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Genomic Imprinting: Significance in Development and Diseases and the Molecular Mechanisms

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Certain mammalian genes are expressed exclusively from either the paternal or the maternal chromosome because of a differential marking process that occurs during gametogenesis. This epigenetic marking is called genomic imprinting. Monoallelic expression of imprinted genes is responsible for the inability of uniparental mammalian embryos to develop normally and for the abnormal phenotypes observed with particular chromosomal disomies. Many of the imprinted genes identified to date are involved in the regulation of cell proliferation and differentiation and, together with other pieces of evidence, they are suggested to play a role in tumorigenesis. Here we discuss how imprinted genes cause diseases and tumors and summarize the recent advances of studies on the molecular basis of this epigenetic phenomenon. In particular, we focus on two well-characterized imprinted chromosomal regions, namely the human Prader-Willi/Angelman syndrome region and the mouse *INS2/IGF2/H19* region. The correlations between the differential gene activity and the changes in DNA methylation, higher order chromatin structure and replication timing, will shed light on gene regulation at the level of the chromosomal domain.

Key words: chromosomal domain, DNA methylation, loss of imprinting, replication timing, uniparental disomy.

Genomic imprinting or parental imprinting describes a biological marking process that modifies the genome differently in the male and female germline, leading to differential activity of the parental genomes in the offspring. This definition of imprinting could sound peculiar because, in a sense, it violates the Mendelian view of gene action that both parental genes of a diploid organism contribute to the phenotypes equally. Another important feature of this phenomenon is that the modifications, or imprints, can be reversed and re-established in each generation: thus imprinting is epigenetic. As expected from these features, mutations within imprinted genes can behave like non-Mendelian traits as described below. Despite these unusual characteristics, imprinting seems widespread among species: imprinting or related phenomena have been documented in plants, insects, and mammals. In this review, we specifically focus on imprinting in mammals, and discuss how imprinted genes affect embryonic development and cause genetic diseases and tumors. Our present understanding of the molecular basis of this epigenetic phenomenon is also summarized in view of the long-range regulation of chromosomal domains. Further or related information can be obtained from other recent reviews (*1-5*).

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Abbreviations: AS, Angelman syndrome; BWS, Beckwith-Wiedemann syndrome; LOH, loss of heterozygosity; LOI, loss of imprinting; PWS, Prader-Willi syndrome; UPD, uniparental disomy.

Mouse embryology and genetics

The first indication that the paternal and the maternal mammalian genomes are functionally non-equivalent comes from the observations made in mouse embryology. Experimental manipulation of one-cell embryos showed that the presence of both a paternal and a maternal genome is essential for normal development (*6-8*). When gynogenetic embryos, which carry two female pronuclei, are produced by nuclear transfer, the reconstituted embryos themselves develop relatively well but the trophoblastic tissues develop only poorly. Diploid parthenogenetic embryos, which have been activated without fertilization, are phenotypically very similar to gynogenetic embryos. Opposing effects are seen with androgenetic embryos, which contain two paternal genomes without a maternal complement: poorly developed embryos and large placentae. None of these uniparental embryos can develop to term. Secondly, non-equivalence of the parental autosomes was explored by genetic studies utilizing chromosomal translocations. Cattanach and colleagues systematically produced a series of uniparental disomies (UPDs), which have two copies of a particular autosomal region from one parent but none from the other, and observed various abnormalities including embryonic and neonatal lethality, growth disturbance, and behavioral disorder (*9, 10*). The study identified a total of eight autosomal regions which exhibit differential effects depending on their parental origin (*11*) (Fig. 1). Thirdly, the X chromosome also retains a parental legacy since the paternal X is preferentially inactivated in the extraembryonic membranes of female

