Normal development and neoplasia: the imprinting connection

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ABSTRACT The observation that a number of autosomal genes are expressed in a parent of origin-dependent monoallelic manner has fuelled a frantic research effort into the underlying mechanisms and biological functions of this phenomenon, termed genomic or parental imprinting. The level of intrigue associated with this subject has been heightened by the discovery that the "transcriptional phenotype" of some imprinted genes shows developmental and tissue-specific variation, and that some imprinted genes are expressed biallelically in tumors. Here we describe some further examples of variation in the allele-specific transcription of an imprinted gene, human *IGF2*. Analysis of different sub-clones of an established tumor cell line (Jeg-3) revealed examples of both a switch from monoallelic to biallelic expression, as well as monoallelic expression from the opposite parental allele. Examination of *IGF2* expression in adult human liver clearly demonstrated that the functional imprinting is manifested in a promoter-specific manner. The P1 promoter produced biallelically derived transcripts, whereas the remaining three promoters were utilized in a complex pattern of mono- and biallelic expression which varied from sample to sample. These observations emphasize the need to re-examine the imprinting phenomenon and its plasticity in terms of the *cis* elements and *trans*-acting factors involved in the transcriptional regulation of these genes both in the normal and pathological contexts.

KEY WORDS: *genomic imprinting, development, cancer, transcriptional regulation*

Introduction

It is now more than ten years ago since the intriguing observation was made that the presence of both parental genomes is necessary for normal mouse embryonic development. (McGrath and Solter, 1984; Surani *et al.*, 1984; reviewed in Surani, 1986). This is graphically illustrated by the severely perturbed development exhibited by uniparental diploid embryos. In parthenogenetic conceptuses, the extra-embryonic tissues remain rudimentary whereas the embryo proper can develop as far as the 25 somite stage (Surani, 1986). By contrast, androgenetic conceptuses develop extra-embryonic membranes relatively well, but produce a poorly-developed embryo proper, which only reaches the 6-8 somite stage at best. Such reciprocal uniparental phenotypes have been attributed to reflect abnormal gain or loss of functions encoded by genes which are transcriptionally controlled in a parent of origin-dependent fashion (Walsh *et al.*, 1994). This phenomenon of preferential expression of certain genes in a parent of origin-dependent manner has been termed "imprinting".

The proportion of genes which may be regulated by imprinting is not known at present. The production of F1 mice showing phenotypic effects when paternally or maternally disomic for different chromosomal regions has generated an imprinting map (Beechey *et al.*, 1990). By using this and other approaches, a dozen or so loci have been documented to be expressed in a parent of origin-dependent manner (Efstratiadis, 1994). Of these, the *IGF2* and *H19* loci are of particular interest, since they are expressed from opposite parental alleles, despite a close physical linkage. The two genes also show a striking similarity in their expression patterns during mouse (Lee *et al.*, 1990; Poirier *et al.*, 1991) and human (Ohlsson *et al.*, 1994) prenatal development. *IGF2* produces the insulin-like growth factor II, which is an important growth factor for a large variety of cell types (Rotwein, 1991). The enigmatic *H19* gene produces a transcript with no conserved open reading frame or detectable protein product (Pachnis *et al.*, 1988) and may, therefore, function at the RNA level (Brannan *et al.*, 1990). Clues to possible biological functions come from the experimental over-expression of *H19*, which is lethal during embryogenesis (Brunkow and Tilghman, 1991) and indicates tumor suppressor properties in malignant cells (Hao *et al.*, 1993).

A number of human diseases are now thought to involving genes which exhibit parental imprinting. The Beckwith-Wiedemann syndrome (BWS), for example, has been genetically linked to chromosome 11 p15.5 which includes both *IGF2* and *H19* (Ping *et al.*, 1989; Feinberg, 1993; but see Nyström *et al.*, 1994). Hence, the overgrowth symptoms of this disease can be

