REVIEW

Genomic Imprinting and Chromatin Insulation in Beckwith-Wiedemann Syndrome

*John M. Greally**

Abstract

Genes are recognized as undergoing genomic imprinting when they are capable of being expressed only from the paternal or only from the maternal chromosome. The process can occur coordinately within large physical domains in mammalian chromosomes. One interesting facet of the study of genomic imprinting is that it offers insight into the regulation of large chromosomal regions. Understanding this regulation involves elucidating the *cis*-acting regulators of gene expression and defining the elements that maintain chromatin insulation, both required for understanding more practically applicable areas of biological research, such as efficient transgene production. This review is focused on the regulation of the imprinted domain of human chromosome 11p15.5, responsible for Beckwith-Wiedemann syndrome (BWS). Recent findings indicate that the maintenance of imprinting within this domain is critically dependent on the stable maintenance of chromatin insulation.

Index Entries: Genomic imprinting; Beckwith-Weidemann syndrome; chromatin; CpG islands; chromosome abnormalities; insulin-like growth factor II; heterochromatin.

1. Genomic Imprinting

As a cell differentiates, the chromatin organization of its nucleus undergoes reorganization. Sets of *cis*-acting elements wax or wane in influence, inducing different patterns of transcription that define the cell's differentiated state. Typically the process involves identical effects at the same locus on both chromosomes in a diploid cell. For some loci, however, the epigenetic reorganization does not allow transcription on one chromosome. If the choice of chromosome is random with respect to parental origin, the maternal locus is inactivated as frequently as the paternal locus and allelic exclusion is said to occur *(1,2)*. If, on the other hand, the choice of chromosome depends on its parental origin, so that some loci are inactive only on the maternal chromosome, others on the paternal chromosome, genomic imprinting is occurring *(3–6)*.

The most striking feature of genomic imprinting is the longevity of the "imprint." The imprinting event must occur when the parental chromosomes are capable of being epigenetically organized distinctively. Gametogenesis is therefore the likely time of this event. This epigenetic organization survives the extensive, heterogenous chromatin remodeling inherent to the differentiation of the large number of mammalian cell types. It is therefore not surprising that some genes imprint in a developmental stage and cell-type specific manner *(7)*, and disruption of imprinting can occur in conditions of general perturbation of chromatin organization, such as neoplasia *(8–16)* and the Immunodeficiency/Centromeric instability/Facial anomalies (ICF) syndrome *(17)*.

Failure to maintain an imprint can therefore be a normal response to certain *trans*-acting environments, or part of an abnormal process usually rec-

*Address to which all correspondence and reprint requests should be addressed: Department of Genetics, SHM I-134, Yale University School of Medicine, 333 Cedar Street, P.O. Box 208005, New Haven, CT 06520-8005. E-mail: john.greally@yale.edu.

 1073–6085/1999/11:2/159–173/$13.75

ognized by being involved in human disease. Genetic malformation syndromes of childhood *(18)*, cancer predisposition syndromes *(11,18–20)*, neurodevelopmental syndromes *(21)*, and an increasing number of neoplasms *(13,16,22–25)* are recognized as involving dysregulation of imprinted genes.

The ability to establish and maintain an imprint is therefore critical in preventing human genetic disease. This review takes one such disease, Beckwith-Wiedemann syndrome (BWS), and discusses the likely mechanisms establishing and maintaining imprinting of its causative genes.

2. Beckwith-Wiedemann Syndrome

Beckwith-Wiedemann syndrome (BWS: OMIM number #130650) not only causes a pathognomic set of physical malformations, but also predisposes the affected to the biochemical abnormality of neonatal hypoglycaemia and a range of childhood tumors *(18)*. These malignancies include Wilms' tumor *(15)*, as well as hepatoblastoma, rhabdomyosarcoma, neuroblastoma, and adrenocortical carcinoma **(14)**. Overgrowth also characterizes many of the physical malformations, whether generalized or localized to certain organs, such as the liver, kidneys, adrenal cortex, and pancreas *(26)*. Omphalocoele is a typical finding *(18)*, owing to failure of the abdominal wall to fuse around the outlet of the umbilical cord, possibly reflecting overgrowth of the abdominal contents at a critical stage of embryogenesis.

The first indication that the chromosomal region responsible for BWS was subject to genomic imprinting came from studies of loss of heterozygosity (LOH) in Wilms' tumor DNA. LOH was found to occur in the 11p15.5 region and to have the unusual feature of always removing the maternal alleles *(27)*. The characteristic cytogenetic duplications of 11p15.5 were found to be of paternal origin *(28)*, whereas the maternal chromosome was invariably involved in the reciprocal translocations into 11p15.5 associated with BWS *(28–31)*. These observations suggested that a gene (or genes) from 11p15.5 was subject to the parent-of-origin effects of genomic imprinting.

Two imprinted genes from 11p15.5 appear to contribute strongly to the pathogenesis of BWS. The $p57^{KIP2}$ gene was found to be mutated in a subset of BWS patients *(32)*. $p57^{KIP2}$ is involved in cell cycle regulation, acting as an inhibitor of the cyclin-dependent kinases that mediate progression of a cell from G1 into S phase *(33)*. Mutation of $p57^{KIP2}$ by homologous recombination in mice leads to a phenotype similar to human BWS. The phenotypes induced by different knockouts differ in their ability to induce the BWS-like phenotype *(34,35)*. The $p57^{KIP2}$-null phenotype lacks some characteristics of BWS both in humans and mice (discussed in *[36]*). It appears that the full BWS syndrome is owing to the additional effects of overexpressing the insulin-like growth factor 2 (*IGF2*) gene. *IGF2* is a protein important for fetal growth *(37)*, found to be dysregulated in BWS *(38–41)* and a variety of human neoplasms *(42–54)*, including those associated with BWS *(38,39,55)*. Overexpression of *IGF2* in mice leads to a phenotype similar to, but again incompletely representative of, BWS in humans *(56)*. The phenotypically similar Simpson-Golabi-Behmel syndrome also involves regulation of *IGF2*, but at the posttranslational stage, the glypican gene responsible for acting to regulate intracellular availability of *IGF2* protein *(57)*. The phenotypes associated with $p57^{KIP2}$ mutation and *IGF2* overexpression appear to complement each other to give rise to a nearly complete set of features comprising BWS *(36)*. The pattern of imprinting of these two genes correlates well with the mutations responsible for BWS. The growth-promoting *IGF2* gene is expressed from the usually over-represented paternal chromosome *(58)*, the presumed tumor suppressive $p57^{KIP2}$ from the under-represented maternal chromosome *(59,60)*.

Although genomic imprinting is important in the physiological regulation of the 11p15.5 domain, skewing the patterns of mutations in a distinctive manner, the mechanism by which mutations act to dysregulate the imprinted domain remains uncertain. An approach to ascertaining this mechanism is to draw parallels between 11p15.5 and the regulation of 15q11–q13, a better-understood

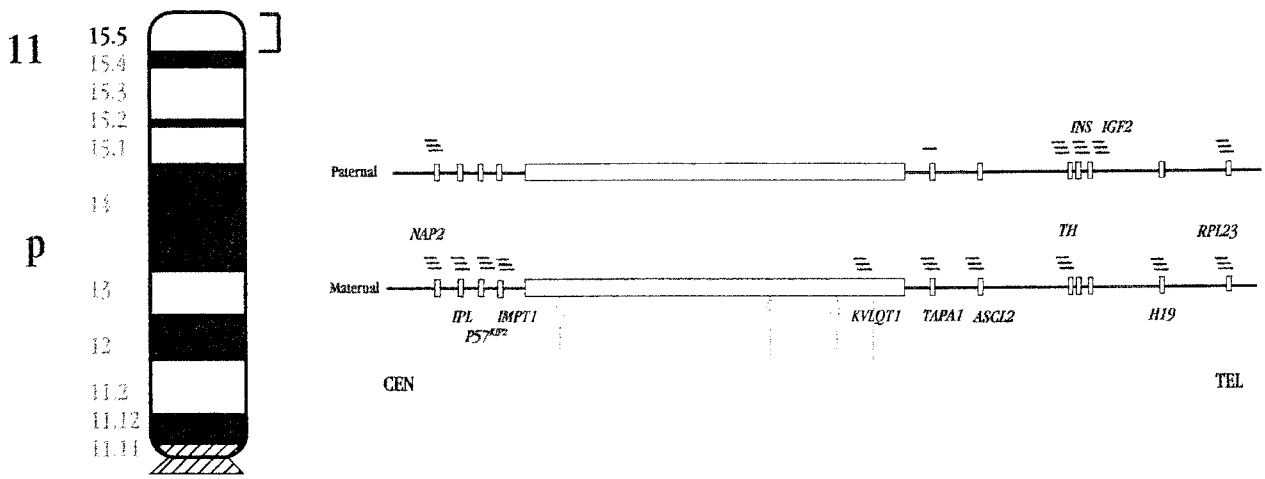

Fig. 1 Triple horizontal lines above genes define transcriptional activity from that allele. Vertical arrows show cytogenetic breakpoints defining BWSCR1 *(125)*. *NAP2* and *RPL23* are biallelically expressed flanking the domain; within the domain most genes are maternally expressed/paternally repressed apart from the *INS* and *IGF2* where expression is paternal and *TH* where expression appears always to be biallelic.

imprinted domain responsible for the neurodevelopmental Prader-Willi and Angelman phenotypes.