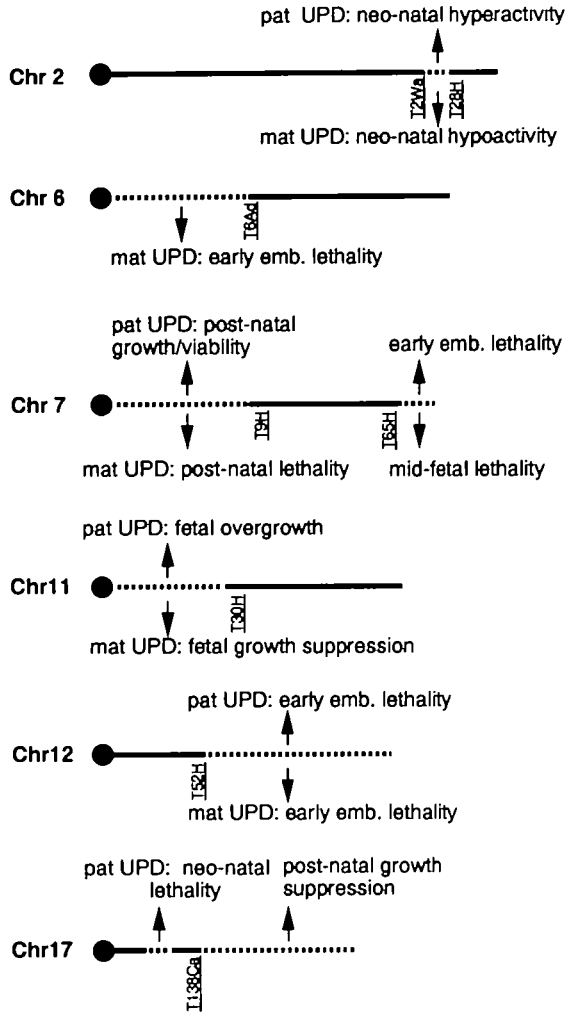

Fig. 1. **Imprinted mouse chromosomal regions identified by genetic tests.** Established imprinted regions are shown by interrupted lines and the phenotype of each UPD is indicated. Translocations used for the tests are indicated at the positions of their breakpoints and underlined. emb., embryo. Adapted from Ref. *11* by permission of B.M. Cattanach.

mice (*12*). Lastly, a mutation called *hairpin-tail* shows an unusual transmission: the effects are more severe upon maternal transmission than upon paternal transmission (*13*). These four lines of evidence collectively suggested that some mouse genes are expressed differently on the paternal and the maternal chromosomes.

Imprinted genes

Since the first identification of the mouse genes coding for insulin-like growth factor 2 (*IGF2*) and its receptor (*IGF2R*) as imprinted genes in 1991, the number of imprinted genes has been ever increasing. (Human gene symbols are used throughout this review.) To date, 17 imprinted genes have been reported: 5 are maternally expressed and 12 paternally expressed (Table I). Estimation of the total number of imprinted genes is difficult at present, but it may be between 100 to 200 (*3*). Although our knowledge on imprinted genes is still limited, below we summarize the characteristics of these genes in terms of their expression pattern, chromosomal location, and biological function.

First, although all imprinted genes are characterized by allele-specific expression, the imprinted expression is subject to complex regulation (*2, 3, 5*). For example, *IGF2, H19*, insulin 1 & 2 (*INS 1 & 2*) and Wilms' tumor 1 (*WT1*) are imprinted in tissue-specific ways. In the case of *IGF2*, imprinted expression is seen in many embryonic tissues, but the gene is expressed biallelically in the choroid plexus and leptomeninges. Imprinting of human *IGF2* is also changed during liver development: monoallelic expression in the fetus switches to biallelic expression after birth. In the cases of *IGF2R, INS2*, and *SP2/U2af* binding protein related sequence 1 (*U2afbp-rs1*), imprinting is observed in mouse, but not in human, indicating that imprinting is not necessarily conserved through evolution. Furthermore, human *IGF2R* and *WT1* have been reported to be imprinted in only a minority of individuals, suggesting "polymorphic imprinting" (*14, 15*).

Second, imprinted genes appear to be clustered in specific regions of the genome. More than half of the imprinted genes listed in Table I lie in two regions on mouse chromosome 7, the *INS/IGF2/H19* and the PWS/AS (see below)-homologous regions, which are syntenic to regions on human chromosome 11 and 15, respectively. The *IGF2R/MAS* region appears to constitute a third imprinted domain and lies on mouse chromosome 17 and human chromosome 6. The clustering of imprinted genes and the presence of oppositely imprinted genes within the same domain suggest that a common regulatory element within each cluster might control the differential transcription through unique mechanisms (*5, 16-18*) (see below).