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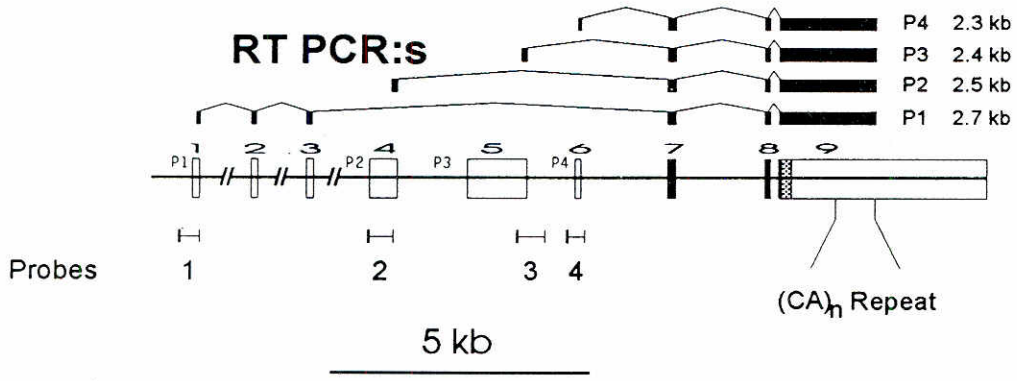

Fig. 1. A schematic view of the *IGF2* transcriptional unit with the four different promoters and polymorphic (C-A)$_n$ repeat indicated. *The promoter-specific cDNA transcripts indicated in the Figure were obtained by reverse transcribing total RNA primed at the 3'-end of the (C-A)$_n$ repeat. Each promoter-specific cDNA was then singled out by PCR amplification using promoter-specific primers paired with a common primer at the 3'-end of the (C-A)$_n$ repeat.*

attributed to excessive *IGF2* activity (Hedborg *et al.*, 1994) which is generated either by a duplication of the active paternal allele (Ohlsson *et al.*, 1993) or by the activation of the normally silent *IGF2* allele (Weksberg *et al.*, 1993). It is of particular interest here that the loss of the active, maternally derived *H19* allele has been suggested to facilitate neoplastic conversion of the kidney to generate Wilms' tumors (Hao *et al.*, 1993) which would be in keeping with its suggested tumor suppressor role.

When the phenomenon of parental imprinting was first discovered, it was assumed (perhaps somewhat naively in retrospect) that the monoallelic expression of imprinted genes would be manifested in every tissue and at all developmental stages. Many of the genes now characterized, however, show monoallelic expression only in certain tissues (DeChiara *et al.*, 1991; Giddings *et al.*, 1994), at certain developmental stages (Kay *et al.*, 1993), or within a subgroup of individuals in the population (Forejt and Gregorová, 1992; Jinno *et al.*, 1994). The normally repressed maternal allele of *Igf-2*, for instance, has been found to be active in the choroid plexus and leptomeninges of the mouse (DeChiara *et al.*, 1991) and man (Ohlsson *et al.*, 1994), giving biallelic expression in these tissues alone. Similarly, the mouse *Ins-1* and *Ins-2* genes are expressed in a parent-of-origin specific manner in the mouse yolk sac, but this is not the case in the pancreas (Giddings *et al.*, 1994). Temporal variation in imprinting status is exhibited by the mouse *Xist* locus, which is paternally expressed in morulae and blastocysts, but from which there is random monoallelic expression from gastrulation onwards (Kay *et al.*, 1993). Some reports also suggest that the imprinted state may depend on the primary sequence of the gene, so that only certain alleles of a polymorphic gene can undergo imprinting. This phenomenon may be seen at the *Tme* locus in mice, which is tightly linked to the *Igf-2r* gene and is expressed primarily from the maternal allele (Barlow *et al.*, 1991). Genetic studies have suggested that the *Tme* locus is only imprinted in certain species of inbred mice (Forejt and Gregorová, 1992), although a final verdict on this has not yet been reached. A similar variability has also been suggested for the human *WT-1* gene, which is biallelically expressed in kidney and some placentas, but is active from the paternally derived allele only in some placental and brain tissues (Jinno *et al.*, 1994). The human *IGF2R* gene also appears to be imprinted in only a sub-population of individuals (Xu *et al.*, 1993). Such variations in the manifestation of the imprint may reflect an incomplete penetrance, or may imply the presence of modifiers, the presence or absence of which can have a profound influence on the epigenetic mark and/or its interpretation. Such modifier genes have been shown to affect the expression of transgenes in mice in a strain-specific fashion (Sapienza *et al.*, 1989; Allen *et al.*, 1990; Engler *et al.*, 1991). These observations point to a certain plasticity in the interpretation of the imprint, to allow tissue and stage-specific reprogramming of the allelic usage of the gene.