3. Molecular Mechanisms of BWS and Comparison with the Imprinted 15q11–q13 Domain

The mutations causing BWS are heterogenous, but each type of mutation affects a chromosome of specific gametic origin. For example, extra copies of chromosome 11p15.5 of paternal origin are associated with BWS *(28)*. Reciprocal translocations into 11p15.5, on the other hand, always involve the maternally-derived chromosome *(28–31)*. The pattern of gene expression within this domain is summarized in **Fig. 1**. Most of the domain is maternally expressed and paternally repressed, with a minority of genes expressed solely from the paternal chromosome. One gene, tyrosine hydroxylase, is biallelically expressed in spite of being located within the otherwise imprinted domain *(61–63)*. The *CD81* gene is also biallelically expressed in most situations, although it shows relatively higher expression from the maternal chromosome early in development *(64)*. In individuals with paternal uniparental disomy (UPD) for 11p15.5, there is a combination of extra paternal 11p15.5 material and an absence of a maternally inherited 11p15.5 contribution. This should allow both increased *IGF2* expression and decreased $p57^{KIP2}$ expression. However, in patients with trisomy for 11p15.5 involving extra paternal chromosomal material, $p57^{KIP2}$ expression should remain unaltered on the remaining intact maternal chromosome. In these patients, the pathogenetic effects are presumably solely owing to paternally expressed genes, possibly *IGF2* alone.

No deletions of 11p15.5 have been associated with BWS, unlike the 15q11–q13 imprinted domain, where deletions of either parental chromosome give rise to phenotypes *(65)*. This is surprising, given the ability of mutations of the $p57^{KIP2}$ gene to cause a recognizable phenotype *(32)*. For the 15q11–q13 domain, deletions of the maternal chromosome remove the causative maternally expressed *UBE3A* gene *(66–68)* to give rise to Angelman syndrome. Deletions of the maternal 11p15.5 domain involving the $p57^{KIP2}$ locus should have an effect similar to $p57^{KIP2}$ mutations, because functional nullisomy for this locus results in both instances. A possible reason may be the presence of a closely linked maternally expressed gene that causes embryonic lethality when deleted, and therefore deletions are

unlikely to occur at substantial frequency, sparing this hypothetical neighboring locus while deleting $p57^{KIP2}$. The immediate $p57^{KIP2}$ region is proving to be very gene-rich *(69–72)*, so an essential embryonic function may yet be found for one of these flanking genes.

In patients with Prader-Willi and Angelman syndromes, UPD can also cause disease *(21)*, although regional trisomy does not cause these phenotypes. BWS is caused by mutations causing paternal UPD or by regional trisomy with the extra copy of paternal origin *(28)*. In addition, there exists a small subset of Prader-Willi and Angelman patients in whom the molecular mechanism for disease is the failure of imprint switching (reviewed in *[73,74]*). This occurs when a maternally derived chromosome 15q11–q13 in a male is processed during spermatogenesis but fails to switch the maternal epigenetic organization to a new paternal pattern. A child born with this unswitched 15q11–q13 has a normal maternally organized 15q11–q13 from the oocyte-derived chromosome and a maternally-organized 15q11–q13 on the sperm-derived chromosome. Maternally expressed genes are active on both chromosomes, but paternally expressed genes are silent. The resulting failure of a functional paternal 15q11–q13 genetic contribution leads to Prader-Willi syndrome. The converse has been found in Angelman syndrome, where a failure to reset the paternal epigenetic organization in oogenesis leads to Angelman syndrome. These imprint switching abnormalities have been found to be owing to microdeletions at the *SNRPN* locus, defining this region as the "imprinting centre" (IC) for the 15q11–q13 domain *(73,74)*.

There is insufficient evidence that a similar process occurs in BWS. The studies to date have focused solely on the expression and methylation patterns of *IGF2* and *H19*. In some cases biallelic expression of *IGF2* is found to be associated with biallelic silencing of *H19 (75)*. The methylation patterns of these two genes were altered to a pattern of generally increased methylation *(75)*, suggesting a paternal epigenetic organization on both chromosomes. These data parallel the 15q11–q13 imprinting switch mutations, as the patterns resemble those that would occur if the paternal chromosome failed to switch to a maternal epigenotype. However, as these two genes are co-regulated by common *cis*-acting elements *(76,77)*, these results are open to the criticism that a primary process affecting the maternal *H19* gene alone should give rise to similar results. This possibility is supported by experiments in mice with deletion of the maternal copy of *H19*. Increased methylation *(78)* and expression *(77,79)* of *Igf2* is found in these animals. Evidence to support the occurrence of imprinting switch mutations would be more convincing if based on analysis of genes from the region other than those previously shown to be co-regulated.

Translocations associated with BWS are always described on the maternally derived chromosome *(28–31)*. Some of these translocation breakpoints occur centromerically to the imprinted domain *(80)*. One such cluster (BWSCR1) occurs within the imprinted domain itself. The locations of the BWSCR1 translocation breakpoints within the *KVLQT1* gene body have also been interpreted to support a parallel with the 15q11–q13 IC *(81)*, because there also exists a transcript through that region *(82)*. However, the 15q11–q13 IC mutations are deletions, not translocations *(74)*, and 15q11–q13 translocations causing disease are not restricted to the maternally derived chromosome *(83–87)*. Although these comparisons suggest that the 11p15.5 maternal translocations are not the equivalent of IC mutations, two caveats are applicable. The first is that two distinct phenotypes occur with 15q11–q13 mutations, whereas only one phenotype is associated with 11p15.5 disruption, possibly explaining the restricted pattern of 11p15.5 translocations. Secondly, no translocations into the 15q11–q13 IC itself have yet been described, so the inability to compare the two mechanisms may possibly reflect a current failure to identify an equivalent mutation in 15q11–q13.

Comparison of the molecular mechanisms of BWS and those of Prader-Willi and Angelman syndromes indicates that significant differences in the patterns of mutations occur. This raises the question of whether the primary mechanism for establishing imprinting is comparable for both

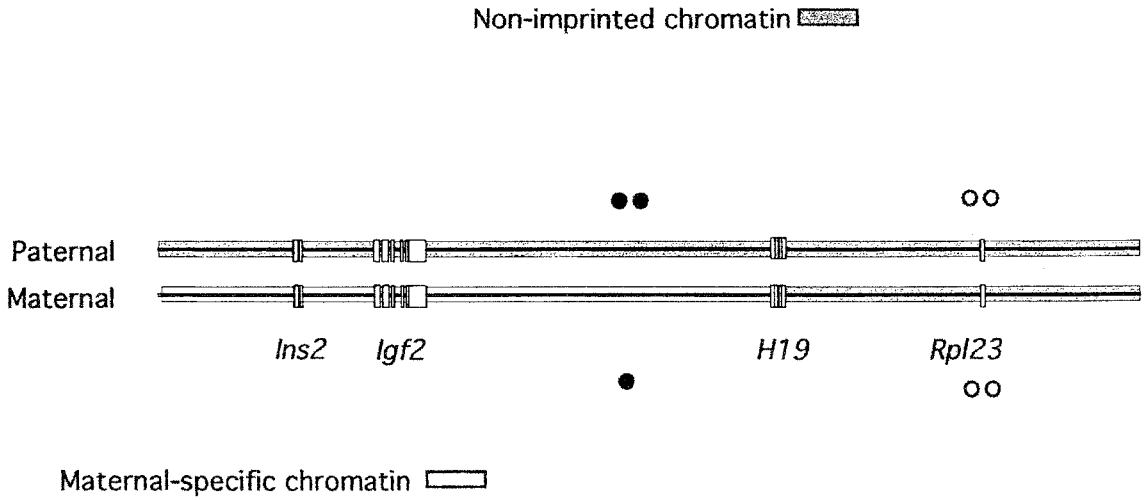

Fig. 2 FISH studies of replication timing patterns define the maternal copy of *H19* as a site of transition from later (single hybridization focus represented by filled circle) to earlier (doublet of foci, open) as illustrated. No difference *in cis* is seen on the paternal chromosome, and this difference *in cis* on the maternal chromosome is lost with deletion of 12.9 kb including the *H19* gene body ***(90)***. It therefore appears that the chromatin organization upstream from paternal *H19* resembles that of the non-imprinted downstream chromatin. This suggests that the gametogenesis-determined 'imprint' in this region is solely oogenesis-determined.

domains. Recent data suggest that imprinting of the $p57^{KIP2}$/*Igf2* domain is critically dependent on maintaining gamete-determined chromatin insulation, a finding which may help to explain some of these differences.

