at *IGF2* is associated with inappropriate silencing and hypermethylation of both *H19* alleles (Steenman et al. 1994; Reik et al. 1995; Catchpoole et al. 1997). How can these LOI phenotypes be viewed, in light of the mouse studies just described?

The first LOI phenotype—biallelic *IGF2*/normal *H19* expression—does present in the mouse, in which the maternal chromosome is inherited in its wild-type form but in which an *H19DMR* deletion occurs late during embryonic development (fig. 2*f*). Thus, by analogy with the mouse, these patients with LOI may be better described as having loss of insulation rather than imprinting. The interesting issue is the molecular basis for loss of insulation in these patients. In mice, this phenotype is caused by a deletion of the insulator element. In most patients, no chromosomal abnormalities or genetic alterations are observed. Intriguingly, however, LOI can be associated with chromosomal inversion/deletions in the *BWSCR1* region, which spans the *KVLQT1* (*KCNQ1*) gene, several hundred kilobases upstream of *IGF2* (Mannens et al. 1994; Hoovers et al. 1995; fig. 1*a*).

Mouse studies predict that the maternal *Igf2* promoter is ready for activation but is silent because of the lack of activation by enhancer elements. If this is also the case for human *IGF2,* there are only two formal explanations for the ability of a mutation to permit activation of the maternal *IGF2*. In the first case, insulator function of *H19DMR* is unaffected, and the normal *IGF2* enhancers remain unable to activate the maternal *IGF2* promoter. Then the effect of BWS mutations must be to somehow "create" a new enhancer element for *IGF2*. To explain molecular data, this new enhancer would need to activate *IGF2* in the appropriate tissues and at levels like that of the endogenous enhancer. Such a mutation was, in fact, engineered in the mouse by removal of the normal endodermal enhancers to a position midway between *H19* and *Igf2* and, thus, on the proximal side of the *Igf2* promoter, relative to the *H19DMR* insulator (Webber et al. 1998; fig. 2*h*). The alternative—and, I think, more likely—scenario is that the *H19DMR* insulator function is abolished in these patients with LOI. This loss of insulation then allows expression of maternal *IGF2* to be driven by the normal enhancers. In this scenario, LOI occurs via an epigenetic or genetic alteration in sequences required to act with the *H19DMR* to organize the maternal *IGF2* into a transcriptionally silent domain. Perhaps the organization of the locus into domains of expressed and unexpressed genes uses paired insulator/boundary domains like the scs/scs′ system described in *Drosophila* (Udvardy 1985; Kellum and Schedl 1991, 1992). Further directed mutagenesis in mice, mimicking the translocation chromosomes of patients with BWS, will help elucidate the true nature of these mutations.

The second type of LOI mutation in BWS shows biallelic *IGF2* associated with loss of expression of the maternal *H19* gene (Brown et al. 1996; Joyce et al. 1997). By analogy with the mouse, this phenotype would be more usefully described as gain of imprinting, because the patient has, in effect, two imprinted chromosomes. No such phenotype is observed in mice, and it is hard to imagine how to generate such a phenotype via genetic alterations. Bestor and Tycko (1996) have described an epigenetic mechanism that could create de novo methylation during DNA synthesis. When imprinted domains pair during mitosis (LaSalle and LaLande 1996), strand exchange that occurs infrequently between methylated and nonmethylated sequences will present hemimethylated sites to the DNMT1 enzyme. DNMT1 maintenance activity thus is used by the organism to create de novo methylation, given an already methylated template. Such a phenomenon has been described in the fungus *Ascobulus* (Colot et al. 1996).

A Second Imprint on 11p15.5?

One clear finding of both mouse and human studies is that imprinting at the *H19/IGF2* locus can be mechanistically separated from imprinting of other genes in the cluster. First, the effect of deletion of the *H19* gene and *H19DMR* is restricted to the *H19, Igf2,* and *Ins2* loci; the deletion has no effect on imprinting of *Mash2, Kvlqt1,* or *p57Kip2* (Leighton et al. 1995*a;* Caspary et al. 1998). Second, the effects of LOI at *IGF2* are, in most cases, restricted to that gene and to *H19* (Reik et al. 1995). Finally, genes in the distal 7 cluster are differentially affected by *Dnmt1* loss-of-function mutations. Together, these findings suggest that there are probably additional cis-acting imprinting centers (ICs) at human 11p15.5/mouse distal 7. The disruption of the

KVLQT1 locus in multiple cases of BWS suggests that *KVLQT1* may harbor these elements (Lee et al. 1997).

