Imprinted Genes as Potential Genetic and Epigenetic Toxicologic Targets

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Genomic imprinting is an epigenetic phenomenon in eutherian mammals that results in the differential expression of the paternally and maternally inherited alleles of a gene. Imprinted genes are necessary for normal mammalian development. This requirement has been proposed to have evolved because of an interparental genetic battle for the utilization of maternal resources during gestation and postnatally. The nonrandom requisite for monoallelic expression of a subset of genes has also resulted in the formation of susceptibility loci for neurobehavioral disorders, developmental disorders, and cancer. Since imprinting involves both cytosine methylation within CpG islands and changes in chromatin structure, imprinted genes are potential targets for dysregulation by epigenetic toxicants that modify DNA methylation and histone acetylation. *Key words*: Angelman syndrome, Beckwith-Wiedemann syndrome, cancer, genetic disorders, genomic imprinting, growth defects, *M6P/IGF2R*, oncogenes, Prader-Willi syndrome, tumor suppressor genes, Wilms tumor. — *Environ Health Perspect* 108(suppl 1):5–11 (2000).
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Genomic imprinting is an epigenetic form of gene regulation that results in monoallelic expression. It is stably inherited during somatic cell division but is reversed when transmitted through individuals of the opposite sex. Imprinting differs from classical Mendelian principles of inheritance because the two parental alleles are unequally expressed despite both parents contributing equal genetic content to their progeny. The expressed allele is also parent-of-origin dependent, unlike the random allele inactivation that occurs at the *Xist* locus in postimplantation embryos (*1–4*). Thus, imprinting is a phenomenon in which the expression of a gene in this generation depends on whether it resided in a male or female in the past generation.

Imprinted genes are normally involved in mammalian embryonic growth and development. They also act as disease susceptibility loci because their functional haploid state makes them vulnerable to being either inactivated or overexpressed. Parental-specific epigenetic events such as DNA methylation at CpG domains and histone acetylation are implicated in the initiation and maintenance of imprinting (*2,5*). Thus, environmental factors can induce imprint gene-dependent disorders and diseases by both genetic and epigenetic alterations (*6,7*). Because the imprinting of genes varies between species, individuals, tissues, cells, and stage of embryonic development (*8–12*), disease susceptibility due to alterations in genomic imprinting represents a substantial epidemiologic and genetic issue that must be addressed.

Imprint Gene Identification

Genomic imprinting was first described in the fly, *Sciara coprophila*, in which the paternal sex chromosome is preferentially lost from the germ line and the soma during embryogenesis (*13*). Imprinting has also been identified in maize (*14*), zebra fish (*15*), and a variety of other insects including *Drosophila melanogaster* in which the phenomenon has been referred to as parental effects (*13,16–19*). The viability of both gynogenic and androgenic flies and zebra fish (*20–22*) indicates, however, that imprinted genes are not as developmentally essential in these species as in mammals (*23–26*).

The existence of imprinted genes in mammals first became apparent when nuclear transplantation experiments demonstrated that diploid androgenotes derived from two male pronuclei and gynogenotes formed from two female pronuclei failed to develop properly during embryogenesis (*24,25*). Similarly, in humans complete hydatidiform moles, which contain only paternal chromosomes, produce primarily placental tissue, whereas dermoid cysts, which contain only maternal chromosomes, produce primarily embryonic tissue (*23,26*). These findings suggested that the mammalian genome contains autosomal genes required for development that are only expressed from either the maternal or paternal allele. These putative imprinted genes were subsequently localized in the mouse genome by the generation of mice with uniparental disomies (UPD) at specific chromosomal locations (*27,28*).

The first gene identified to have parent-of-origin–dependent expression was the autosomal transgene *RsvIgmyc*, which was expressed exclusively from the paternal allele in the heart (*29*). It was not until 1991 that the first endogenous imprinted genes were discovered (*30,31*). DeChiara et al. (*31*) observed that homozygous *Igf2* null mice were approximately 40% smaller than wild-type mice when they were born, consistent with the known growth effects of Igf2. Importantly, the dwarfing phenotype was also unexpectedly observed in heterozygous mice but only when the mutated allele was inherited from the father. This demonstrated that the *Igf2* gene is imprinted and expressed solely from the paternal allele. *IGF2* is also imprinted in human tissues with two notable exceptions: the adult liver, where expression is biallelic because of alternate promoter usage after birth (*10*), and the brain, where a natural loss of imprinting (LOI) results in biallelic expression in both the fetal brain and pons region of the adult brain (*32*).