4. Chromatin Insulation and the *H19* Locus (see Fig. 2)

Three nonimprinted genes were identified downstream from human *H19* ***(88,89)***. At one of these loci, *RPL23* (the gene symbol for the locus published as *L23MRP*), similar methylation patterns were found on the paternal and maternal chromosomes ***(88)***. These results suggested that the end of the imprinted domain was to be found between *H19* and *RPL23*. Because genomic imprinting is manifested not only by expression and methylation, but also nuclease accessibility and DNA replication timing differences between homologous chromosomes ***(5)***, and it was previously suggested that DNA replication timing was less affected by tissue and developmental stage variability affecting the other manifestations ***(61)***, we decided to test whether replication patterns differed at the syntenic mouse *H19* and *Rpl23* loci ***(90)***. We cloned cosmids spanning the *Rpl23* locus and confirmed the presence of the gene by sequence analysis. Fluorescence *in situ* hybridization (FISH) studies were performed, showing a decrease in the proportion of single-double hybridization patterns from the 30–40% range at and upstream from *H19* to less than 20% at *Rpl23*. This added support to the previous observations, suggesting a transition from imprinted to nonimprinted chromatin organization between *H19* and *RPL23* ***(88)***.

We then explored the idea that the chromosomal regions exhibiting distinct replication patterns were located in distinct replicons. Replicons are theoretical subdivisions of the genome in which replication takes place. Their existence is suggested by the discrete punctate signals when fluorescence-labeling is used to detect newly synthesized DNA within the cell nucleus. The limited number of these signals suggests a defined number of clusters of replication enzymes within the nucleus, and it has been suggested that the genome is likewise organized to take advantage of this organization by replicating in a segmental manner as replicons, rather than randomly ***(91)***.

Our approach to this question was to compare replication upstream from *H19* with the replication at *Rpl23* using two-color FISH. The replication patterns for each probe could be compared in the same nucleus. We found that the replication timing on the early-replicating chromosome was indistinguishable *in cis*, whereas the replication on the other chromosome was markedly delayed upstream from *H19* compared with that at *Rpl23* ***(90)***. The data did not support the replicon model: the differences in replication timing were occurring on only one of the two chromosomes, whereas we had expected a distinct difference for both chromosomes. We instead began to focus on the later-replicating chromosome, previously found to be of maternal origin at the *H19* locus ***(61)***.

The later-replicating, maternal chromosome had distinct replication timing patterns upstream and downstream from *H19*. This observation indicated that a partitioning of the replication organization was occurring in the vicinity of maternal *H19*, mediated by a previously uncharacterized regulatory element. In the genome as a whole, functional compartmentalization has been suggested to be mediated by the biochemically-defined nuclear matrix-attachment regions (MARs) ***(92)***, or elements defined by their ability to block the effects of enhancers on promoters, chromatin insulators ***(93)***. The experimental support for MARs mediating genomic compartmentalization has been undermined by the occurrence of intronic MARs ***(94)***, the inability of some MARs to confer position-independent expression upon transgenic constructs ***(95)*** and the failure of some well-characterized chromatin insulators to act as MARs biochemically ***(95)***. Furthermore, our prior analyzis of the mouse *H19* region failed to find any MARs in the area of transition of replication patterns ***(96)***. There is, however, support for the presence of a chromatin insulator at the *H19* locus. Transgenes from this locus were found to express *H19* in a position-independent manner ***(97,98)***, a further characteristic of chromatin insulators ***(99)***. Moreover, deletion of the 3 kb *H19* gene body and 9.9 kb of upstream DNA allowed access of downstream endodermal enhancers to *Igf2* and *Ins2* upstream, suggesting that an enhancer-blocking element had been removed ***(77)***.

We analyzed the mice with the 12.9 kb deletion at *H19* ***(77)*** and found that replication asynchrony was lost in the upstream imprinted region ***(90)***. This equalization of replication timing occurred only in mice inheriting the knockout maternally. The difference in replication timing *in cis* on the maternal chromosome upstream and downstream from *H19* was abolished in these mice. These data support the hypothesis that these mice lost a functional insulator of replication patterns. The element partitioning replication timing is subject to genomic imprinting, in that it acts solely on the maternal chromosome. Because the FISH experiments defined an insulator of replication patterns, the assay cannot be said to have identified a canonical chromatin insulator. However, the FISH studies were prompted by data suggesting the presence of a chromatin insulator at the same locus found to mediate an effect on replication timing. It is therefore unproven but likely that a chromatin insulator is present at the *H19* locus, mediating not only position-independent expression of transgenes ***(97,98)*** and blocking of enhancers ***(77)*** but also partitioning of replication patterns ***(90)***.

5. *H19*: A Locus Required as a Chromatin Insulator?

H19 has proven to be an interesting locus to study in terms of its role in genomic imprinting. The similarity of its expression pattern with that of *Igf2* and their physical proximity led to a focus on a shared mechanism of imprinting ***(100)***. The identification of endodermal enhancers downstream from *H19* ***(101)*** suggested a possible model for reciprocal imprinting of these two genes, a model centered on promoter competition by the two genes for these enhancers ***(100)***. When the promoter of *H19* is inactivated by methylation, as occurs on the paternal chromosome owing to genomic imprinting, the more distant promoter of *Igf2* (and that of *Ins2*) can utilize the enhancers and be expressed. This model was supported by the deletion of the *H19* gene body and upstream DNA referred to previously ***(77)***. The removal of the less

methylated, transcriptionally active maternal *H19* gene allowed the upstream *Igf2* and *Ins2* genes to be expressed from the maternal chromosome, whereas deletion of the normally inactive, methylated paternal copy of *H19* had no effect on gene expression *(77)*. Placing the enhancers approximately equidistantly between the *Igf2* and *H19* genes actually resulted in preferential use by the *Igf2* promoters, indicating that the physiological location of the enhancers closer to *H19* is important for effective competition to occur *(102)*.

The untranslated *XIST* gene product is required to epigenetically inactivate mammalian X chromosomes *(103)*. The absence of a translational product for *H19 (104)* also suggested a possible *XIST*-like role for the locus to establish imprinting *in cis*. This further possibility was explored. A deletion of the *H19* gene body alone was made in mice, and found to allow maternal *Igf2* expression, although to a markedly lesser extent than the larger deletion involving 9.9 kb of upstream DNA. This partial imprinting of *Igf2* occurred with complete imprinting of the neomycin resistance gene inserted into the *H19* gene body *(79)*. The failure to abolish imprinting with deletion of the *H19* gene body indicated that the *H19* transcript does not determine imprinting and therefore does not have an epigenetic effect comparable with that of *Xist*.

Further analysis of this locus suggested that the function of *H19* is not sequence-dependent, as significant divergence of sequence between human and mouse *H19* exists, with preservation of organization in terms of CpG islands *(105)*. The mouse *H19* locus was introduced transgenically into *Drosophila melanogaster* and found to silence the eye-pigment reporter gene in the construct. The silencing activity was narrowed down to a 1210 bp fragment extending to 1690 bp upstream from the transcription start site of *H19 (106)*. This silencing had to be methylation-independent, because *Drosophila* does not methylate its genome at CpG dinucleotides as do mammals *(107)*. This silencer is physically separate from repetitive DNA sequences also 5' to *H19*, proposed as candidates to mediate some aspect of imprinting regulation *(108)*. The ability of the *H19* silencer to act in mammalian cells has not been reported.

These observations can be reinterpreted in the context of a chromatin insulator at *H19*. Most known chromatin insulators have been described in *Drosophila melanogaster (93,109,110)*, with two vertebrate counterparts *(111,112)*. None of these is subject to genomic imprinting. For those characterized in *Drosophila*, genetic experiments have allowed the elucidation of some of the *trans*-acting factors regulating their activity. Most of these factors are recognized chromatin constituents *(113,114)*. An interesting observation is that in a mod(mdg4) mutant background the chromatin insulator of the *gypsy* retrotransposon acts as a silencer *(115)*. The vertebrate chromatin insulators described are the 5'HS-4 of the chicken ß globin locus control region (LCR) *(111)* and the BEAD-1 element at the human T cell receptor α/δ locus *(112)*. The 5'HS-4 insulator activity has been narrowed down to a CpG island within the LCR. This CpG island is physiologically unmethylated and lacks significant promoter activity *(116)*. As well as its defining ability to block enhancers, this element has been reported to allow position-independent expression of transgenes *(117)*. (G + C) richness also defines the cores of the scs and scs' chromatin insulators *(118)*. The sequence analyses of other chromatin insulators have not been described.