Finally, many imprinted genes are likely to play an important role in the regulation of embryonic growth and development. The imprinted genes identified to date can be classified into five major categories according to their biological functions: growth factors and their receptors, transcription factors, splicing factors, cell cycle regulators, and RNAs that do not appear to be translated into proteins. Recent gene-knockout studies demonstrated that a deficiency of *IGF2, IGF2R, H19*, or *MASH2* causes defects in embryonic or placental growth (*3*). Also, a deficiency of *WT1* resulted in embryonic lethality at midgestation. In general, paternally expressed genes tend to promote cell growth while maternally expressed genes have opposite effects, and this may be related to the reasons for the evolution of imprinting in mammals (*19*). However, there may be some exceptions to this rule, such as the case for *MASH2*, which is essential for placental development but expressed from the maternal allele.

Genetic diseases

Evidence for imprinting in human was originally derived from the studies on some chromosome deletion syndromes which are caused by preferential loss of the relevant region of a specific parental origin. A number of diseases are now suspected to be caused by lesions that affect imprinted genes, or at least are related to imprinting phenomenon, as listed in Table IIA (*1, 2*). These include paternal and maternal UPDs, differential mutability between parental chromosomes and expansion of unstable triplet repeats upon transmission through a specific sex. In familial or inherited diseases, phenotypes may be transmitted in an autosomal dominant mode, since one of the alleles is

TABLE I. **Imprinted genes and chromosomal regions in mammals.**

Gene[a]	Imprinting[b]		Expressed allele[c]	Chromosomal location[d]		Function
	Human	Mouse		Human	Mouse	
IGF2	+	+	pat	11p	7	growth factor
INS1 & 2	−	+	pat	11p	7	insulin
H19	+	+	mat	11p	7	RNA, growth suppressor
p57^{KIP2}	nd	+	mat	11p	7	cell cycle regulator
MASH2	nd	+	mat	11p	7	transcriptional factor
WT1	+	nd	mat	11p	2	zinc finger, tumor suppressor
SNRPN	+	+	pat	15q	7	splicing factor
ZNF127	+	+	pat	15q	7	zinc finger protein
PAR5 & PAR1	+	nd	pat	15q	nd	RNA
IPW	+	nd	pat	15q	nd	RNA
PEG3	nd	+	pat	19q	7	zinc finger protein
IGF2R	−	+	mat	6q	17	receptor
MAS	nd	+	pat	6q	17	protooncogene
PEG1/MEST	nd	+	pat	nd	6	hydrolase
CDC25Mm	nd	+	pat	nd	9	Ras activator
SP2/U2afbp-rs1	−	+	pat	nd	11	splicing factor
XIST	nd	+	pat/Random	X	X	RNA, X inactivation

[a] *IGF2*, insulin-like growth factor-2; *INS*, insulin; *WT1*, Wilms' tumor 1; *SNRPN*, small nuclear ribonucleoprotein-associated polypeptide N; *PAR*, Prader-Willi/Angelman region; *IPW*, imprinted gene in the Prader-Willi syndrome region; *PEG3*, paternally expressed gene 3; *IGF2R*, insulin-like growth factor-2 receptor; *U2afbp-rs1*, U2af binding protein related sequence 1; *XIST*, X-inactive specific transcript. [b] +, imprinted; −, not imprinted; nd, no data. [c] mat, maternal allele; pat, paternal allele. [d] nd, no data.

normally repressed by imprinting. Here we take a closer look at the pathogenesis of three well-defined syndromes.

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are distinct clinical phenotypes resulting from opposite patterns of imprinting in chromosome 15q11-q13 (*16, 20*). PWS is characterized by infantile hypotonia, gonadal hypoplasia, moderate mental retardation, neonatal feeding difficulties followed later by hyperphagia with subsequent obesity, and small hands and feet. Approximately 70% of PWS cases have a deletion of 4 megabases on the paternally derived chromosome. With rare exceptions of small deletions, translocations, and duplications, the remainder of PWS patients have maternal UPD. These observations indicate that deficiency of paternally expressed genes including small nuclear ribonucleoprotein associated polypeptide N (*SNRPN*) is responsible for the PWS phenotype (*21*). In contrast, deletions on the maternal chromosome and paternal UPD are associated with the AS phenotype characterized by severe mental retardation, absent speech, seizure, ataxic gate, microcephaly, protruding tongue, and inappropriate laughter. Existence of familial AS cases and the fact that about one-third of sporadic AS exhibit neither deletion nor UPD suggest that deficiency of a single maternally expressed, but yet unidentified gene may cause AS. "Imprinting mutations," characterized by abnormal DNA methylation patterns, have also been reported in some familial PWS and AS cases with microdeletions (*16, 17, 22*).