Opposite allele usage of *IGF2* in human tumor cells: loss of imprinting?

An important recent observation has been that the *IGF2* gene does not appear to be functionally imprinted in tumors. This "loss of imprinting" has been speculated to lead to a growth advantage by allowing transcription from both alleles (Feinberg, 1993; Ogawa *et al.*, 1993; Rainier *et al.*, 1993). A problem inherent in these studies is that the extraction of RNA from whole pieces of tissue gives an averaging effect and so any heterogeneity in the allelic expression patterns of cells within the tissue (due to mosaicism for instance) might be missed. To investigate possible *IGF2* expression heterogeneity in cells of the trophoblast lineage, we wondered whether the allele usage in the Jeg-3 choriocarcinoma cell line is homogeneous, or if variations can be detected in sub-populations of these cells.

We first established a system for evaluating the allele usage of *IGF2* in normal tissues. To discriminate between the parental *IGF2* alleles, we exploited the (C-A)$_n$ repeat polymorphism of exon nine, which is common to all known *IGF2* transcripts, with the exception of one 2.2 kb species (Fig. 1). The *IGF2* genotype was determined by analyzing such (C-A)$_n$ repeats by a polymerase chain reaction (PCR) amplification procedure, followed by RNase protection analysis using ^{32}P-labeled RNA probes generated from either parental allele (schematically shown in Fig. 2) (Ohlsson *et al.*, 1993). In this assay, use of an RNA probe derived from the cDNA of one particular allele will fully protect only the identical corresponding mRNA: transcripts derived from other alleles will be clipped at mismatch sites, giving a banding pattern characteristic for each type of allele. Extensive screening has identified 7 alleles assorting at the polymorphic (C-A)$_n$ repeat locus of *IGF2*. By PCRing the DNA isolated from the same samples, the alleles present in the genome and the total

cellular RNA can be directly compared. Figure 3 shows the allele usage pattern in a family in which the mother was heterozygous and the father homozygous with respect to polymorphic *IGF2* alleles. When the DNA and RNA of the offspring were analyzed, it was clear that only the paternally derived *IGF2* allele was expressed. This approach has been used to more extensively assay the allelic usage of *IGF2* during human pre- and postnatal development. *IGF2* was found to be imprinted in all of the human prenatal and perinatal tissues examined, except in the choroid plexus and leptomeninges of a perinatal patient (Ohlsson *et al.*, 1994). It is not known whether *IGF2* is monoallelically expressed from the paternal chromosome in the precursor cells from which the choroid plexus and leptomeninges cells derive (in which case the imprint must be ignored in their daughter cells), or whether these precursor cells fail to establish the imprint at all.

Primary cultures of fibroblasts have been shown to maintain a monoallelic expression pattern of *IGF2* (Eversole-Cire *et al.*, 1993). Interestingly, this contrasts with the situation for the trophoblast-derived malignant Jeg-3 cells. Here, although an "early" passage (passage 100) of the Jeg-3 cell line (obtained from ATTC) also appears to express *IGF2* mono-allelically, a later passage (passage 150) appears to express both parental alleles. To establish whether the apparently biallelic expression of *IGF2* was due to the culture containing two subpopulations of cells, each expressing a different allele, we subcloned Jeg-3 cells by stable transfection and neomycin selection. A total of 63 resistant clones were isolated and expanded to enable the analysis of *IGF2* allele usage. The surprising outcome of this experiment was that 8% expressed one allele type exclusively, 9% expressed the other allele type exclusively and 83% of the subclones expressed *IGF2* from both parental alleles. Since the Jeg-3 cell line has undergone serial cloning procedures prior to its availability at ATTC, there is a possibility that unusual *in vitro* artefacts occur. If so, the mutability of the allele usage pattern in cultured cells suggests that the epigenetic modification pattern controlling a gene's expression is more susceptible to damage or to change than the DNA itself. If, on the other hand, these results are not due to *in vitro* artefacts or to epigenetic mutation, then an explanation for the allele usage in these cell lines would have to be found within the context of the currently understood principles of imprinting of *IGF2* (and other genes) *in vivo*.