The three candidate regions to study for the presence of a chromatin insulator within the 12.9 kb deletion at *H19* are the conserved DNA sequences at the promoter and gene body *(104)*, the 5' silencer element *(106)* and the upstream *(119)* and gene body *(120)* CpG islands. The silencer may be active as a chromatin insulator in mammals but act as a silencer in *Drosophila* because the genetic background lacks *trans*-acting factors required for activity as a chromatin insulator, in a manner analogous with the mod(mdg4) example described for a native *Drosophila* silencer *(115)*. The CpG islands at the promoter are candidates because of the similar characteristic of the chicken 5'HS-4 chromatin insulator *(116)*. The active insulation of replication patterns is mediated by the unmethylated, maternal *H19* allele. Interestingly,

the chicken 5'HS-4 chromatin insulator is likewise physiologically unmethylated *(116)*. The *H19* gene body deletion *(79)*, removing as it did one CpG island described *(120)*, in this model would have weakened the chromatin insulator allowing incomplete access of the downstream enhancers to *IGF2* and the consequent partial expression of the *IGF2* gene.

It has been proposed that chromatin insulators act as false promoters, attracting enhancers to preclude their use by more distal true promoters *(113)*. As such, the promoter competition model proposed to explain the reciprocal imprinting in this region *(100)* remains consistent with the mechanism of chromatin insulation at *H19*. The *H19* insulator may only differ from that of the chicken 5'HS-4 insulator by its incidental ability to promote transcription, explaining the presence of an untranslated mRNA at this locus *(104)*. This model predicts that the evolutionary pressure at this locus is not directed at the gene itself, but rather to maintain chromatin insulation, perhaps accounting for the conservation of organization in terms of CpG islands but not sequence upstream from *H19 (105)*.

6. Implications of a Model of Chromatin Insulation Determining Genomic Imprinting

The model that imprinting of 11p15.5 requires intact flanking chromatin insulators necessarily makes several predictions. A simple initial prediction is that certain instances of biallelic *IGF2* expression occur owing to the failure of the *H19* chromatin insulator. Because increased methylation and decreased expression of *H19* characterize the inactive insulator on the paternal chromosome, these are features worth analyzing in disease states associated with biallelic *IGF2* expression. Numerous cases of Wilms' tumor *(55,121,122)* and BWS *(41)* have now been described to involve not only biallelic *IGF2* expression but also abnormal expression and methylation of *H19*. A further useful test to determine whether some of these cases are owing to failure of *H19* to act as a chromatin insulator would be to analyze replication timing at *IGF2* in these individuals. Judging from the results of similar studies in the *H19* knockout mice *(90)*, cases owing to loss of insulator function should cause reversion to synchronous replication at *IGF2*.

A second prediction of the model accounts for the pathological effect of translocations into the imprinted domain on the maternal chromosome. The integrity of the imprinted domain is dependent on maintaining the flanking insulator at *H19*, as seen by studies of expression *(77)*, methylation *(78)* and replication timing *(90)* in mice deleted for *H19*. If a translocation into the domain can bypass the flanking insulators to influence chromatin structure *in cis*, the effects would be expected to differ on each parental chromosome. On the paternal chromosome, the *IGF2* gene is already influenced by non-imprinted chromatin because the insulator at *H19* is inactive (**Fig. 2**). Disruption of the silencing of $p57^{KIP2}$ on the paternal chromosome would not be expected to be pathogenic. Translocation into the maternal chromosome, on the other hand, would be expected to mimic the effect of deletion of *H19* by allowing derepression of *IGF2 (77)* and possibly also inducing a chromatin environment less favorable for $p57^{KIP2}$ expression (**Fig. 3**). FISH studies of the *IGF2* locus in cells with such rearrangements have shown a perturbation of replication timing *(26,40)*, consistent with an effect *in cis* to dysregulate the domain.

The other major possibility allowed by this model is that of an imprinted domain not containing an IC. Imprinting of a chromosomal region could occur in a theoretical situation where two imprinted chromatin insulators flank the region and are active on the same chromosome. The intervening domain would be subject to position-effect *in cis* on the chromosome on which the insulators are inactive but shielded from such effects on the chromosome bearing the active insulators. Position-effect on chromatin organization and consequently gene expression would then differ in these intervening areas, leading to what would be recognized as genomic imprinting. In this model (**Fig. 4**), the components of the domain with all the *cis*-elements capable of conferring imprinting are the flanking regions. Therefore the

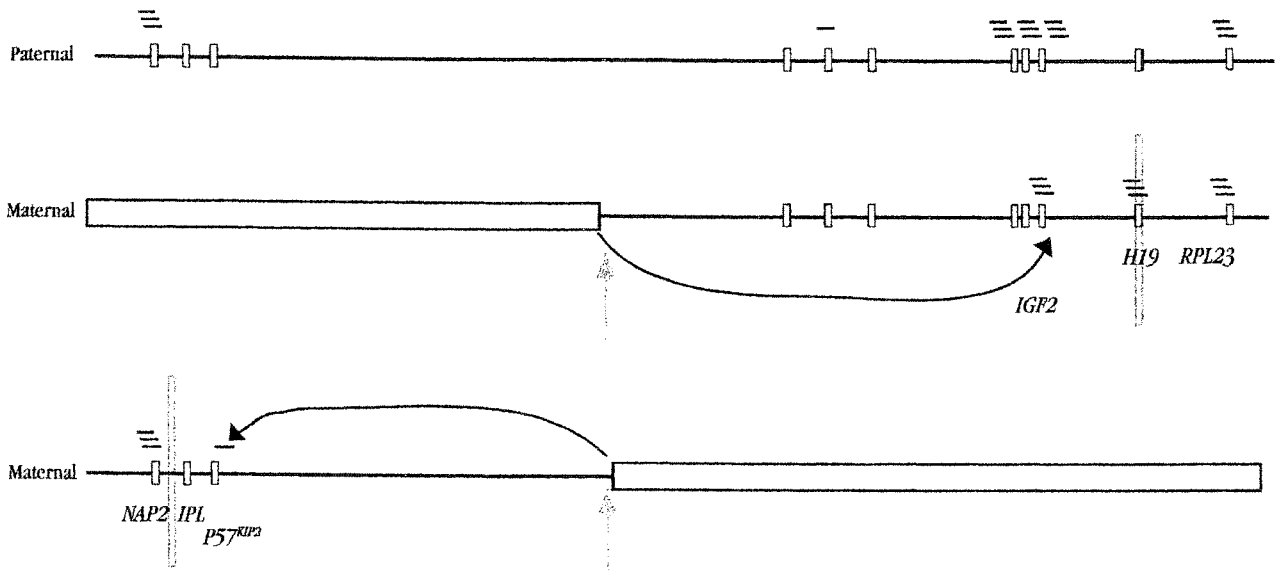

Fig. 3 Translocation of exogenous DNA (open bar) into the imprinted domain at a known translocation breakpoint within BWSCR1 (vertical arrow) is proposed to give rise to disruptive effects *in cis* (represented by curving arrows) either to promote *IGF2* transcription or to diminish $p57^{KIP2}$ transcription. The intact chromatin insulators being bypassed by the translocation are represented by grayed vertical bars at *H19* and within the centromeric area of transition from nonimprinted to imprinted gene expression. The translocations into the maternal chromosome are proposed to cause disease by bypassing chromatin insulation rather than disrupting an 11p15.5 imprinting centre.

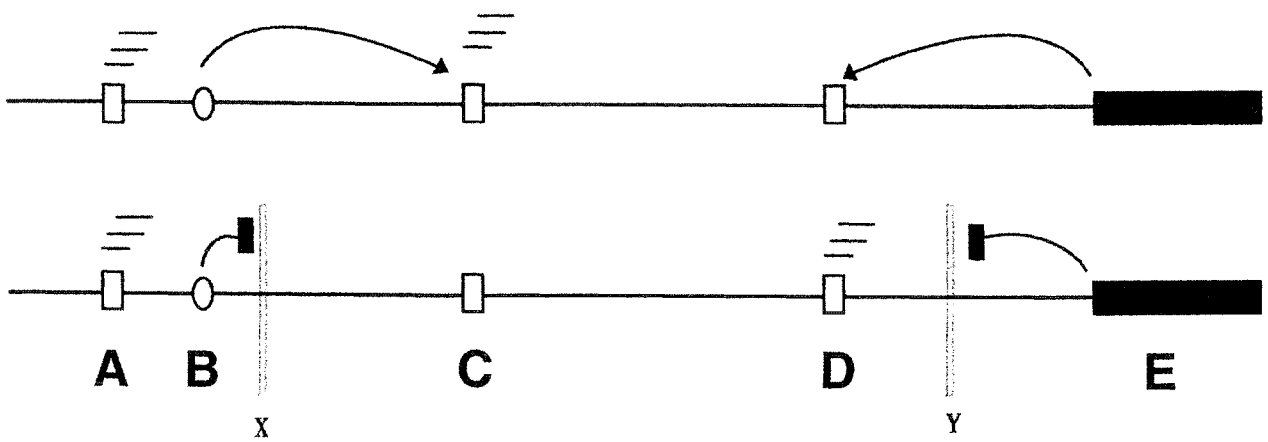

Fig. 4 A model for imprinting due to imprinted chromatin insulators, not requiring the presence of an imprinting centre. A pair of homologous chromosome are represented. A region circumscribed by the imprinted chromatin insulators X and Y contains two genes, C and D. A positive influence (enhancer B) and a negative influence (heterochromatin E) are shown outside the "domain." As chromatin insulators are defined by their capacities to block enhancer action *(93)* and confer position-independence from nearby heterochromatin *(99)*, an imprinted insulation model predicts that some genes will be insulated from positive influences *in cis* (C), while others will be shielded from negative influences (D). Uninsulated genes outside the domain are represented by A. The patterns observed for genes C and D would be recognized as imprinting, but would be occurring in the absence of an imprinting centre.