Beckwith-Wiedemann syndrome (BWS) is characterized by prenatal overgrowth, macroglossia, umbilical hernia, hemihypertrophy, and an increased incidence of embryonic tumors such as Wilms' tumor, hepatoblastoma, and rhabdomyosarcoma (*23*). Although the majority of BWS cases occur sporadically with normal karyotype, less than 20% show abnormalities in chromosome 11p15, where *IGF2*, *H19*, and other imprinted genes exist. The changes include paternal UPD and trisomy with two paternal and one maternal chromosome. There is evidence that a double dosage of *IGF2* and defective *H19* are in part responsible for the BWS phenotype.

UPD is caused primarily by meiotic nondisjunction, which appears to be related with advanced maternal age, followed by monosomic conception with subsequent chromosome gain, or trisomic conception followed by chromosome loss (*24, 25*). In addition to the above diseases, UPDs have been demonstrated in several other human phenotypes as listed in Table IIA.

Tumorigenesis

Genomic imprinting is also implicated in the genesis of tumors listed in Table IIB. An extreme case involving the whole genome is hydatidiform mole, an androgenetic placental tumor, in which two entire sets of chromosomes are of paternal origin. The opposite genome constitution is observed in a subset of ovarian teratomas which arise from parthenogenetically activated eggs. The phenotypes of these tumors fit well with the features of androgenetic and parthenogenetic mouse embryos, respectively.

It is widely accepted that either or both of the following two events are involved in tumorigenesis: inactivation of tumor suppresser or growth-repressing genes and activation of oncogenic or growth-promoting genes (*23*). Knudson's two-hit model proposes that one allele of a tumor suppresser is first mutated and subsequently the other is lost by a chromosomal deletion. The process is known as loss of heterozygosity (LOH) and the resulting chromosomal constitutions can be determined using polymorphic markers. Preferential LOH of a specific parental allele has been observed in many tumors, suggesting a role for imprinting in the two-hit model. Allele-specific deletions are predominantly observed for maternally derived chromosomes but there are exceptions such as paraganglioma (*26*) and acute myelogenous leukemia (*27*). It is, however, unclear whether this bias arises from a higher incidence of mutation during male gametogenesis, or from imprinting effects manifesting after fertilization. More recent reports suggest a possible link between tumor progression and loss of imprinting (LOI) or abnormal imprinting (*28, 29*). Altered DNA methylation in cancer cells is thought to be closely coupled with abnormal imprinting. The LOH and

TABLE II. **Human diseases related with genomic imprinting.**

(A) Genetic diseases

Disease[a]	Chromosome[b]	Mutation[c]	Gene[d]	Affected allele[e,f]
Well-defined syndromes:				
Prader-Willi syndrome	15	del,UPD^{m}, imp	*SNRPN*,?	pat
Angelman syndrome	15	del,UPD^{p}, imp	—	mat
Beckwith-Wiedemann syndrome	11	UPD^{p}, others	*IGF2*, *H19*,?	mat
Uniparental disomies:				
Short stature	7	UPD^{m}	—	pat
Multiple congenital anomalies	14	UPD^{p}	—	mat
Short stature	14	UPD^{m}	—	pat
Transient neonatal diabetes	6	UPD^{p}	—	mat
Unstable triplet repeat diseases:				
Fragile X(A) syndrome	X	$(CCG)_n$	*FMR1*	mat
FRAXE	X	$(CCG)_n$	*FRAXE*	mat
DRPLA	12	$(CAG)_n$	Atrophin	pat
Huntington disease	4	$(CAG)_n$	Huntingtin	pat
Kennedy disease	X	$(CAG)_n$	*Androgen R*	pat
Spinocerebellar ataxia I	6	$(CAG)_n$	*Ataxin*	pat
Myotonic dystrophy	19	$(CTG)_n$	*DMPK*	mat
Parental origin differences in transmission:				
Insulin-dependent diabetes mellitus	11	—	—	pat
Neurofibromatosis I	17	—	—	mat
Neurofibromatosis II	22	—	—	mat
Narcolepsy	6	—	—	mat
Malignant hyperthermia	19	—	—	mat
Polycystic kidney	16,2	—	—	mat,pat
Atopic hypersensitivity	11	—	—	mat
Albright hereditary osteodystrophy	20	—	—	mat
Bipolar affective disorder	—	—	—	mat
Tourette's syndrome	—	—	—	mat,pat
Adams Oliver syndrome	—	—	—	mat
Alzheimer disease	—	—	—	pat
Cleft lip	—	—	—	pat
Cogenital heart disease	—	—	—	mat
Goldenhar syndrome	—	—	—	mat
Idiopathic hypertrophic subaortic stenosis	—	—	—	pat
Neural tube defect	—	—	—	mat
Polycystic ovary	—	—	—	mat
Psoriasis	—	—	—	pat
Seizures	—	—	—	mat
Tuberous sclerosis	—	—	—	mat