The *mannose 6-phosphate/insulin-like growth factor 2 receptor* (*M6p/Igf2r*) was also shown to be imprinted but maternally expressed (*30*). The *M6p/Igf2r* maps to the T-associated maternal effect locus on mouse chromosome 17 (*30*) and is the gene responsible for this maternally inherited lethal effect (*33*). The *M6P/IGF2R* encodes for a receptor in mammals that binds both M6P-containing glycoproteins and IGF2 through independent binding sites (*34*). The primary functions of this receptor are the intracellular trafficking of phosphomannosyl glycoproteins from the Golgi apparatus to the lysosomes, and the internalization of IGF2 and other extracellular ligands to the lysosomes for degradation (*34*). IGF2 signaling is not mediated by the M6P/IGF2R, but rather it occurs principally through the IGF1 receptor (*35*) and the insulin receptor isoform A (*36*).

Imprinting characteristics are becoming apparent as more imprinted genes are identified. Imprinted genes are not randomly distributed throughout the genome but rather are frequently found clustered in imprinted domains. Two imprinted domains in humans that have been extensively investigated reside at human chromosomes 11p15.5 and 15q11-q13 (syntenic to the distal and central region of mouse chromosome 7, respectively). Imprinted genes have been identified within

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these domains that encode both translated and untranslated RNA (*37,38*) as well as antisense RNA that may be involved in imprint control (*39,40*). Imprinted genes such as *H19* and *IGF2* show coordinate regulation (*41*), and higher order regulation is imposed upon domains by imprint control centers (*42*). Imprinted genes also often reside in chromosomal regions that undergo asynchronous replication (*43,44*). Thus, the meiotic recombination frequencies in these regions may differ between the male and female germ cells (*45*). Another characteristic of imprinted genes is allele-specific cytosine methylation of CpG dinucleotides that appears to distinguish the parental alleles (*46–48*). Tandem, repetitive sequence elements associated with the areas of differential methylation have also been identified in several imprinted genes (i.e. *H19, M6p/Igf2r, U2afbp-rs* and $p57^{KIP2}$) (*49–53*).

Presently, more than 20 human imprinted genes have been detected, and it is postulated that 100–500 imprinted genes may exist (*54*). Identifying the full complement of imprinted genes may therefore represent a daunting task. Some of the unique characteristics of imprinted genes described above have provided a means to systematically screen for new imprinted genes. Positional cloning coupled with candidate gene testing has been used to identify novel human imprinted genes located in imprinted domains (*55–58*). Parental differences in DNA methylation and expression have also been used to detect imprinted genes (*59,60*). Subtractive hybridization or differential display techniques utilizing cDNA from gynogenotes, androgenotes, and fertilized embryos have yielded novel imprinted genes. These include the paternally expressed genes *Peg1/Mest,* a mesoderm restricted hydrolase at mouse chromosome 6; *Peg3,* a zinc-finger protein on proximal mouse chromosome 7; and *Peg5/Nnat,* located on mouse chromosome 2 (*61,62*). *Grf1* and *U2afl-rs1* were identified by a genomewide screen termed restriction landmark genome scanning using methylation-sensitive restriction enzymes (*60,63*). The *GABAA* receptor subunit genes *GABRB3, GABRA5,* and *GABRG3* were shown to be exclusively expressed from the paternal allele by microcell-mediated chromosome transfer (*64*). Clearly, the identification of new imprinted genes needs to be a top priority to further our understanding of the molecular mechanisms underlying both their expression and association with genetic disorders and diseases.