These observations suggest interesting parallels with the gene which seems to initiate X chromosome inactivation, *Xist* (Kay *et al.*, 1993). *Xist* is first expressed from the paternal allele only, then from about gastrulation onwards, from one parental allele which is randomly-chosen in any given cell, a so-called chromosome counting mechanism. Here, the imprint on the gene may only serve to bias a basically random process under a certain developmental time period, after which the information as to parental origin represented by the imprint is ignored. It is interesting to speculate that this might be true of other imprinted genes, too. A certain leakiness in the bias might then be expected, such that the "wrong" parentally-derived allele might be selected for activation or repression in a sub-population of cells. This scenario would imply a "committed step" in the choice of active and inactive alleles following cell division, which on the evidence of the clonal *IGF2* expression patterns in Jeg-3 cells, may possess some degree of self-propagation. This might

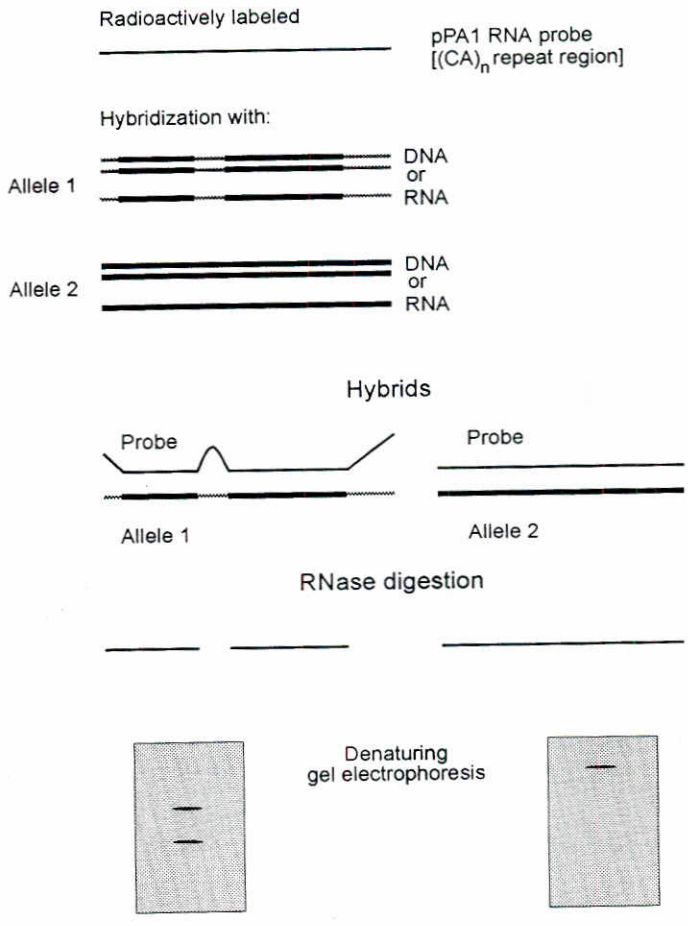

Fig. 2. The principle of RNase protection to assess *IGF2* allelic usage directly at the RNA level. *The allele-specific fragmentation of the ^{32}P-labeled RNA probe (encompassing the polymorphic (C-A)$_n$ repeat within exon 9), is obtained when either total cellular RNA or PCR-amplified exon 9 is analyzed. A comparison of the band patterns identifies the active allele. Reproduced from Nyström et al. (1994) with kind permission from the Publisher.*

explain such results as the allele switching and biallelic expression seen in some tissues for *H19* (Zhang *et al.*, 1993) and the observation of monoallelic expression of *WT1* in only some placentas (Jinno *et al.*, 1994). Although the Jeg-3 cell line was established by serial cloning, it is not possible to exclude an initial heterogeneity in the population with regard to *IGF2* allele usage. Even if only a tiny fraction of the cells initially expressed the maternal allele (or both alleles simultaneously), the serial cloning and selection could fix this allele usage in subsequent cell cultures. Alternatively, a relaxation of the monoallelic expression may also account for the transition from monoallelic to biallelic expression for the population of cells as a whole. It has been noted that the "loss of imprinting" of *IGF2* seen in several tumor types, which has been suggested to represent the relaxation of monoallelic control, might equally well reflect a heterogeneity in the precursor cell population with respect to allele usage (Ogawa *et al.*, 1993).

