Promoter-specific IGF2 imprinting status and its plasticity during human liver development

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SUMMARY

IGF2 has been shown to be expressed preferentially from the paternally derived allele, although the maternal allele can be found active during both prenatal and postnatal development as well as in neoplastic tumours in humans. We addressed here whether or not the biallelic expression patterns that can be seen during postnatal human liver development reflected a coordinated change in the activities of the four promoters of human IGF2. We show here that the P2, P3 and P4 promoters, but not the P1 promoter, display monoallelic activity in embryonic, neonatal and younger infant liver specimens. The P2, P3 and P4 promoters can, however, be found active either monoallelically or biallelically or even monoallelically on opposite parental alleles in older infant and adult liver specimens. In contrast, H19, which is closely linked to IGF2, is monoallelically expressed in all postnatal liver samples analysed. We conclude that the functional imprinting status of IGF2 during postnatal liver development appears to be promoter/enhancer-specific and either partly or completely independent of H19.

Key words: IGF2, human, liver, promoter, imprinting

INTRODUCTION

An increasing number of both murine and human autosomal loci are found to be imprinted, i.e. expressed in a parent-of-origin-dependent manner (Efstratiadis, 1994). This asymmetric expression pattern may underly the loss or gain of gene expression in several pathological conditions (Ogawa et al., 1993; Rainier et al., 1993; Walsh et al., 1994). Disruption of imprinting of IGF2, for example, has been implicated in subtypes of the Beckwith-Wiedemann syndrome (Wekberg et al., 1993) and Wilms’ tumours (Ogawa et al., 1993; Rainier et al., 1993). In these instances, both parental alleles are transcriptionally active, presumably generating an overall increase in IGF2 expression levels, as only the paternally derived allele is preferentially expressed normally (Ogawa et al., 1993; Rainier et al., 1993; Ohlsson et al., 1993; Giannoukakis et al., 1993). The activity of the maternal IGF2 allele in the Wilms’ tumours has been suggested to be linked to the silencing of the closely linked and maternally expressed H19 locus (Zhang and Tycko, 1993; Moulton et al., 1994; Steenman et al., 1994). Such data is consistent with the notion that some form of common control exists over the expressivity of the neighbouring, imprinted IGF2 and H19 loci (Bartolomei et al., 1993; Efstratiadis, 1994). In addition, IGF2 has been found to be expressed biallelically during normal development: notably in the choroid plexus/leptomeninges of both mouse (DeChiara et al., 1991) and man (Ohlsson et al., 1994) and postnatal human livers (Kalscheuer et al., 1993; Ohlsson et al., 1994; Davis, 1994). It remains to be established, however, whether or not the biallelic expression patterns of IGF2 observed during normal and abnormal development are generated by a similar mechanism(s).

Expression of IGF2 is controlled by four different promoters, as well as alternate splicing patterns and poly(A) addition sites (van Dijk et al., 1991; Sussenbach et al., 1991). While the P2, P3 and P4 promoters have their counterparts in the rodents, the P1 promoter appears to be unique to humans. The P2, P3 and P4 promoters are generally perceived as being active primarily during embryonic/fetal development although a number of adult tissues express transcripts derived from these promoters (Rechler and Nissley, 1990; Glaser et al., 1992; Ekström et al., 1995). The spatial expression patterns directed from the P2, P3 and P4 promoters appear to be coordinated during human prenatal and early postnatal development (Ohlsson et al., 1994). In addition, the postnatal choroid plexus/leptomeninges and liver express P1 promoter-derived transcripts (Ohlsson et al., 1994). To explore whether or not there was a correlation between P1 promoter usage and biallelic expression of IGF2, we employed a reverse transcriptase-PCR approach for each IGF2 promoter independently. We document here that the P1 promoter directs expression from both parental alleles in prenatal as well as postnatal liver specimens. Surprisingly, the P2, P3 and P4 promoters were either monoallelically or biallelically expressed in complex patterns indicative of a promoter-specific imprinting status as well as a postnatal
mechanism that either neutralises, ignores or reinterpretes the gametic imprint. Finally, we also show that H19 is monoallelically expressed in all postnatal liver samples examined. We discuss these observations with respect to the current understanding of *IGF2/H19* imprinting.