Evolution of Imprinting

The functional haploid state of imprinted genes eliminates inherent protection from deleterious recessive mutations. Therefore, genomic imprinting appears to be a risky method for regulating gene expression, particularly since imprinted genes are involved in such critical aspects of embryogenesis (i.e., growth control and behavioral development). Explanations for why and when imprinting evolved is therefore a hotly debated topic (*65,66*). Numerous theories have been proposed to explain the presence of imprinted genes. Their presence blocks parthenogenesis, guaranteeing a continued role for the male in mammalian reproduction. Although this may be comforting to the male gender, it is unlikely to be the driving force for imprinting, as it does not explain why both maternally and paternally imprinted genes exist in the genome; neither does the suggestion that imprinting developed to protect the cell against aneuploidy (*67*), which would predict a random distribution for imprinted genes rather than discrete domains. Imprinted genes are also postulated to have arisen to protect the female from ovarian germ cell tumors (*68*); however, again this does not explain why both maternally and paternally imprinted genes exist in the genome. An alternative proposal for imprinting suggests that the cytosine methylation involved in imprint regulation evolved as a defense mechanism for the inactivation of parasitic sequences such as transposable elements and proviral DNA (*69*). This is supported by the finding that 5-azacytidine, (5-azaC), an inhibitor of cytosine DNA methyltransferase, activates silent retroviruses (*70*). Nevertheless, it does not provide a compelling explanation for the reason this host defense system was used to create genes that are functionally haploid.

The reciprocal imprinting of the *Igf2* and *M6p/Igf2r* genes suggests that the evolution of genomic imprinting may have resulted from an interparental conflict to control intrauterine fetal growth (*71*). This parental tug-of-war model of Haig predicts that "...multiple paternity of a female's offspring, in combination with postzygotic maternal care, favors differential expression of maternal and paternal alleles in offspring such that the expression of paternal alleles increases the cost of the offspring to its mother, whereas the expression of maternal alleles reduces the cost to the mother" (*65*). Thus, paternally expressed genes are predicted to promote prenatal and postnatal growth, whereas maternally expressed genes would suppress growth. In support of this theory, the gene encoding the fetal growth factor IGF2 is paternally expressed, whereas *H19,* which encodes for an untranslated RNA involved in silencing IGF2 expression, is maternally expressed (*31,72,73*). The genes that encode for the M6P/IGF2R, which degrades IGF2, and MEG1/GRB10, which inhibits IGF2 signaling, are both maternally expressed (*30,34,74*). Finally, inactivation of *Peg1/Mest* and *Peg3* in mice results in growth retardation during embryogenesis, demonstrating that these two paternally expressed genes stimulate growth (*75,76*).

A number of predictions are made by this genetic conflict model. If the genetic interests of the male are to promote the growth of his offspring over those of competing males, monogamous species should not require such genetic mechanisms to guarantee potential fitness. Tilghman and colleagues (*77*) tested this postulate by generating crosses between the monogamous rodent species *Peromyscus polionotus* and the polyandrous *Peromyscus maniculatis*. Although the two species are similarly sized, the offspring generated from these crosses exhibited striking growth defects, consistent with the idea that *P. polionotus* does not harbor imprinted genes. Surprisingly, genomic imprinting was maintained in both parental species but was widely disrupted in the F_1 hybrids. These results do not necessarily differ with the prediction of the genetic conflict model because if monogamous behavior developed in *P. polionotus* after imprinting evolved in an ancestral parent, it may still persist even though a parental conflict no longer exists.

The parent-offspring conflict is also predicted to be absent when the parental genes are unable to influence the amount of nutrients the offspring receive from their mother during gestation. Thus, a second correlate of the genetic conflict model is that imprinting will play an important role in development in viviparous animals but not in oviparous animals. The platypus, *Ornithorhynchus anatinus*, is a monotreme that appears to be a eutherian mammal–avian hybrid. It is the closest oviparous relative of eutherian mammals. A conflict between the maternal and paternal genomes over control of intrauterine fetal growth and allocation of maternal resources should not exist in the platypus because its offspring hatch from an egg. Therefore, support for the genetic conflict model would also be generated by data showing a lack of imprinting in this oviparous species. Such a finding would also suggest that genomic imprinting is unique to eutherian mammals, where fetal growth occurs *in utero.* We are presently investigating these intriguing postulates.