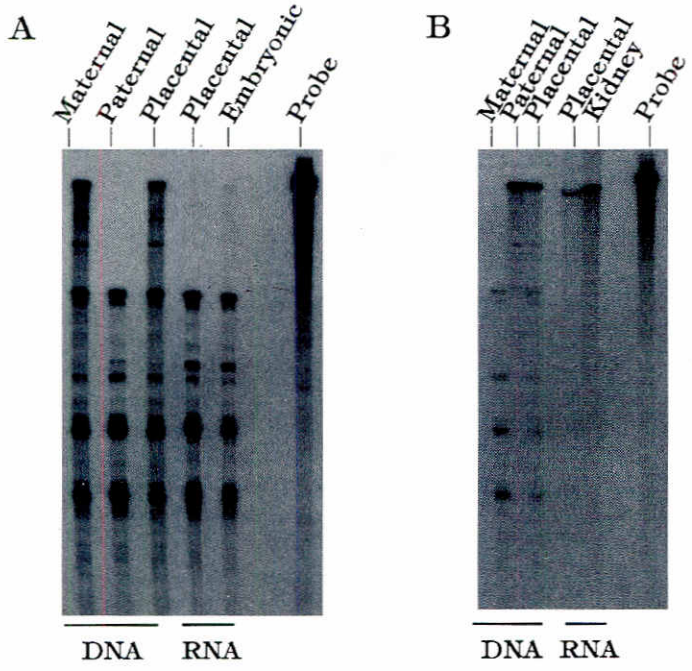

Fig. 3. *IGF2* is imprinted in the index patient of a familial form of the Beckwith-Wiedemann syndrome and in first trimester placenta and embryo. *RNA and DNA depict RNase protection analysis of total cellular RNA and the PCR-amplified polymorphic (C-A)$_n$ repeat region within exon 9 DNA, respectively. Reproduced from Ohlsson et al. (1993) with kind permission from the Publisher.*

The loss of *IGF2* imprinting during human liver postnatal development reflects promoter-specific allele usage

Recent work suggests that a reversal to a paternal epigenotype on the maternal 11p15.5 in humans causes activation of the silent *IGF2* allele and repression of the active *H19* allele (Moulton *et al.*, 1994; Steenman *et al.*, 1994). Tumorigenesis in these tissues has therefore been speculated to be a consequence of the events leading to biallelic expression of *IGF2*. We have obtained data suggesting that the allelic usage of *IGF2* is temporally controlled during human liver development. Hence, while overall expression is monoallelic during embryonic, fetal and neonatal liver development, samples from later developmental time points show overall biallelic expression (Ekström *et al.*, 1995). These results might reflect a temporal relaxation of imprinting by the erasing or neutralization of parental imprints during human postnatal development. Alternatively, the allelic activity of the four promoters could have been modified so that one promoter is active from one parental allele while other promoters could be active from the other parental allele. To investigate this possibility we elaborated on a RT-PCR and RNase protection technique which we had developed earlier. By combining a primer 3' of the polymorphic (C-A)$_n$ repeat in exon 9 with a 5' primer for either exon 1, 4, 5 or 6 (specific for promoters 1, 2, 3 and 4, respectively), each promoter-specific transcript can be analyzed individually (Fig. 1). Subsequent RNase protection experiments allowed us to address the imprinting status of each specific promoter.

The results of these investigations are schematically summarized in Fig. 5, which shows that in all the informative liver specimens investigated (covering a wide range of developmental timepoints) the P1 promoter directed expression from both parental alleles. Because expression from the P1 promoter is very low prenatally, an examination of the pool of all the *IGF2* transcripts in the prenatal stages shows an overwhelming predominance of paternal transcripts, explaining the apparent contradiction between the findings using promoter-specific and non-specific probes. The P4 promoter-derived transcripts were from one allele only in all liver cases examined. The picture becomes more complex with the P2 and P3 promoters. The P3 promoter-derived transcripts came from both parental alleles in three out of four adult specimens, as well as in the 18-month-old liver sample. Hence, the P3 promoter allelic usage can be relaxed during liver development. We also found that the parental allele transcribed via the P2 and P3 promoters was sometimes the opposite to the one used by the P4 promoter (Fig. 4). This could, of course, reflect mosaicism in allele usage among the population of cells in the liver. This issue was addressed by hybridizing ^{35}S-labeled antisense RNA probes for exons 4 and 6 (specific for P2 and P4 derived transcripts, respectively) to adjacent thin sections of one liver which expressed the P2 and P4 transcripts from opposite alleles. This revealed that the P2 and P4 promoters direct expression from opposite parental alleles in the same cell (data not shown). It therefore seems that the parental chromosome from which transcription will occur can be specified independently for each promoter.