regions capable of autonomously imprinting as transgenes should be solely the flanking regions, whereas transgenes containing the intervening domain would not imprint. The *H19* region can imprint autonomously, both as large YAC transgenes *(123)* and in smaller genomic constructs *(97,98,124)*. A transgene containing the *Mash2* gene (mouse homolog of human *ASCL2*) has been reported not to be capable of imprinting *(64)*. The *H19* locus is not an IC equivalent, as its removal has no effect on imprinting of *Mash2*, *Kvlqt1*, or $p57^{Kip2}$ *(64)*. Therefore, in spite of lacking an IC in these transgenic constructs, *H19* has the necessary *cis*-acting regulators to imprint efficiently *(97,98,124)*. This observation alone raises doubt as to whether an IC is present in this domain, separate from and required for *H19* imprinting. The need to identify a second candidate chromatin insulator at the centromeric end of the domain is needed to begin to test this prediction. The recent narrowing of the likely region in which this insulator should occur to 15 kb indicates that identification of such an element should soon be possible *(70)*.

7. Conclusions

This review has focused on the domain organization of the human chromosome 11p15.5 region that undergoes genomic imprinting and contains genes giving rise to human diseases such as BWS. It is not clear that the the attempted parallels being made between imprinting of 11p15.5 and that of 15q11–q13 will be useful in elucidating the mechanism of imprinting of 11p15.5. With a novel emphasis on chromatin insulation, the organization of 11p15.5 can be framed in terms that are consistent with the patterns of mutations leading to human disease. By transgenic analysis, many components of this model will be testable. It is clear that if further evidence is found to support this model, 11p15.5 will be an excellent model region for study of the complex topic of circumscribed chromatin domains. That the region undergoes imprinting adds an extra attractive facet to such studies: comparable, differently epigenetically organized sequences are present in the same nucleus. The 11p15.5 imprinted domain has the potential to be a useful resource to exploit in the quest to understand complex chromatin regulatory elements such as chromatin insulators.

Acknowledgments

Diana Starr and Steven Hwang contributed insightful critical appraisal of the manuscript. Li-qun Song and Sharon Zemel are gratefully acknowledged for their ongoing collaboration to study this region. The author is funded by a grant from the National Institutes of Health.

References

1. Holliday, R. (1990) Genomic imprinting and allelic exclusion. *Dev. Suppl.* 125–129.
2. Chess, A. (1998) Expansion of the allelic exclusion principle? *Science* **279,** 2067–2068.
3. Glenn, C. C., Driscoll, D. J., Yang, T. P., and Nicholls, R. D. (1997) Genomic imprinting: potential function and mechanisms revealed by the Prader-Willi and Angelman syndromes. *Mol. Hum. Reprod.* **3,** 321–332.
4. Tycko, B., Trasler, J., and Bestor, T. (1997) Genomic imprinting: gametic mechanisms and somatic consequences. *J. Androl.* **18,** 480–486.
5. Feil, R. and Kelsey, G. (1997) Genomic imprinting: a chromatin connection. *Am. J. Hum. Genet.* **61,** 1213–1219.
6. Bartolomei, M. S. and Tilghman, S. M. (1997) Genomic imprinting in mammals. *Annu. Rev. Genet.* **31,** 493–525.
7. Giddings, S. J., King, C. D., Harman, K. W., Flood, J. F., and Carnaghi, L. R. (1994) Allele specific inactivation of insulin 1 and 2, in the mouse yolk sac, indicates imprinting. *Nature Genet.* **6,** 310–313.
8. Tycko, B. (1994) Genomic imprinting: mechanism and role in human pathology. *Am. J. Pathol.* **144,** 431–443.
9. Rainier, S. and Feinberg, A. P. (1994) Genomic imprinting, DNA methylation, and cancer. *J. Natl. Cancer Inst.* **86,** 753–759.
10. Junien, C. and Henry, I. (1994) Genetics of Wilms' tumor: a blend of aberrant development and genomic imprinting. *Kidney Int.* **46,** 1264–1279.
11. McCaffrey, T. V., Meyer, F. B., Michels, V. V., Piepgras, D. G., and Marion, M. S. (1994) Familial paragangliomas of the head and neck. *Arch. Otolaryngol. Head Neck Surg.* **120,** 1211–1216.
12. Feinberg, A. P., Rainier, S., and DeBaun, M. R. (1995) Genomic imprinting, DNA methylation, and cancer. *J. Natl. Cancer Inst. Monogr.* **17,** 21–26.
13. Ohlsson, R. and Franklin, G. (1995) Normal development and neoplasia: the imprinting connection. *Int. J. Dev. Biol.* **39,** 869–876.
14. Squire, J. and Weksberg, R. (1996) Genomic imprinting in tumors. *Semin. Cancer Biol.* **7,** 41–47.

15. Feinberg, A. P. (1996) Multiple genetic abnormalities of 11p15 in Wilms' tumor. *Med. Pediatr. Oncol.* **27,** 484–489.
16. Fleming, S. (1997) Genetics of renal tumors. *Cancer Metast. Rev.* **16,** 127–140.
17. Schuffenhauer, S., Bartsch, O., Stumm, M., Buchholz, T., Petropoulou, T., Kraft, S., Belohradsky, B., Hinkel, G. K., Meitinger, T., and Wegner, R. D. (1995) DNA, FISH and complementation studies in ICF syndrome: DNA hypomethylation of repetitive and single copy loci and evidence for a trans acting factor. *Hum. Genet.* **96,** 562–571.
18. Li, M., Squire, J. A., and Weksberg, R. (1997) Molecular genetics of Beckwith-Wiedemann syndrome. *Curr. Opin. Pediatr.* **9,** 623–629.
19. van der Mey, A. G., Maaswinkel-Mooy, P. D., Cornelisse, C. J., Schmidt, P. H., and van de Kamp, J. J. (1989) Genomic imprinting in hereditary glomus tumors: evidence for new genetic theory. *Lancet* **2,** 1291–1294.
20. Heutink, P., van der Mey, A. G., Sandkuijl, L. A., van Gils, A. P., Bardoel, A., Breedveld, G. J., van Vliet, M., van Ommen, G. J., Cornelisse, C. J., Oostra, B. A., et al. (1992) A gene subject to genomic imprinting and responsible for hereditary paraganglioma maps to chromosome 11q23-qter. *Hum. Mol. Genet.* **1,** 7–10.
21. Nicholls, R. D. (1993) Genomic imprinting and uniparental disomy in Angelman and Prader-Willi syndromes: a review. *Am. J. Med. Genet.* **46,** 16–25.
22. Pedone, P. V., Tirabosco, R., Cavazzana, A. O., Ungaro, P., Basso, G., Luksch, R., Carli, M., Bruni, C. B., Frunzio, R., and Riccio, A. (1994) Mono- and bi-allelic expression of insulin-like growth factor II gene in human muscle tumors. *Hum. Mol. Genet.* **3,** 1117–1121.
23. Montagna, M., Menin, C., Chieco-Bianchi, L., and D'Andrea, E. (1994) Occasional loss of constitutive heterozygosity at 11p15.5 and imprinting relaxation of the IGFII maternal allele in hepatoblastoma. *J. Cancer Res. Clin. Oncol.* **120,** 732–736.
24. Walsh, C., Miller, S. J., Flam, F., Fisher, R. A., and Ohlsson, R. (1995) Paternally derived *H19* is differentially expressed in malignant and nonmalignant trophoblast. *Cancer Res.* **55,** 1111–1116.
25. Vu, T. H., Yballe, C., Boonyanit, S., and Hoffman, A. R. (1995) Insulin-like growth factor II in uterine smooth-muscle tumors: maintenance of genomic imprinting in leiomyomata and loss of imprinting in leiomyosarcomata. *J. Clin. Endocrinol. Metab.* **80,** 1670–1676.
26. Weksberg, R. and Squire, J.A. (1996) Molecular biology of Beckwith-Wiedemann syndrome. *Med. Pediatr. Oncol.* **27,** 462–469.
27. Reeve, A. E., Housiaux, P. J., Gardner, R. J., Chewings, W. E., Grindley, R. M., and Millow, L. J. (1984) Loss of a Harvey ras allele in sporadic Wilms' tumor. *Nature* **309,** 174–176.
28. Weksberg, R., Teshima, I., Williams, B. R., Greenberg, C. R., Pueschel, S. M., Chernos, J. E., Fowlow, S. B., Hoyme, E., Anderson, I. J., Whiteman, D. A., et al. (1993) Molecular characterization of cytogenetic alterations associated with the Beckwith-Wiedemann syndrome (BWS) phenotype refines the localization and suggests the gene for BWS is imprinted. *Hum. Mol. Genet.* **2,** 549–556.
29. Sait, S. N., Nowak, N. J., Singh-Kahlon, P., Weksberg, R., Squire, J., Shows, T. B., and Higgins, M.J. (1994) Localization of Beckwith-Wiedemann and rhabdoid tumor chromosome rearrangements to a defined interval in chromosome band 11p15.5. *Genes Chrom. Cancer* **11,** 97–105.
30. Mannens, M., Hoovers, J. M., Redeker, E., Verjaal, M., Feinberg, A. P., Little, P., Boavida, M., Coad, N., Steenman, M., Bliek, J., et al. (1994) Parental imprinting of human chromosome region 11p15.3-pter involved in the Beckwith-Wiedemann syndrome and various human neoplasia. *Eur. J. Hum. Genet.* **2,** 3–23.
31. Hoovers, J. M., Kalikin, L. M., Johnson, L. A., Alders, M., Redeker, Law, D. J., Bliek, J., Steenman, M., Benedict, M., and Wiegant, J. (1995) Multiple genetic loci within 11p15 defined by Beckwith-Wiedemann syndrome rearrangement breakpoints and subchromosomal transferable fragments. *Proc. Natl. Acad. Sci. USA* **92,** 12,456–12,460.
32. Hatada, I., Ohashi, H., Fukushima, Y., Kaneko, Y., Inoue, M., Komoto, Y., Okada, A., Ohishi, S., Nabetani, A., Morisaki, H., Nakayama, M., Niikawa, N., and Mukai, T. (1996) An imprinted gene p57KIP2 is mutated in Beckwith-Wiedemann syndrome. *Nature Genet.* **14,** 171–173.
33. Matsuoka, S., Edwards, M. C., Bai, C., Parker, S., Zhang, P., Baldini, A., Harper, J. W., and Elledge, S. J. (1995) p57KIP2, a structurally distinct member of the p21CIP1 Cdk inhibitor family, is a candidate tumor suppressor gene. *Genes Dev.* **9,** 650–662.
34. Zhang, P., Liegeois, N. J., Wong, C., Finegold, M., Hou, H., Thompson, J. C., Silverman, A., Harper, J. W., DePinho, R. A., and Elledge, S. J. (1997) Altered cell differentiation and proliferation in mice lacking p57KIP2 indicates a role in Beckwith-Wiedemann syndrome. *Nature* **387,** 151–158.
35. Yan, Y., Frisen, J., Lee, M. H., Massague, J., and Barbacid, M. (1997) Ablation of the CDK inhibitor p57Kip2 results in increased apoptosis and delayed differentiation during mouse development. *Genes Dev.* **11,** 973–983.
36. Eggenschwiler, J., Ludwig, T., Fisher, P., Leighton, P. A., Tilghman, S. M., and Efstratiadis, A. (1997) Mouse mutant embryos overexpressing IGF-II exhibit phenotypic features of the Beckwith-Wiedemann and Simpson-Golabi-Behmel syndromes. *Genes Dev.* **11,** 3128–3142.
37. DeChiara, T. M., Efstratiadis, A., and Robertson, E. J. (1990) A growth-deficiency phenotype in heterozy-