Imprinting and Genetic Disorders

Regardless of which theory correctly accounts for the presence of paternally and maternally imprinted loci, a functional consequence of genomic imprinting includes the cellular loss of protection from deleterious recessive mutations. Ironically, this has led to an increased susceptibility to developmental defects, behavioral disorders, and cancer.

Beckwith-Wiedemann Syndrome

There are a number of human genetic disorders associated with imprinting defects (*78,79*). Beckwith-Wiedemann syndrome (BWS) maps to 11p15 and is characterized by general overgrowth, with symptoms including hemihypertrophy, macroglossia, and visceromegaly. Individuals with this disease also have an increased incidence of Wilms tumor, a childhood kidney malignancy (*80*). The most common molecular event occurring in BWS patients who do not have cytogenetic abnormalities is the biallelic expression of IGF2 due to LOI (*81,82*). LOI at the *IGF2* locus may be accompanied by the methylation and silencing of the active maternal allele of *H19* (*83,84*). Translocations in BWS patients may also lead to LOI at the *IGF2* locus but without loss of *H19* imprinting (*85*). These translocations affect imprinting by disrupting a gene involved in imprint control, or by altering the function of an imprinting center. It is possible that multiple genes within this imprinted cluster (e.g., *IGF2, H19, $p57^{KIP2}$, KvLQT1*) contribute to the etiology of BWS. However, the available evidence suggests that this syndrome results from the dysregulation of *IGF2* imprinting, since transgenic mice that overexpress Igf2 develop symptoms similar to BWS (*86*).

Prader-Willi and Angelman Syndromes

Two clinically distinct genetic disorders associated with genomic imprinting on chromosome 15q11-q13 are the Prader-Willi syndrome (PWS) and the Angelman syndrome (AS). Each syndrome is associated with deficiencies in sexual development and growth, as well as behavioral and mental problems including retardation (*3,87*). PWS and AS are autosomal dominant disorders showing parent-of-origin effects. The preferential loss of the paternal or maternal allele in PWS and AS, respectively, suggests the involvement of imprinted genes (*3*).

PWS is a developmental and neurobehavioral disorder that results from the loss of a paternally expressed imprinted gene(s) (*87*). The identity and number of genes involved in the etiology of PWS are unknown, but *SNPRN* (*small nuclear ribonucleoprotein N*) is the best-characterized candidate (*87–91*). This genetic locus appears to play a key role in regulating imprinting throughout 15q11-q13. Microdeletions in the 5′ end of *SNRPN* alter promoter methylation, prevent expression of the paternal allele of *SNPRN,* and result in silencing of other paternally expressed genes in this imprinted domain (*88–91*). These microdeletions in PWS disrupt one component of a bipartite imprinting center and block the maternal-to-paternal switch of the entire 2-Mb imprinted domain that occurs normally in the paternal germline (*87,88,90*). This leads to the inappropriate downstream effect of both parental alleles being imprinted, with subsequent silencing of the domain. SNURF (SNRPN upstream reading frame), a protein transcribed along with SmN from the *SNURF–SNRPN* bicistronic transcript, may be involved in this imprint-switching process (*92*). Offspring inheriting these microdeletions from their mother exhibit no apparent deleterious phenotype; however, a subsequent paternal transmission can result in PWS (*87,88,90*).

AS patients lacking a chromosomal deletion harbor a variety of mutations in *UBE3A,* a gene encoding for E6-AP ubiquitin–protein ligase involved in protein turnover (*93–95*). *UBE3A* maps to human chromosome 15q11-q13. It is maternally expressed in the human brain (*96,97*) and in the hippocampus and cerebellum of the mouse (*98*). Abnormalities in maternal-specific UBE3A expression during brain development are proposed to cause AS. A small percentage of AS patients also have microdeletions in the bipartite imprinting center at the 5′ end of *SNRPN* (*88,99,100*). These deletions are distinct and upstream from those involved in PWS and result in the loss of a novel 5′ exon of *SNRPN* (u5) (*101*). They block the paternal-to-maternal imprint switch that occurs normally in the maternal germline and may therefore define sequences that are involved in the regulation of the imprinting center. Consequently, progeny inheriting these microdeletions from their father do not develop AS. However, maternal transmission results in AS, presumably due to the aberrant paternal epigenotype of the maternal allele, which leads to transcriptional silencing of *UBE3A* and possibly other maternally expressed genes within this domain (*99*).