We conclude that the biallelic expression of *IGF2* seen during postnatal human liver development may be the sum of many effects: i) an increase in P1 promoter activity on both alleles during development; ii) a frequent switch from monoallelic to biallelic transcription from P3 and iii) the opposite allele usage seen for P2, P3 and P4 in some individuals. These results indicate that imprinting at the *IGF2* locus is a complex phenomenon, involving different allele usage patterns for each promoter, which can themselves show further variation over developmental time. With such varied parental allele usage occurring at the different *IGF2* promoters, it seems reasonable to imagine that the imprint must be specified in a promoter-autonomous fashion. This would seem to contradict the proposal that there might be a single locus which specifies the imprinting pattern of both *IGF2* and the neighboring *H19* gene in an interdependent manner (Bartolomei *et al.*, 1993; Brandeis *et al.*, 1993; Surani, 1993). It is possible to imagine, however, that the allele usage patterns of the *IGF2* and *H19* genes are initially synchronized, but that this relationship is uncoupled during the later stages of development. The ability of the cell to grow and divide changes during development and it may be that the replication status of the cells can affect the ability to maintain the imprint. It has been shown that imprinted loci are replicated asynchronously on the two parental chromosomes (Kitsberg *et al.*, 1993), in contrast to the synchronous replication of the parental alleles at most loci. The liver specimens of older patients are likely to be dominated by non-replicating cells which, nonetheless, express *IGF2*. It may be that it is harder for a cell to maintain the original imprint when it is not replicating, although this is, of course, very speculative.

Discussion

It is clear from the data presented in this and other reports that the functional read-out of the imprinting process can exhibit both tissue and promoter specificity during normal development. In addition, loss of imprinting has been suggested to be of importance in an increasing number of human neoplastic diseases (Ogawa *et al.*, 1993; Rainier *et al.*, 1993; Li *et al.*, 1995). What are the mechanisms that regulate these processes and is the loss of imprinting in cancer cells a normal but deregulated process? Any answer to these questions will obviously first require a much clearer understanding of the molecular mechanisms which control the "normal" monoallelic expression of the imprinted loci. It appears clear that imprinting can be divided into three main stages: i) initiation, ii) establishment and iii) maintenance and propagation. Whereas it is widely assumed that the parent of origin-dependent expression patterns are initiated during male and female gametogenesis, secondary imprints may be established postzygotically. While there is no evidence that the initial gametic imprint can be reversed in somatic cells, it is possible that the parental-specific epigenotype may be switched during neoplasia by strategically placed gene conversion or somating cross-over events. Such hypothesis would depend on the existence of imprinting centers which could coordinate or even induce secondary imprinting patterns over replicon-sized (i.e. several mb) regions. Such possibility may be hinted at by the observation that the specific loss of a CpG island in a patient suffering from PWS correlates with a loss of methylation over at least 300 kb (Sutcliffe *et al.*, 1994). These and other data (Brandeis *et al.*, 1993) highlight the fact that functional imprinting involves secondary modifications, probably triggered postzygotically by the gametic imprint. While a mutation within an imprinting center could prevent a reversal of the primary imprint, the total loss of the imprinting center could erase the imprinting status of the entire locus. Together with the demonstration that clusters of imprinted loci are asynchronously replicated, with the paternal alleles always being replicated first, it is clear that imprinting hierarchies must exist. Whether or not the same regulatory element can control both the imprinting status and the asynchronous replication in these examples is currently not known. Since the clusters of imprinted genes are interrupted by non-imprinted genes, it is likely that such long-ranging cis elements would directly or indirectly interact with locus-specific parental imprints. Our observations that the *IGF2* promoters can determine the allele usage is in keeping with this possibility. Let us then consider the role of transcriptional regulatory elements in the imprinting phenomenon.