**MATERIALS AND METHODS**

**Tissue samples**

Human embryonic liver from a first trimester pregnancy (7 weeks of gestation) was obtained by a therapeutic termination carried out by the Huddinge university hospital, with the permission of the local ethical committee. The postnatal liver specimens were obtained from the departments of Pathology at the Karolinska hospital and Uppsala university hospital. The 9-month (female) and 18-month (male) infant livers as well as the 3-year liver (male) were recovered in association with surgery for hepatoblastoma from patients treated with chemotherapy. The perinatal liver came from a child who died at birth from pneumonia. This patient otherwise appeared normal. The 21-year old patient (female) suffered from a liver adenoma, and the tissue was taken from a normal part of the liver during surgery. The 28-year old patient (female) suffered from a focal nodular hyperplasia of the liver, and normal liver tissue was taken during surgery. The 46-year liver was from a patient who died of an aortic aneurysm. The 61-year patient (male) suffered from adenocarcinoma whereas the 62-year old patient (male) died from a heart attack. Two liver specimens (one of each case) was isolated and purified using Quiex (Quiagen).

Since we have used tissue samples that were collected either during surgery or autopsy, it could be argued that our results presented below were influenced by the pathological condition of some of the patients as well as the chemotherapy treatment that these patients received. However, in one case, we have been able to show that the P2, P3 and P4 promoters directed expression of only the paternally derived allele in the macro- and microscopically normal liver, as is the case in embryonic and perinatal liver specimens. Hence, the substantial chemotherapy treatment of this patient apparently did not modulate the *IGF2* allele usage of the morphologically normal liver. Perhaps more importantly, the complex allele activities of the downstream *IGF2* promoters could be seen in one case (the 62-year-old patient) with no known history of liver disease or chemotherapy treatment.

**Production of *IGF2*-specific cDNA**

10 µg of DNase 1-treated (Ogawa et al., 1993), total RNA was precipitated with 20 µl of primer corresponding to a sequence downstream of the polymorphic (C-A) repeat region of exon 9 (primer sequence was: GCATCTCTGTCATGGTGGAAAG). The precipitate was dissolved in 10 µl H2O, denatured at 75°C for 5 minutes and allowed to slowly cool to room temperature. Reaction components and AMV reverse transcriptase were added according to the manufacturers protocol (Promega) and incubated at 42°C for 1 hour. The mixture was extracted with phenol/chloroform and the water phase passed through a G-50 Sephadex column to exclude any remaining primer. Following ethanol precipitation, the pellet was dissolved in 50 µl H2O.

**Amplification by thermocycling**

For amplifying the polymorphic (C-A) repeat region in exon 9 from genomic DNA, the procedure described previously was employed (Ohlsson et al., 1993). For amplification of specific promoter-derived transcripts, 1 µl of cDNA (see above) was used in a 50 µl reaction with the following conditions; 5 µl 10× pfu buffer (#2 Stratagene), 5 µl DMSO, 0.48 µM primer 1 (immediately downstream of the polymorphic region which was common to all reactions, GCCTGATCATACAGATATCG), 0.48 µM of primer 2, 2 µl of 2 mM dNTP, 20 µl H2O and 2.5 U pfu polymerase (Stratagene). Exon primers specific for the four promoters were as follows.

- **Promoter 1**: CACTCGACCGGTAGAGAC.
- **Promoter 2**: CGTGAAGCAACTGGATTTGG.
- **Promoter 3**: GGACAATGACGAAATTTCC.
- **Promoter 4**: CCTTCTCTGTAAGAGACTTC.

The thermo cycling was performed as follows: 2 cycles of 2 minutes at 95°C, 1 minute at 52°C and 3 minutes at 72°C. This was followed by 35 cycles with 1 min at 94°C, 1 minute at 52°C and 3 minutes at 72°C. The reaction mixtures were phenol/chloroform-extracted once, ethanol precipitated and analysed on a 1% ethidium bromide-stained agarose gel. The major band (at the expected size in each case) was isolated and purified using Quiex (Quiagen).

**Preparation of DNA and RNA probes**

cRNA probes were prepared using T3 and T7 RNA polymerase from Promega with 32P-UTP (Amersharm) according to the manufacturers protocoll. For P1-specific transcripts, a PCR fragment covering exon 1 was cloned into the pBluescript SK vector, and used with T3 RNA polymerase. For P2 transcripts, a PstI-Sacl subclone in pBluescript SK, covering most of exon 4 was used with T3 RNA polymerase. For P3 transcripts, an EcoRI-PsrI fragment covering the 3′-end of exon 5 was used for preparation of a 32P-labeled DNA probe using the Mutli-kit (Amersharm). For P4 transcript, a 32P-labeled RNA probe was generated from a Smal-PsrI clone in pBluescript SK, covering all of exon 6 using T3 RNA polymerase (Ohlsson et al., 1994). The simultaneous detection of *H19* and *IGF2* transcript probes (specific activity approximately 900 Ci/mmol) was performed as has been described (Ohlsson et al., 1994). A 32P-labelled antisense probe (specific activity approximately 50 Ci/mmol) for the internal marker, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA, was generated according to the protocol of the manufacturer (TRIGAPDH, Ambion).