gous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* **345,** 78–80.
38. Weksberg, R., Shen, D. R., Fei, Y. L., Song, Q. L., and Squire, J. (1993) Disruption of insulin-like growth factor 2 imprinting in Beckwith-Wiedemann syndrome. *Nature Genet.* **5,** 143–150.
39. Ogawa, O., Becroft, D. M., Morison, I. M., Eccles, M. R., Skeen, J. E., Mauger, D. C., and Reeve, A. E. (1993) Constitutional relaxation of insulin-like growth factor II gene imprinting associated with Wilms' tumor and gigantism. *Nature Genet.* **5,** 408–412.
40. Brown, K. W., Villar, A. J., Bickmore, W., Clayton-Smith, J., Catchpoole, D., Maher, E. R., and Reik, W. (1996) Imprinting mutation in the Beck with-Wiedemann syndrome leads to biallelic IGF2 expression through an H19-independent pathway. *Hum. Mol. Genet.* **5,** 2027–2032.
41. Joyce, J. A., Lam, W. K., Catchpoole, D. J., Jenks, P., Reik, W., Maher, E. R., and Schofield, P. N. (1997) Imprinting of IGF2 and H19: lack of reciprocity in sporadic Beckwith- Wiedemann syndrome. *Hum. Mol. Genet.* **6,** 1543–1548.
42. Vu, T. H., Yballe, C., Boonyanit, S., and Hoffman, A.R. (1995) Insulin-like growth factor II in uterine smooth-muscle tumors: maintenance of genomic imprinting in leiomyomata and loss of imprinting in leiomyosarcomata. *J. Clin. Endocrinol. Metab.* **80,** 1670–1676.
43. Zhan, S., Shapiro, D. N., and Helman, L. J. (1995) Loss of imprinting of IGF2 in Ewing's sarcoma. *Oncogene* **11,** 2503–2507.
44. Douc-Rasy, S., Barrois, M., Fogel, S., Ahomadegbe, J. C., Stehelin, D., Coll, J., and Riou, G. (1996) High incidence of loss of heterozygosity and abnormal imprinting of H19 and IGF2 genes in invasive cervical carcinomas. Uncoupling of H19 and IGF2 expression and biallelic hypomethylation of H19. *Oncogene* **12,** 423–430.
45. McCann, A. H., Miller, N., O'Meara, A., Pedersen, I., Keogh, K., Gorey, T., and Dervan, P. A. (1996) Biallelic expression of the IGF2 gene in human breast disease. *Hum. Mol. Genet.* **5,** 1123–1127.
46. Kinouchi, Y., Hiwatashi, N., Higashioka, S., Nagashima, F., Chida, M., and Toyota, T. (1996) Relaxation of imprinting of the insulin-like growth factor II gene in colorectal cancer. *Cancer Lett.* **107,** 105–108.
47. Uyeno, S., Aoki, Y., Nata, M., Sagisaka, K., Kayama, T., Yoshimoto, T., and Ono, T. (1996) IGF2 but not H19 shows loss of imprinting in human glioma. *Cancer Res.* **56,** 5356–5359.
48. Wu, H. K., Weksberg, R., Minden, M. D., and Squire, J. A. (1997) Loss of imprinting of human insulin-like growth factor II gene, IGF2, in acute myeloid leukemia. *Biochem. Biophys. Res. Commun.* **231,** 466–472.
49. Nonomura, N., Miki, T., Nishimura, K., Kanno, N., Kojima, Y., and Okuyama, A. (1997) Altered imprinting of the H19 and insulin-like growth factor II genes in testicular tumors. *J. Urol.* **157,** 1977–1979.
50. Wu, H. K., Squire, J. A., Catzavelos, C. G., and Weksberg, R. (1997) Relaxation of imprinting of human insulin-like growth factor II gene, IGF2, in sporadic breast carcinomas. *Biochem. Biophys. Res. Commun.* **235,** 123–129.
51. Oda, H., Kume, H., Shimizu, Y., Inoue, T. and Ishikawa, T. (1998) Loss of imprinting of IGF2 in renal-cell carcinomas. *Int. J. Cancer* **75,** 343–346.
52. Kim, K. S. and Lee, Y. I. (1997) Biallelic expression of the H19 and IGF2 genes in hepatocellular carcinoma. *Cancer Lett.* **119,** 143–148.
53. Wu, M. S., Wang, H. P., Lin, C. C., Sheu, J. C., Shun, C. T., Lee, W. J., and Lin, J. T. (1997) Loss of imprinting and overexpression of IGF2 gene in gastric adenocarcinoma. *Cancer Lett.* **120,** 9–14.
54. Randhawa, G. S., Cui, H., Barletta, J. A., Strichman-Almashanu, L. Z., Talpaz, M., Kantarjian, H., Deisseroth, A. B., Champlin, R. C., and Feinberg, A. P. (1998) Loss of imprinting in disease progression in chronic myelogenous leukemia. *Blood* **91,** 3144–3147.
55. Rainier, S., Johnson, L. A., Dobry, C. J., Ping, A. J., Grundy, P. E., and Feinberg, A. P. (1993) Relaxation of imprinted genes in human cancer. *Nature* **362,** 747–749.
56. Sun, F. L., Dean, W. L., Kelsey, G., Allen, N. D., and Reik, W. (1997) Transactivation of IGF2 in a mouse model of Beckwith-Wiedemann syndrome. *Nature* **389,** 809–815.
57. Pilia, G., Hughes-Benzie, R. M., MacKenzie, A., Baybayan, P., Chen, E. Y., Huber, R., Neri, G., Cao, A., Forabosco, A., and Schlessinger, D. (1996) Mutations in GPC3, a glypican gene, cause the Simpson-Golabi-Behmel overgrowth syndrome. *Nature Genet.* **12,** 241–247.
58. DeChiara, T. M., Robertson, E. J., and Efstratiadis, A. (1991) Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* **64,** 849–859.
59. Hatada, I. and Mukai, T. (1995) Genomic imprinting of p57KIP2, a cyclin-dependent kinase inhibitor, in mouse. *Nature Genet.* **11,** 204–206.
60. Matsuoka, S., Thompson, J. S., Edwards, M. C., Bartletta, J. M., Grundy, P., Kalikin, L. M., Harper, J. W., Elledge, S. J., and Feinberg, A. P. (1996) Imprinting of the gene encoding a human cyclin-dependent kinase inhibitor, p57KIP2, on chromosome 11p15. *Proc. Natl. Acad. Sci. USA* **93,** 3026–3030.
61. Kitsberg, D., Selig, S., Brandeis, M., Simon, I., Keshet, I., Driscoll, D. J., Nicholls, R. D., and Cedar, H. (1993) Allele-specific replication timing of imprinted gene regions. *Nature* **364,** 459–463.
62. Zhou, Q. Y., Quaife, C. J., and Palmiter, R. D. (1995) Targeted disruption of the tyrosine hydroxylase gene reveals that catecholamines are required for mouse fetal development. *Nature* **374,** 640–643.