Transcription and imprinting

Since imprinting involves the parental allele-specific *transcription* of genes, it would be expected that DNA sequences involved in transcriptional regulation (promoters, enhancers, silencers, locus control regions etc.) would represent the targets for the imprinting mechanism. It may well be, therefore, that genes which are associated with several cell type-specific or developmental stage-specific promoters or enhancers will not exhibit the same imprinted status in all situations. Special care should, therefore, be taken in the evaluation of any potentially imprinted gene which may be driven by multiple promoters. This

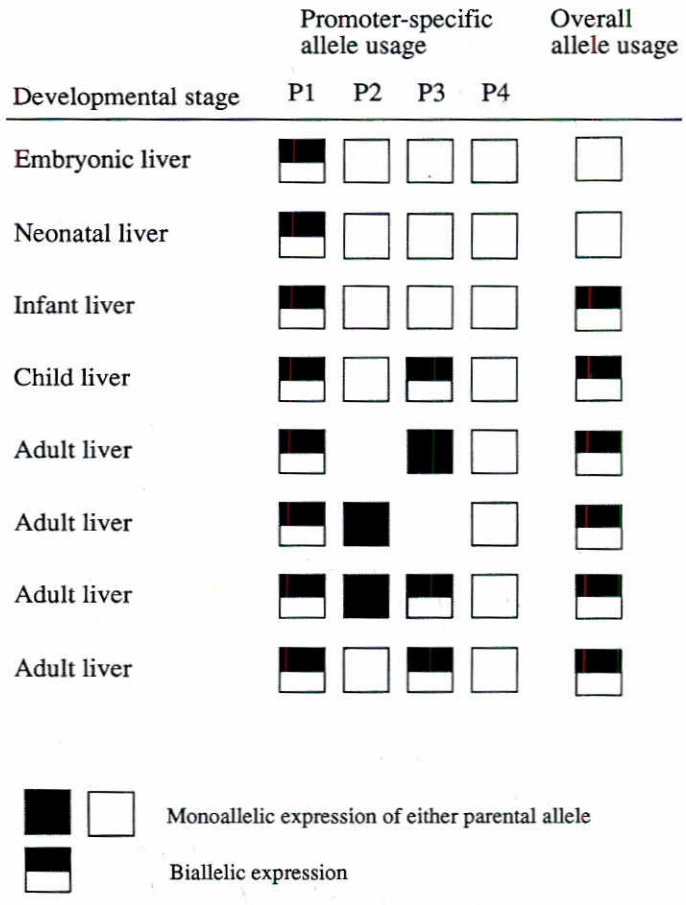

Fig. 4. Schematic summary of the promoter-specific *IGF2* allele usage during human liver development. *Open or filled boxes depict opposite parental alleles, respectively.*

is particularly important with respect to the region of the mRNA targeted for RNase protection assays, where alternative choices of probe could result in very different conclusions, as seen here with regard to *IGF2*.

The plasticity of imprinting, as exhibited by *IGF2* in human liver, could be the result of either a change in the epigenetic imprint, or a change in the response of the transcriptional machinery to that imprint. Changes in the availability of the various components of the complex transcription apparatus (Buratowski, 1994) whether in a cell type-specific, developmentally regulated or disease-related situation, could be envisaged to produce a new transcriptional response by "imprinted" alleles. For example, if the "imprint" is targeted to a particular protein-binding DNA sequence whose function becomes redundant in response to a new repertoire of available factors, then that cell could "ignore" the original imprinting signal. This is all the more plausible in the light of the "modular" nature of transcriptional control elements (Dynan, 1989) i.e., that promoters and enhancers are known to be made up of multiple modules that represent discrete protein binding sites which can interact to produce a different overall effect of the element, depending on which of the potential element-binding factors are available

(Tijan and Maniatis, 1984). It is possible that an imprint would be limited to one such key regulatory module within a promoter or enhancer and that plasticity in imprinting would not therefore be limited to genes which are regulated by multiple promoters and/or enhancers.