Imprinting and Behavior Development

The paternally expressed human *MEST* (mesoderm specific transcript) gene maps to 7q32, a region where maternal UPD is associated with intrauterine and postnatal growth retardation (*62,102*) and allelic loss with cancer (*103*). Recently, a targeted deletion was introduced into the coding sequence of the mouse homolog of *MEST, Peg1/Mest,* to determine its function (*76*). *Peg1/Mest*-deficient mice were viable and fertile when the deletion was paternally derived; however, they exhibited growth retardation and increased lethality as predicted by the genetic conflict model for imprint evolution (*65,71*). Decreased reproductive fitness in the females was also observed when the targeted disruption was inherited from their father. Maternal behavioral deficiencies included failure to ingest the extra-embryonic tissues (a normal behavior in most mammals), reduced rate of nest building, and pup neglect, compared to wild-type control mice. This effect was not based on the genotype of the progeny but rather was due to an abnormal nurturing behavior of the mutant parturient females.

Similarly, a mutation in the paternally expressed gene *Peg3* resulted in growth retardation as well as a striking impairment of maternal behavior that frequently resulted in death of the offspring (*75*). It is presently unknown whether inactivation of these genes in humans has a detrimental effect on maternal nurturing behavior. These results clearly demonstrate that epigenetic regulation of imprinted gene dosage can significantly alter mammalian growth and behavior. They are also consistent with the hypothesis that genomic imprinting arose in mammals over a parental genetic conflict to control distribution of maternal resources (*104*). They further suggest that the increased cost of progeny to the mother arising from paternally expressed genes does not end at parturition, as these same genes either directly or indirectly influence the nurturing capacity of the mothers toward their offspring.

Evidence for imprinting effects in human disorders associated with mental abnormalities includes the aforementioned Prader-Willi and Angelman syndromes. Skuse et al. (*105*) reported that an imprinted X-linked locus is potentially responsible for differences in cognitive function of females with Turner syndrome. In normal females (46, XX), one of the two inherited X chromosomes is inactivated. Turner syndrome results when all or part of one X chromosome is deleted in females and is manifested by a higher incidence of social difficulties (*106,107*). Evidence for imprinting came from the finding that maternally inherited X chromosome abnormalities (45, X^m) in Turner syndrome generally result in more behavioral difficulties than paternally inherited X-chromosome abnormalities (45, X^p) (*105*). Based on cytogenetic analysis it was determined that the putative imprinted locus escapes X inactivation and potentially lies in Xp11.23–Xqter. Interestingly, Miller and Willard (*108*) have recently identified a 5.5-megabase region on human Xp11.21–p11.22 that contains eight expressed sequences that escape X inactivation; an imprinted gene(s) in this region has yet to be identified.

Parent-of-origin effects involved in other behavioral and brain disorders have also been reported. Included among these are bipolar affective disorder (*109–111*), schizophrenia (*112,113*), and autism (*114*). The involvement of genomic imprinting in these examples remains to be elucidated. For an extensive summary of parent-of-origin effects in human disease, consult Morison and Reeve (*115*).

Imprinting and Cancer

Imprinted genes are normally involved in embryonic growth and behavioral development. When imprinting is disrupted, some of these genes can also either lose tumor suppressor function or gain oncogenic potential. Modifications in DNA methylation are proposed to have a mechanistic role in carcinogenesis (*116*). In the case of imprinted genes, such epigenetic alterations may be more immediately evident because of disruption to their normal functionally haploid state. Loss of heterozygosity or UPD at an imprinted locus may result in the deletion of the only functional copy of an imprinted tumor suppressor gene (*6,54*). Alternatively, LOI or UPD at an imprinted locus may result in increased expression of an imprinted protooncogene. Furthermore, genetic or epigenetic inactivation of an imprint control center could lead to abnormal expression of multiple imprinted protooncogenes and/or tumor suppressor genes, as imprinted genes often occur in chromosomal domains (*5,117*). Imprinted genes now known to be involved in carcinogenesis include *WT1, p57KIP2, p73, NOEY2* and the functionally related *IGF2,* and the *M6P/IGF2R* (*7,54*).