[a]FRAXE, fragile X(E) syndrome; DRPLA, dentatorubral-pallidoluysian atrophy. [b-d] —, no data. [c]del, deletion; UPD^{m}, maternal uniparental disomy; UPD^{p}, paternal uniparental disomy; imp, imprinting mutation. [d]*SNRPN*, small nuclear ribonucleoprotein-associated polypeptide N; *IGF2*, insulin-like growth factor-2; *FMR1*, fragile X mental retardation-1; *Androgen R*, androgen receptor; *DMPK*, myotonic dystrophy protein kinase. [e]mat, maternal allele; pat, paternal allele. [f]Affected allele is defined as a parental allele which transmits mutations including chromosomal loss in UPD.

(B) Tumorigenesis

Disease	Chromosome	Mutation[a]	Gene[b]	Affected allele[c,d]
Hydatidiform mole	all	androgenote	—	—
Ovarian teratoma	all	parthenogenote	—	—
Wilms' tumor	11p	LOH	*IGF2*	—
Wilms' tumor	11p	LOI	*IGF2,H19*	matIGF2, patH19
Rabdoid tumor	11p	LOI	*IGF2,H19*	matIGF2, patH19
Rabdomyosarcoma	11	LOH	—	mat
Rabdomyosarcoma	11p	LOI	*IGF2*	—
Leiomyosarcoma	11p	LOI	*IGF2*	mat
Hepatoblastoma	11p	LOH	—	mat
Hepatoblastoma	11p	LOI	*IGF2,H19*	mat,—
Testicular germ cell tumor	11p	LOI	*IGF2,H19*	matIGF2, patH19
Testicular germ cell tumor	11p	LOH	—	—
Choriocarcinoma	11p	LOI	*IGF2,H19*	matIGF2, patH19
Lung cancer	11p	LOI	*IGF2,H19*	matIGF2, patH19
Cervical carcinoma	11p	LOI	*IGF2,H19*	matIGF2, patH19
Cervical carcinoma	11p	LOH	*IGF2,H19*	—
Ewing's sarcoma	11p	LOI	*IGF2*	mat
Esophageal cancer	11p	LOI	*H19*	pat
Paraganglioma	11q	LOH	—	pat
Retinoblastoma	13q	LOH	*Rb*	mat
Osteosarcoma	13q	LOH	*Rb*	mat
Hepatocellular tumor	6q	LOH	*IGF2R*	—
Acute myelogenous leukemia	7	LOH	—	pat
Chronic myelogenous leukemia	9q,22q	tr	*ABL,BCR*	patABL, matBCR
Neuroblastoma	1p	LOH	—	mat
Neuroblastoma	2	amp	*N-myc*	pat

[a]LOH, loss of heterozygosity; LOI, loss of imprinting; tr, translocation; amp, amplification. [b]*IGF2*, insulin-like growth factor-2; *Rb*, retinoblastoma; *IGF2R*, insulin-like growth factor-2 receptor; —, no data. [c]mat, maternal allele; pat, paternal allele; —, no data. [d]Affected allele is defined as a parental allele which transmits mutations including activation of an imprinted unexpressed gene.

LOI of two oppositely imprinted genes, *IGF2* and *H19*, have been described for a broad range of tumors, especially in those occurring in childhood. Examples of allele-specific alterations in oncogenic genes are the preferential translocation between paternal *ABL* and maternal *BCR* in chronic myelogenous leukemia (*30*), and the preferential amplification of the paternal *N-myc* gene in neuroblastoma (*31*). It should be noted, however, that some conflicting evidence exists for the preferential LOH in neuroblastoma (*31*) and retinoblastoma, and for the allele-specific translocation in chronic myelogenous leukemia. Establishment of a causal link between imprinting and tumorigenesis requires further evidence.