63. Kobayashi, K., Morita, S., Sawada, H., Mizuguchi, T., Yamada, K., Nagatsu, I., Hata, T., Watanabe, Y., Fujita, K., and Nagatsu, T. (1995) Targeted disruption of the tyrosine hydroxylase locus results in severe catecholamine depletion and perinatal lethality in mice. *J. Biol. Chem.* **270,** 27,235–27,243.
64. Caspary, T., Cleary, M. A., Baker, C. C., Guan, X.-J., and Tilghman, S. M. (1998) Multiple mechanisms regulate imprinting of the mouse distal chromosome 7 gene cluster. *Mol. Cell. Biol.* **18,** 3466–3474.
65. Nicholls, R. D. (1994) New insights reveal complex mechanisms involved in genomic imprinting. *Am. J. Hum. Genet.* **54,** 733–740.
66. Albrecht, U., Sutcliffe, J. S., Cattanach, B. M., Beechey, C. V., Armstrong, D., Eichele, G., and Beaudet, A. L. (1997) Imprinted expression of the murine Angelman syndrome gene, Ube3a, in hippocampal and Purkinje neurons. *Nature Genet.* **17,** 75–78.
67. Rougeulle, C., Glatt, H., and Lalande, M. (1997) The Angelman syndrome candidate gene, UBE3A/E6-AP, is imprinted in brain. *Nature Genet.* **17,** 14–15.
68. Vu, T. H. and Hoffman, A. R. (1997) Imprinting of the Angelman syndrome gene, UBE3A, is restricted to brain. *Nature Genet.* **17,** 12–13.
69. Qian, N., Frank, D., O'Keefe, D., Dao, D., Zhao, L., Yuan, L., Wang, Q., Keating, M., Walsh, C., and Tycko, B. (1997) The IPL gene on chromosome 11p15.5 is imprinted in humans and mice and is similar to TDAG51, implicated in Fas expression and apoptosis. *Hum. Mol. Genet.* **6,** 2021–2029.
70. Lee, M. P. and Feinberg, A. P. (1998) Genomic imprinting of a human apoptosis gene homologue, TSSC3. *Cancer Res.* **58,** 1052–1056.
71. Dao, D., Frank, D., Qian, N., O'Keefe, D., Vosatka, R. J., Walsh, C. P., and Tycko, B. (1998) IMPT1, an imprinted gene similar to polyspecific transporter and multi-drug resistance genes. *Hum. Mol. Genet.* **7,** 597–608.
72. Schwienbacher, C., Sabbioni, S., Campi, M., Veronese, A., Bernardi, G., Menegatti, A., Hatada, I., Mukai, T., Ohashi, H., Barbanti-Brodano, G., Croce, C. M., and Negrini, M. (1998) Transcriptional map of 170-kb region at chromosome 11p15.5: identification and mutational analyzis of the BWR1A gene reveals the presence of mutations in tumor samples. *Proc. Natl. Acad. Sci. USA* **95,** 3873–3878.
73. Horsthemke, B. (1997) Structure and function of the human chromosome 15 imprinting center. *J. Cell Physiol.* **173,** 237–241.
74. Horsthemke, B., Dittrich, B., and Buiting, K. (1997) Imprinting mutations on human chromosome 15. *Hum. Mutat.* **10,** 329–337.
75. Reik, W., Brown, K. W., Schneid, H., Lebouc, Y., Bickmore, W., and Maher, E. W. (1995) Imprinting mutations in the Beckwith-Wiedemann syndrome suggested by an altered imprinting pattern in the IGF2-H19 domain. *Hum. Mol. Genet.* **4,** 2379–2385.
76. Leighton, P. A., Saam, J. R., Ingram, R. S., Stewart, C. L., and Tilghman, S. M. (1995) An enhancer deletion affects both H19 and IGF2 expression. *Genes Dev.* **9,** 2079–2089.
77. Leighton, P. A., Ingram, R. S., Eggenschwiler, J., Efstratiadis, A., and Tilghman, S. M. (1995) Disruption of imprinting caused by deletion of the H19 gene region in mice. *Nature* **375,** 34–39.
78. Forné, T., Oswald, J., Dean, W., Saam, J. R., Bailleul, B., Dandolo, L., Tilghman, S. M., Walter, J., and Reik, W. (1997) Loss of the maternal H19 gene induces changes in IGF2 methylation in both cis and trans. *Proc. Natl. Acad. Sci. USA* **94,** 10,243–10,248.
79. Ripoche, M. A., Kress, C., Poirier, F., and Dandolo, L. (1997) Deletion of the H19 transcription unit reveals the existence of a putative imprinting control element. *Genes Dev.* **11,** 1596–1604.
80. Mannens, M., Alders, M., Redeker, B., Bliek, J., Steenman, M., Wiesmeyer, C., de Meulemeester, M., Ryan, A., Kalikin, L., Voute, T., De Kraker, J., Hoovers, J., Slater, R., Feinberg, A., Little, P., and Westerveld, A. (1996) Positional cloning of genes involved in the Beckwith-Wiedemann syndrome, hemihypertrophy, and associated childhood tumors. *Med. Pediatr. Oncol.* **27,** 490–494.
81. Lee, M. P., Hu, R. J., Johnson, L. A., and Feinberg, A. P. (1997) Human KVLQT1 gene shows tissue-specific imprinting and encompasses Beckwith-Wiedemann syndrome chromosomal rearrangements. *Nature Genet.* **15,** 181–185.
82. Dittrich, B., Buiting, K., Korn, B., Rickard, S., Buxton, J., Saitoh, S., Nicholls, R. D., Poustka, A., Winterpacht, A., Zabel, B., and Horsthemke, B. (1996) Imprint switching on human chromosome 15 may involve alternative transcripts of the SNRPN gene. *Nature Genet.* **14,** 163–170.
83. Schulze, A., Hansen, C., Skakkebaek, N. E., Brondum-Nielsen, K., Ledbeter, D. H., and Tommerup, N. (1996) Exclusion of SNRPN as a major determinant of Prader-Willi syndrome by a translocation breakpoint. *Nature Genet.* **12,** 452–454.
84. Burke, L. W., Wiley, J. E., Glenn, C. C., Driscoll, D. J., Loud, K. M., Smith, A. J., and Kushnick, T. (1996) Familial cryptic translocation resulting in Angelman syndrome:implications for imprinting or location of the Angelman gene? *Am. J. Hum. Genet.* **58,** 777–784.
85. Sun, Y., Nicholls, R. D., Butler, M. G., Saitoh, S., Hainline, B. E., and Palmer, C. G. (1996) Breakage in the SNRPN locus in a balanced 46,XY,t(15;19) Prader-Willi syndrome patient. *Hum. Mol. Genet.* **5,** 517–524.
86. Greger, V., Knoll, J. H., Wagstaff, J., Woolf, E., Lieske, P., Glatt, H., Benn, P. A., Rosengren, S. S., and Lalande, M. (1997) Angelman syndrome associated with an inversion of chromosome 15q11.2q24.3. *Am. J. Hum. Genet.* **60,** 574–580.