Two labs have identified a region near *KVLQT1* exon 10, *KvDMR,* that is hypermethylated specifically on the maternal chromosome (Lee et al. 1999; Mitsuya et al. 1999; Smilinich et al. 1999). Preliminary evidence supports the notion that this methylation is a true imprint, in that it appears to be oocyte specific and not acquired developmentally in response to allele-specific transcription. The functional significance of *KvDMR* is not yet clear. LOI at the site is associated with a number of cases of BWS, including cases that also show LOI at *IGF2* but, more generally, cases that are normal for *IGF2* function. Lee et al. (1999) speculate that *KvDMR* might be a regulatory site working analogously to the *H19DMR,* whose methylation permits expression of the downstream *KVLQT1* and *p57KIP2* promoters.

If independent mechanisms determine imprinting for discrete clusters of genes within the 11p15.5/distal 7 supercluster, it remains puzzling why the clusters are then grouped in the first place. One explanation is that the region contains sequences that make the whole region permissive for imprinting but that individual IC centers are further necessary to implement the imprint. This description does not account for the ability of relatively small *H19* transgenes to direct their own imprinting at a wide range of chromosomal integration sites. Imprinting of transgenes has not been demonstrated for other genes in the cluster.

Imprinting at a Distance: 15q11-q13

A second well-characterized cluster of imprinted genes is located on human chromosome 15q11-q13 (central mouse 7) (fig. 1*b*). PWS and AS are two clinically distinct neurobehavioral disorders that are each most commonly caused by an identical 4-Mbp deletion of this region. However, the deletion is always paternal in origin for PWS but maternal in origin for AS (for reviews, see Jiang et al. 1998; Nicholls et al. 1998). The maternally expressed *UBE3A* gene maps toward the telomeric end of the deletion, and loss of *UBE3A* is likely to explain AS (Nakao et al. 1994; Rougeulle et al. 1997; Vu and Hoffman 1997). At least six paternal-specific transcripts—*ZNF127, NDN, SNURF/SNRPN, PAR5, IPW,* and *PAR1*—have been identified, and all map to the centromeric end of the deletion (Ozcelik et al. 1992; Sutcliffe et al. 1994; Wevrick et al. 1994; MacDonald and Wevrick 1997; Sutcliffe et al. 1997; Gray et al. 1999; Jong et al. 1999; fig. 1*b*). More recently, eight novel imprinted transcripts have been mapped to the locus, but their regulation by the IC mutations (see below) has not yet been tested (Lee and Wevrick 2000). The mapped imprinted genes span >2 Mb. A region in the middle of the cluster that encompasses the *SNURF/SNRPN* promoter is hypermethylated on the maternal chromosome at all stages of development and is therefore a good candidate for a gametic imprint (Sutcliffe et al. 1994; Glenn et al. 1996). This imprint is conserved in the mouse (Shemer et al. 1997; Gabriel et al. 1998*a*).

One genetically interesting class of patients with PWS have biparental inheritance of chromosome 15, but both chromosomes behave maternally—that is, are hypermethylated at the *SNURF/SNRPN* locus and express only *UBE3A* (Reis et al. 1994; Buiting et al. 1995; Saitoh et al. 1996). In rare patients with PWS who have microdeletions causing this imprinting defect, the PWS imprinting center (*PWS-IC*) has been mapped to a 4.3-kbp region spanning the *SNURF/SNRPN* promoter and coincident with the methylation imprint (Ohta et al. 1999*a;* fig. 1*b*). These *PWS-IC* mutations can be transmitted silently over multiple generations, with the phenotype apparent only when the imprint needs to be reset from maternal to paternal. Thus, it has been suggested that, at this locus in humans and in mice, *PWS-IC* controls switching in the male germline (Yang et al. 1998). However, recent experiments demonstrate that *PWS-IC,* in both humans and mice, is required for maintainance of the paternal identity of the chromosome during early embryogenesis (Bielinska et al. 2000).