Molecular mechanisms

Three epigenetic mechanisms, namely DNA methylation, chromatin structure, and asynchronous replication, have been suggested to play a role in the imprinting process. Involvement of methylation was first suggested by the studies on some transgenes, which are differentially methylated depending on their parental origin (*32*). Differential methylation between parental alleles was then detected in a number of endogenous imprinted genes. More recently, targeted disruption of the DNA methyltransferase (methylase) gene showed that genome-wide hypomethylation causes loss of imprinting (*33*). This study convincingly showed that methylation is at least involved in the maintenance of imprints. Two distinct imprinted chromo-

somal regions have been characterized to some detail and, hence, we will summarize our present knowledge on these regions below.

Human PWS/AS region

More than half of the patients with PWS and AS have a deletion of a common 4 megabases (indicated by two jagged lines in Fig. 2A) on paternal and maternal chromosome 15, respectively (*20, 34*). Translocations and smaller deletions have narrowed the PWS and AS critical regions and a deletion breakpoint in a Japanese kindred provided a boundary between the two regions. The PWS region centromeric to the breakpoint encompasses five paternally expressed sequences: *SNRPN, PAR-5, PAR-1, IPW*, and *ZNF127*. Higher DNA methylation on the maternal than on the paternal chromosome was reported at four loci including SNRPN, Y48.5, PW71, and ZNF127. In contrast, genes identified in the AS region [E6-associated protein, *PAR-2*, gamma aminobutyric acid receptor subunit (*GABRB3* and *GABRA5*), and *P* (human homologue of a pink-eyed dilution gene in mouse)] gave no evidence of imprinting (*35*). For most loci in the PWS/AS region, the paternally derived allele replicates earlier than the maternal allele. There is, however, a small region near the *GABRA5* gene, where the opposite pattern of allele-specific replication timing has been reported (*36, 37*). More recently, physical association between the allelic PWS/AS regions in the late S phase was reported in normal lymphocytes, but not in PWS and AS cells, suggesting that normal imprinting could involve mutual recognition and *trans* sensing for the maintenance of allelic differences during cell divisions (*38*).

Interestingly, some familial PWS and AS cases with microdeletions upstream of the *SNRPN* gene suggested the presence of an "imprinting center," which may be capable of regulating methylation, replication, chromatin structure, and gene activity of the entire region (*16, 17*). A number of unique direct repeats are also found in the CpG islands of *SNRPN* just as for the other imprinted genes (*39*).

Mouse *INS2/IGF2/H19* region

An imprinted region in the distal portion of mouse chromosome 7 has also been characterized extensively. There are three imprinted genes in this region: *INS2* and *IGF2* are paternally expressed (*40*) and *H19* maternally expressed (*41*) (Fig. 2B). Two other imprinted genes, *MASH2* and $p57^{KIP2}$, are also mapped close to this region although their exact locations have not been reported. The *INS2/IGF2/H19* region is flanked by two non-imprinted genes: tyrosine hydroxylase (*TH*) is located upstream of *INS2* and L23 (mitochondrial)-related protein (*L23mrp*) is about 40 kb downstream of *H19* (*42*, and unpublished, Mohamad Zubair and H.S.). As reported for the human PWS/AS region, differential DNA methylation between the parental chromosomes is observed in some small regions including the CpG-rich promoter of *H19* (*18, 43-45*). Nuclease-resistant, condensed chromatin structure has also been reported in the inactive paternal *H19* allele (*18, 44*). In *IGF2*, the promoters are not methylated and assume open chromatin structure even on the inactive chromosome (*43*). However, differential methylation has been found upstream of the gene and in the body of the gene (*43, 44*). In this case, increased methylation occurs on the active allele. Asynchronous replication is also seen in the region (*46*).