87. Conroy, J. M., Grebe, T. A., Becker, L. A., Tsuchiya, K., Nicholls, R. D., Buiting, K., Horsthemke, B., Cassidy, S. B., and Schwartz, S. (1997) Balanced translocation 46,XY,t(2;15)(q37.2;q11.2) associated with atypical Prader-Willi syndrome. *Am. J. Hum. Genet.* **61,** 388–394.
88. Tsang, P., Gilles, F., Yuan, L., Kuo, Y.-H., Lupu, F., Samara, G., Moosikasuwan, J., Goye, A., Zelenetz, A.D., Selleri, L., and Tycko, B. (1995) A novel L23-related gene 40 kb downstream of the imprinted H19 gene is biallelically expressed in mid-fetal and adult human tissues. *Hum. Mol. Genet.* **4,** 1499–1507.
89. Yuan, L., Qian, N., and Tycko, B. (1996) An extended region of biallelic gene expression and rodent-human synteny downstream of the imprinted H19 gene on chromosome 11p15.5. *Hum. Mol. Genet.* **5,** 1931–1937.
90. Greally, J. M., Starr, D. J., Hwang, S., Song, L., Jaarola, M., and Zemel, S. (1998) The mouse H19 locus mediates a transition between imprinted and non- imprinted DNA replication patterns. *Hum. Mol. Genet.* **7,** 91–96.
91. Jackson, D. A. and Pombo, A. (1998) Replicon clusters are stable units of chromosome structure: evidence that nuclear organization contributes to the efficient activation and propagation of S phase in human cells. *J. Cell Biol.* **140,** 1285–1295.
92. Laemmli, U. K., Kas, E., Poljak, L., and Adachi, Y. (1992) Scaffold-associated regions: cis-acting determinants of chromatin structural loops and functional domains. *Curr. Opin. Genet. Dev.* **2,** 275–285.
93. Kellum, R. and Schedl, P. (1992) A group of scs elements function as domain boundaries in an enhancer-blocking assay. *Mol. Cell. Biol.* **12,** 2424–2431.
94. Cockerill, P. N. and Garrard, W. T. (1986) Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. *Cell* **44,** 273–282.
95. Poljak, L., Seum, C., Mattioni, T., and Laemmli, U.K. (1994) SARs stimulate but do not confer position independent gene expression. *Nucleic Acids Res.* **22,** 4386–4394.
96. Greally, J. M., Guinness, M. E., McGrath, J., and Zemel, S. (1997) Matrix-attachment regions in the mouse chromosome 7F imprinted domain. *Mamm. Genome* **8,** 805–810.
97. Pfeifer, K., Leighton, P. A., and Tilghman, S. M. (1996) The structural H19 gene is required for transgene imprinting. *Proc. Natl. Acad. Sci. USA* **93,** 13,876–13,883.
98. Elson, D. A. and Bartolomei, M. S. (1997) A 5' differentially methylated sequence and the 3'-flanking region are necessary for H19 transgene imprinting. *Mol. Cell. Biol.* **17,** 309–317.
99. Kellum, R. and Schedl, P. (1991) A position-effect assay for boundaries of higher order chromosomal domains. *Cell* **64,** 941–950.
100. Zemel, S., Bartolomei, M. S., and Tilghman, S. M. (1992) Physical linkage of two mammalian imprinted genes, H19 and insulin-like growth factor 2. *Nature Genet.* **2,** 61–65.
101. Yoo-Warren, H., Pachnis, V., Ingram, R. S., and Tilghman, S. M. (1988) Two regulatory domains flank the mouse H19 gene. *Mol. Cell. Biol.* **8,** 4707–4715.
102. Webber, A. L., Ingram, R. S., Levorse, J. M., and Tilghman, S. M. (1998) Location of enhancers is essential for the imprinting of H19 and IGF2 genes. *Nature* **391,** 711–715.
103. Panning, B., Dausman, J., and Jaenisch, R. (1997) X chromosome inactivation is mediated by Xist RNA stabilization. *Cell* **90,** 907–916.
104. Brannan, C. I., Dees, E. C., Ingram, R. S., and Tilghman, S. M. (1990) The product of the H19 gene may function as an RNA. *Mol. Cell. Biol.* **10,** 28–36.
105. Jinno, Y., Sengoku, K., Nakao, M., Tamate, K., Miyamoto, T., Matsuzaka, T., Sutcliffe, J. S., Anan, T., Takuma, N., Nishiwaki, K., Ikeda, Y., Ishimaru, T., Ishikawa, M., and Niikawa, N. (1996) Mouse/human sequence divergence in a region with a paternal-specific methylation imprint at the human H19 locus. *Hum. Mol. Genet.* **5,** 1155–1161.
106. Lyko, F., Brenton, J. D., Surani, M. A., and Paro, R. (1997) An imprinting element from the mouse H19 locus functions as a silencer in Drosophila. *Nature Genet.* **16,** 171–173.
107. Wines, D. R., Talbert, P. B., Clark, D. V., and Henikoff, S. (1996) Introduction of a DNA methyltransferase into Drosophila to probe chromatin structure in vivo. *Chromosoma* **104,** 332–340.
108. Pfeifer, K. and Tilghman, S. M. (1994) Allele-specific gene expression in mammals: the curious case of the imprinted RNAs. *Genes Dev.* **8,** 1867–1874.
109. Hagstrom, K., Muller, M., and Schedl, P. (1996) Fab-7 functions as a chromatin domain boundary to ensure proper segment specification by the Drosophila bithorax complex. *Genes Dev.* **10,** 3202–3215.
110. Roseman, R. R., Pirrotta, V., and Geyer, P. K. (1993) The su(Hw) protein insulates expression of the Drosophila melanogaster white gene from chromosomal position-effects. *EMBO J.* **12,** 435–442.
111. Chung, J. H., Whiteley, M., and Felsenfeld, G. (1993) A 5' element of the chicken beta-globin domain serves as an insulator in human erythroid cells and protects against position effect in Drosophila. *Cell* **74,** 505–514.
112. Zhong, X.-P. and Krangel, M. S. (1997) An enhancer-blocking element between α and δ gene segments within the human T cell receptor α/δ locus. *Proc. Natl. Acad. Sci. USA* **94,** 5219–5224.
113. Geyer, P. K. (1997) The role of insulator elements in defining domains of gene expression. *Curr. Opin. Genet. Dev.* **7,** 242–248.

114. Gerasimova, T. I. and Corces, V. G. (1998) Polycomb and trithorax group proteins mediate the function of a chromatin insulator. *Cell* **92,** 511–521.
115. Gerasimova, T. I., Gdula, D. A., Gerasimov, D. V., Simonova, O., and Corces, V. G. (1995) A Drosophila protein that imparts directionality on a chromatin insulator is an enhancer of position-effect variegation. *Cell* **82,** 587–597.
116. Chung, J. H., Bell, A. C., and Felsenfeld, G. (1997) Characterization of the chicken beta-globin insulator. *Proc. Natl. Acad. Sci. USA* **94,** 575–580.
117. Wang, Y., DeMayo, F. J., Tsai, S. Y., and O'Malley, B. W. (1997) Ligand-inducible and liver-specific target gene expression in transgenic mice. *Nature Biotech.* **15,** 239–243.
118. Vazquez, J. and Schedl, P. (1994) Sequences required for enhancer blocking activity of scs are located within two nuclease-hypersensitive regions. *EMBO J.* **13,** 5984–5993.
119. Tremblay, K. D., Duran, K. L., and Bartolomei, M. S. (1997) A 5' 2-kilobase-pair region of the imprinted mouse H19 gene exhibits exclusive paternal methylation throughout development. *Mol. Cell. Biol.* **17,** 4322–4329.
120. Ferguson-Smith, A. C., Sasaki, H., Cattanach, B. M., and Surani, M. A. (1993) Parental-origin-specific epigenetic modification of the mouse H19 gene. *Nature* **362,** 751–755.
121. Steenman, M. J., Rainier, S., Dobry, C. J., Grundy, P., Horon, I. L., and Feinberg, A. P. (1994) Loss of imprinting of IGF2 is linked to reduced expression and abnormal methylation of H19 in Wilms' tumor. *Nature Genet.* **7,** 433–439.
122. Taniguchi, T., Sullivan, M. J., Ogawa, O., and Reeve, A. E. (1995) Epigenetic changes encompassing the IGF2/H19 locus associated with relaxation of IGF2 imprinting and silencing of H19 in Wilms tumor. *Proc. Natl. Acad. Sci. USA* **92,** 2159–2163.
123. Ainscough, J. F., Koide, T., Tada, M., Barton, S., and Surani, M. A. (1997) Imprinting of IGF2 and H19 from a 130 kb YAC transgene. *Development* **124,** 3621–3632.
124. Bartolomei, M. S., Webber, A. L., Brunkow, M. E., and Tilghman, S. M. (1993) Epigenetic mechanisms underlying the imprinting of the mouse H19 gene. *Genes Dev.* **7,** 1663–1673.
125. Reid, L. H., Davies, C., Cooper, P. R., Crider-Miller, S. J., Sait, S. N., Nowak, N. J., Evans, G., Stanbridge, E. J., deJong, P., Shows, T. B., Weissman, B. E., and Higgins, M. J. (1997) A 1-Mb physical map and PAC contig of the imprinted domain in 11p15.5 that contains TAPA1 and the BWSCR1/WT2 region. *Genomics* **43,** 366–375.