IGF2 encodes for a growth factor that has oncogenic potential when overexpressed (*118–120*). Direct genetic evidence linking tumorigenesis and aberrant imprinting was shown for Wilms tumor, in which 70% were found to have biallelic expression of IGF2 (*121–123*). LOI for *IGF2* was also found in both normal mucosa and colonic tumor tissues of patients having colorectal carcinoma (*118*). This indicates that increased IGF2 expression due to LOI is an extremely early event in oncogenesis. Deregulation of *IGF2* imprinting has now been shown to occur in over 20 different tumor types, demonstrating its fundamental mechanistic importance in carcinogenesis (*54*).

The *M6P/IGF2R,* at human chromosome 6q26, is inactivated in a variety of tumors at the earliest stage of transformation (*124–127*). It is mutated in 60% of dysplastic liver lesions and hepatocellular carcinomas of patients with or without hepatitis virus infection (*124,126–128*). The *M6P/IGF2R* is also mutated in rat liver tumors induced with the genotoxic agent diethylnitrosamine (*129*). The gene contains a poly-G region that is a common mutational target in colon, gastric, and endometrial tumors with mismatch repair deficiencies and microsatellite instability (*130–132*). Moreover, the *M6P/IGF2R* is mutated in human gliomas that do not contain mutations in the *transforming growth factor b type II receptor* or *Bax* genes (*130*), and in 30% of human breast tumors (*125*). Thus, the *M6P/IGF2R* is frequently mutated in a number of cancers, suggesting that this multifunctional receptor normally serves as a tumor suppressor.

Although gene imprinting is often conserved between mammalian species, the imprint status of the *M6P/IGF2R* in humans and rodents is strikingly different. The *M6p/Igf2r* is imprinted in mice (*30*) and rats (*129*), but imprinting at this locus appears to be a polymorphic trait in humans postnatally, with most individuals having biallelic expression (*12,133,134*). The existence of individuals with an imprinted *M6P/IGF2R* tumor suppressor suggests that they may have increased susceptibility to tumor development because of aberrant imprint control. This postulate is supported by Xu et al. (*135*), who reported partial imprinting of the *M6P/IGF2R* in 50% of Wilms tumor patients. Furthermore, only one inactivating event, or hit, rather than the two hits postulated by Knudson to be requisite for oncogenesis (*136*), would be needed to inactivate the tumor suppressor function of the M6p/Igf2r in mice. This may in part explain why mice are more sensitive to tumor formation than humans. It also suggests that transgenic mice with directed biallelic expression of the M6p/Igf2r may be better human surrogates for carcinogen risk assessment.

Regulation of Imprinting

A cellular consequence of genomic imprinting includes increased susceptibility to inappropriate gene expression through inactivation of functionally haploid loci. Importantly, both genetic and epigenetic targets associated with imprinted gene expression add to the inherent susceptibility of these loci to phenotypic abnormalities. These include the primary nucleotide sequence of the imprinted gene, regulatory sequences directing gene transcription activity, and imprinting control centers, which exert multigenic influence over domains. Cellular factors that are required to establish, maintain, and read the imprint marks are also potential targets for perturbation of the imprinting process. These cellular components include the enzymatic machinery required to both induce and sustain chromatin structure. Therefore, toxic agents capable of altering any one of these targets have the potential to elicit disease in the recipient. Depending on the particular allele affected, this may also generate a heritable, deleterious (epi)genotype that is inapparent until passed through the germline of the opposite sex.

Although there is compelling evidence for the role of sequence-specific elements in the control of imprinted gene expression (*87,90,137*), it is increasingly apparent that these regulatory elements are also highly dependent upon the context of their chromosomal location (*138*). In *Drosophila,* for example, translocation of euchromatic sequence into a region of heterochromatin induces condensation and gene silencing, a phenomenon referred to as position effect variegation (*16,139*). Similarly, imprinted expression of the mouse transgene *RsvIgmyc* contrasts with biallelic expression of the endogenous gene (*2,140*). These contextual effects may be induced by the interactions of multiple cis-acting regulatory elements; however, a more likely scenario is that they are induced by alterations in chromatin structure.