An interesting picture emerged when small regions containing the *H19* gene or its downstream enhancers was deleted by gene targeting. A deletion of *H19* itself and its 5′-flanking region caused an activation of the otherwise silent *IGF2* and *INS2* genes on the maternal chromosome (*47*). This deletion did not show any effects when paternally derived. When the endoderm-specific downstream enhancers were deleted, all three genes on the same chromosome became silent, irrespective of the parental origin (*48*). Thus it appears that the enhancers have a potential to interact with all three genes but with only one or two at once. This is consistent with the "enhancer competition model," in which imprinting is explained by alternative enhancer-gene interactions on the parental chromosomes

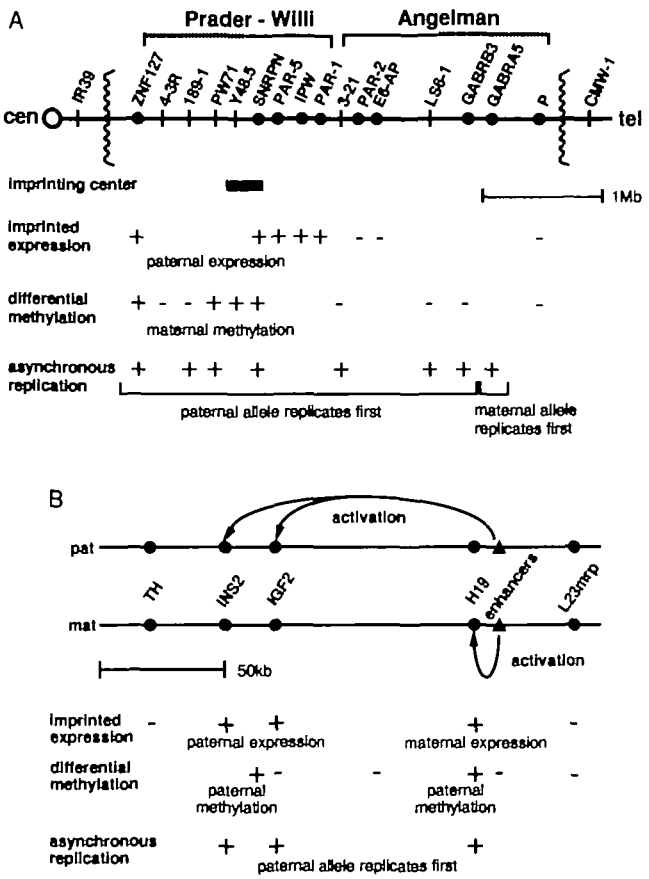

Fig. 2. **Structure and epigenetic status of two imprinted chromosomal domains.** (A) Human PWS/AS region on chromosome 15q11-q13. Loci (vertical lines) or genes (closed circles) are listed from the centromere (cen) to telomere (tel), and the common deletion intervals are indicated by two jagged lines. The critical regions for PWS and AS are deleted on the paternal and the maternal chromosome, respectively. The presence (+) or absence (−) of imprinted expression, differential methylation, and asynchronous replication is shown at each locus. The presence of an imprinting center (horizontal bold line) is proposed, which may regulate the DNA methylation, DNA replication, chromatin structure, and gene activity in the region. (B) Mouse *INS2/IGF2/H19* region on chromosome 7, which is syntenic to human 11p15.5. Interactions between the endoderm-specific enhancers and the three imprinted genes are also shown for each parental chromosome. An approximate scale is included in each panel.

(*43, 45*) (Fig. 2B). Perhaps, the enhancers preferentially interact with *H19* (because of the affinity or the proximity) and the other genes can use the enhancers only when *H19* is highly methylated and condensed. However, some conflicting evidence has been reported for other occasions, especially in human (*49, 50*).

An important implication of the above two domains is that these regions may serve as models to study functional chromosomal domains. For example, both cases suggest that the regulation by imprinting is achieved through long-range interactions between the genes and their regulatory elements. Each imprinted domain could be demarcated by boundary elements which insulate the action of a putative "imprinting center" or enhancers involved in imprinting. Alternatively, a single imprinted domain could encompass both imprinted and non-imprinted genes, and primary structure such as direct repeats could mark each imprinted gene (*39*). It will be interesting to identify such functional sequences and know the detailed organization of chromosomal domains.

Conclusion

Genomic imprinting is crucial for normal mammalian development and is apparently associated with some chromosome abnormality syndromes, although establishment of causal links between imprinting and other human diseases including tumors needs further evidence. Molecular studies of imprinted genes and imprinted chromosomal regions have revealed the importance of long-range mechanisms in gene regulation, and provide us with an opportunity to understand the structure and function of chromosomal domains.

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