At a superficial level, *PWS-IC* thus resembles *H19DMR*. Methylation of either element results in silencing of linked genes—either *SNURF/SNRPN* and other paternal genes at 15q11-q13 or *H19* at 11p15.5. In both cases, they are certainly required during early embryogenesis, and probably also in germ cells, to mark the silent chromosome. Of course, *PWS-IC* is able to silence transcription of genes that are orders of magnitude distant, relative to the effects of *H19DMR*. The mechanism by which the silent state is transmitted over such long distances is not understood. The overlap of the IC with *SNURF/SNRPN* is intriguing, and it is not clear whether this is coincidence or whether expression of that promoter and/or the RNA product itself are somehow required to keep the paternal chromosome transcriptionally active. However, at least some insertional mutations at the *SNURF/SNRPN* locus do not disrupt imprinting (Yang et al. 1998; Tsai et al. 1999).

The ability of the imprint to activate maternal *UBE3A* is perhaps explained by the recent discovery of a paternally expressed antisense transcript overlapping *UBE3A* but proximal to it, relative to *PWS-IC* (Rougeulle et al. 1998; fig. 1*b*). The authors who reported this discovery proposed that *UBE3A* imprinting might be the indirect effect of paternal-specific expression of the antisense transcript. In keeping with this hypothesis, *UBE3A* is biallelic in all tissues in which the antisense tissue is not expressed, but it is monoallelic in the brain, where the antisense transcript is expressed.

The previous discussion of imprinting at 15q11-q13

is oversimplified, however, because it has not accounted for the presence of an *AS-IC* in addition to *PWS-IC*. This 1.15-kb element is ~40 kb upstream of the *SNURF/SNRPN* promoter (fig. 1*b*) (Ohta et al. 1999*b*) and is defined by the microdeletions that cause maternally inherited chromosomes to show a paternal phenotype—that is, hypomethylation at the SNRPN promoter, expression of paternal transcripts, and loss of maternal-specific transcription of *UBE3A*. This situation appears to be very different from the *H19/Igf2* system, in which it is presumed that the chromosome is imprinted in only one germline and passes through the other unmarked.

Several models describe mechanisms for gamete-specific imprinting events at 15q11-q13 (Buiting et al. 1995; Dittrich et al. 1996; Burger et al. 1997; Ohta et al. 1999*a*). One unifying model proposes that the role of both IC elements is to regulate expression of paternal-specific genes (Brannan and Bartolomei 1999). *PWS-IC* is a cis-regulatory element required for activation of the paternal expression pattern, possibly via its role in initiating or maintaining transcription of *SNURF/SNRPN*. Thus, loss of *PWS-IC* prevents activation of the paternal program. In the female germline, the imprint, which is dependent on some transcript or sequence from *AS-IC,* is established at the *SNURF/SNRPN* promoter. This imprint prevents *SNURF/SNRPN* transcription and, thereby, halts the rest of the paternal program. As predicted by this model, deletion of *PWS-IC* is epistatic to deletion of *AS-IC*.

Summary

Although imprinting remains largely mysterious, recent experiments have made considerable progress in elucidating some of the mechanisms for monoallelic expression of imprinted genes. These results indicate that, although DNA methylation plays a crucial role, its direct effect on transcription will vary from locus to locus. At the *H19/Igf2* locus, methylation represses transcription of the paternal *H19* allele by directly blocking the activation of the *H19* promoter but activates expression of *Igf2* by its simultaneous inactivation of a transcriptional insulator. On human 15q11-q13, methylation appears to directly block activation of the maternal *SNURF/SNRPN* promoter, perhaps analogous to its effect on paternal *H19*. However, it activates *UBE3A* expression, possibly indirectly, by blocking expression of an antisense RNA. These examples of imprinting effects on promoter activation, insulator function, and long-range chromatin structure suggest that imprinting has evolved in the mammal by using conventional mechanisms of transcriptional regulation. Thus, through clever engineering, the cell is able to use methylation imprints as both positive and negative signals. Dissecting monoallelic expression pathways will therefore contribute toward an understanding of normal gene regulation and of the molecular basis for diseases associated with disregulation at imprinted loci.

Electronic-Database Information

The URL and accession numbers for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for BWS [MIM 130650], PWS [MIM 176270], AS [MIM 105830], Russell Silver syndrome [180860], Albright hereditary osteodystrophy [MIM 103580], Wilms tumor [MIM 194070], *Igf2* [MIM 147470], *H19* [MIM 103280], *Snrpn* [MIM 182279], and Igf2R [MIM 147280])

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