All imprinted genes identified thus far have exhibited differential methylation of parental alleles. Methylation, therefore, likely plays a key role in discriminating between the two alleles and transmittance of information for imprint reading (*2*). Differential methylation occurs primarily in CpG islands, which are roughly 1 kilobase in length and are rich in the CpG dinucleotide. The cytosine residue in this context is recognized by (5-cytosine) DNA methyltransferases, which add a methyl group to the 5-carbon position (*141–143*). CpG islands are found throughout the genome and are predominantly associated with genes (*144*). The importance of DNA methylation for the proper expression of imprinted genes was first demonstrated with the use of methyltransferase null mice (*145*). It was subsequently shown that methyltransferase activity inhibition, through treatment of cells with 5-azaC, also led to biallelic expression of IGF2 (*146*).

For genes not imprinted, CpG islands are primarily unmethylated and the genes are transcriptionally functional. Methylated CpG islands are normally heterochromatic and induce transcriptional silencing when associated with the promoter of an imprinted allele (*144*). Conversely, unmethylated CpG islands located 3′ of the promoter on the opposite allele are associated with transcriptional activity, often producing antisense RNAs (*3,147–149*). The antisense RNA produced from the *M6p/Igf2* imprinting box, located within an intragenic CpG island (*149*), is thought to function in *cis* to repress gene expression (*2*). This may be similar to the mechanism by which Xist RNA reportedly randomly inactivates the additional X chromosome in human females by first coating the length of the targeted chromosome, and then inducing heterochromatin spreading in *cis* (*2*).

Like unmethylated CpG islands, acetylated histones are associated with euchromatin (*150*). Therefore, differential histone acetylation between parental alleles may provide another means by which imprinted genes are regulated (*151*). In support of this postulate, treatment with histone deacetylase inhibitors results in loss of imprinting for both IGF2 (*151*) and H19 (*152*). The evidence indicates that imprinting is influenced by chromatin structure resulting from the status of both DNA methylation and histone acetylation.

Recently, a provocative connection between these two epigenetic modifications was established. The methyl-binding protein 2 binds specifically to methylated DNA and also forms a complex with histone deacetylase, providing a link between the two in the establishment of chromatin structure (*150,153,154*). It has been suggested that DNA methylation and histone acetylation (*155*) together serve as layers for epigenetic gene silencing, with methylation acting to commit genes into a transcriptionally repressed state (*153,156, 157*). Although a number of chemical agents disrupt DNA methylation (*158–161*) and histone deacetylation (*150,156,162*), their influence on genomic imprinting is largely unknown. It is therefore crucial to determine if these agents or other environmental toxicants are capable of causing alterations in genomic imprinting. Such a finding would have far-reaching ramifications both for our understanding of the disorders of genomic imprinting and for the design of potential preventative or therapeutic measures.

In summary, epigenetic mechanisms of gene inactivation involved in the progression to a disease state are becoming more widely recognized (*144*). Imprinted genes, therefore, need to be considered as toxicologic targets for both genetic and epigenetic alterations. Unfortunately, apart from the few studies showing that inhibitors of histone deacetylation and DNA methylation disrupt imprinting (*146,151,152*), little is presently known about the ability of physical and chemical agents to perturb the status of an imprinted gene, cluster, or domain.

Conclusions

Genomic imprinting has evolved in eutherian mammals as an elaborate mechanism to control gene expression. Imprint establishment, maintenance, and reading appear to involve both genetic (e.g., CpG islands, imprint boxes, primary sequence elements) and epigenetic mechanisms (e.g., chromatin structure, as a result of cytosine methylation and histone acetylation). The inherent plasticity of the imprinting system implies that deleterious alterations in such genes may result in a gradation of phenotypical effects. Furthermore, inheritance of an imprinting mutation may not be evident if the sex of the carrier does not switch, as is seen in Prader-Willi and Angelman syndromes (*99,163,164*). An additional complication is that some genes exhibit polymorphic imprinting (*11,165–167*). Thus, genomic imprinting not only creates serious complications for genetic counseling of afflicted families, but also for human risk assessment. It is necessary to now directly test the ability of environmental pollutants to alter imprinting and disease susceptibility.

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