The epigenetic status of the alleles of imprinted loci is generally thought to be of great importance in controlling their transcription, but the cause and effect relationship between epigenetic modification and transcription is not entirely clear. For example, it is known that not all transcription factors are inhibited by chromatin structure and that many such factors might actually function by actively displacing nucleosomes from their binding sites (Workman and Buchman, 1993). This could represent one way in which the repertoire of transcription factors might ignore or re-interpret the primary imprint. It is also possible that the epigenetic status of the alleles of an imprinted gene might be determined by their transcriptional status and not vice-versa, as is often assumed. Hints that this type of regulatory hierarchy might exist can be seen in *Drosophila* studies, where the spatially-determined "on" or "off" transcriptional status of the *Ubx* gene at a crucial developmental time point defines whether or not a stable DNA/protein complex forms on the promoter and maintains the corresponding transcriptional state of the promoter through multiple cell divisions (Chan *et al.,* 1994). It has also been proposed that non-processive transcriptional "idling" may play a role in maintaining open chromatin configurations of some genes, such as c-*myc* (Krumm *et al.,* 1993). This may apply to the *IGF2* locus in which both parental transcriptional units display an open chromatin structure (Sasaki *et al.,* 1992). Another intriguing recent observation with regard to epigenetic modification and imprinting is that the immunoglobulin κ-chain enhancer can induce cell-specific and developmentally regulated active de-methylation of its promoter (Lichtenstein *et al.,* 1994).

Mechanisms underlying biallelic expression of imprinted loci

The observation that the monoallelic expression patterns of imprinted genes can vary through developmental time may not, therefore, be so surprising, given that the response to the imprint may change in concert with the variations in the transcription factor composition. In addition, it is worth considering what the "biallellic" expression of imprinted loci in early development really represents. It is almost impossible to make any sensible quantitative comparisons between the transcriptional output of the two alleles expressed in very early development and the monoallelic expression in the later stages on a "per cell" basis. It equally difficult, therefore, to evaluate whether the biallelic to monoallelic switch represents the selective repression of one of two highly active alleles, the activation of one of two very weakly expressed alleles, or a combination of both these effects. It is possible that the biallelic expression of some imprinted loci detected in very early development derives from a "neutral", basal level of transcription which is not subject to the positive and negative regulatory mechanisms which apply at all later stages of development. Recent research has indeed indicated that some of the fundamental mechanisms involved in transcriptional regulation may change over developmental time: both the requirement for an enhancer to stimulate high level transcription (Majumder *et al.,* 1993) and the requirement for a TATA box in activated transcription (Majumder and DePamphilis, 1994) have been reported to be developmentally acquired in the mouse embryo. This may be of some relevance to the initially biallelic expression of the *Xist* gene during early mouse development. The precise characterization of any such developmental-dependent changes in transcriptional mechanisms which coincide with a switch in biallelic to monoallelic expression could obviously be of great value in pinpointing the mechanism of the imprinting process itself.

The plasticity in the imprinting status in both normal and neoplastic cells should perhaps be seen from these points of view. In addition, the allele-specific asynchronous replication of imprinted genes adds to the complexity of this situation. It is possible, for example, that the biallelic expression of *IGF2* in the Jeg-3 choriocarcinoma cells reflects the loss of asynchronous allele replication. An alternative explanation could invoke deregulated activity of transcription factors that are normally active only during one part of the cell cycle, during which time they interact exclusively with the replicating allele. The E2F factor is an interesting candidate, since it interacts with the Rb protein which inhibits its activity in a cell cycle-specific manner, such that the transcription factor is active only during the G1/S transition period (La Thangue, 1994). Hence, a mutation in Rb could be envisaged to relieve this inhibition, so that E2F would be active during the entire S phase, potentially overriding the effect of an asynchronously replicated locus and generating biallelic expression. A model in which cell cycle-specific transcription factors, regulated by tumor suppressor proteins, contribute to the monoallelic expression of certain genes in normal cells by specific interaction with one of the asynchronously replicated alleles may be particularly inviting, given the "loss" of imprinting described in cancer cells. This scenario would not require the cell cycle-specific expression of the imprinted gene itself, since the hypothesized interaction could represent a "priming" event (such as transcription-dependent chromatin changes, as discussed previously) that would lead to stable transcriptional activity until the following cell cycle. How this situation would apply to non-replicating cells is another matter, of course. It is possible that once "primed" during the final cell division, the monoallelic transcription is maintained. This might underlie the previously proposed "difficulty" for non-replicating cells to maintain monoallelic expression, since there could be no cell cycle-dependent re-priming to rescue the biallelic expression in such cells. It is clear that the mysteries of genomic imprinting will probably require a more integrated approach in the future, demanding a deeper understanding of the genetic, epigenetic and transcriptional factors involved, before the final denouement can be achieved.

Acknowledgments

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