**Analysis of monoallelic expression**

Total cellular RNA, extracted as has been described (Chomczynsky and Sacchi, 1987), was subjected to RNase protection analysis over the polymorphic (C-A)n repeat region within *IGF2* as has been described (Ohlsson et al., 1993). The genotypes of informative samples were characterised by RNase protection of PCR-amplified DNA, covering the polymorphic (C-A)n repeat as has been described (Ohlsson et al., 1993). Analysis of *H19* allelic usage was performed by reverse transcribing DNase 1-treated (Ogawa et al., 1993) total RNA, followed by PCR amplification, exploiting an *RsaI* polymorphism in exon 5 as has been described (Zhang and Tycko, 1992).

**Southern blot hybridization of promoter-specific transcripts**

Promoter-specific transcripts from P1-P4 isolated as described above, were run out separately on a 1% agarose gel in four sets, followed by blotting onto nylon membranes. Each set of blot was prehybridized and hybridized to P1-, P2-, P3- and P4-specific probes, respectively, as described previously (Ohlsson et al., 1988).
Plasticity of IGF2 imprinting status

range of developmental time points, using RNase protection analysis. Since IGF2 and H19 display strikingly similar expression patterns during human prenatal development (Ohlsson et al. 1994), H19 expression was also examined. To match the RNA input levels, GAPDH expression was used as an internal marker. Since the RNase protected fragments obtained were different in sizes for all three transcripts (the H19 transcript is fragmented due to allelic differences between the 32P-labeled antisense RNA probe and substrate), they could be simultaneously detected by combining the corresponding 32P-labeled, antisense RNA probes (Fig. 1). The results show that following birth, IGF2 mRNA steady-state levels remain high for at least two decades (Fig. 1). Thereafter, from 28 to at least 62 years of age, IGF2 expression appears to be significantly reduced. Interestingly, the developmental profiles of IGF2 and H19 expression were very similar while GAPDH expression remained essentially constant. This result may indicate that the transcription factors regulating IGF2 expression are shared with H19.

Apparent loss of IGF2 imprinting status during human liver development

It has previously been shown that IGF2 is biallelically expressed in postnatal human livers (Kalscheuer et al., 1993; Ohlsson et al., 1994; Davis, 1994). Since IGF2 is preferentially expressed from the paternally derived allele during human prenatal development, we set out to examine the possibility of a temporally controlled loss of imprinting during liver development. To this end, we analysed the allele usage of IGF2 in human liver specimens representative for various phases of human development by exploiting the polymorphic (C-A)n repeat motif within exon nine of IGF2. This method exploits polymorphic sequence differences between a 32P-labeled RNA probe and other allele types. A near identity between the probe and PCR-amplified DNA covering the (C-A)n repeat would be represented by one single protected band, with occasional minor bands reflecting allele variants with minor sequence differences. Other allele types will be fragmented in characteristic patterns dependent on the extent of sequence differences (Ohlsson et al., 1993; Nyström et al., 1994). After screening a large number of specimens, a total of eight human livers of various ages were scored as informative for this (C-A)n repeat. Each of these liver samples was judged to be morphologically normal on macroscopic and microscopic examination. Fig. 2 shows that in the embryonic, neonatal and a 9-month-old infant livers, there was preferential expression from one of the parental alleles (the preferential expression of the paternally derived allele could be confirmed for the 9-month liver, data not shown). In contrast, biallelic expression of IGF2 could be demonstrated in an 18-month-old infant liver, as well as in four adult liver specimens. Similar data has recently been presented by Davis (1994). We conclude that in the liver IGF2 is biallelically expressed for a majority of a human’s life. This observation might reflect a temporal relaxation of imprinting by the erasing or neutralisation of epigenetic imprints during human postnatal development. Alternatively, the regulation of transcription by each of the four promoters could have been modified so that one promoter is active from one parental

Fig. 1. Developmental profile of IGF2 and H19 expression during human postnatal liver development. 32P-labeled antisense RNA probes specific for the IGF2, H19 and GAPDH transcripts were annealed to RNA substrates either singly or in combination. Each of the protected bands, specific for either transcript, are indicated. Whereas the protected IGF2 probe appears as a discrete collection of bands at around 130 bases, nibbled by the RNases, the protected H19 probe is fragmented into several bands owing to polymorphic differences between the probe and the RNA substrate (Ohlsson et al., 1994). The size of the protected GAPDH RNA probe fragment is approximately 316 bases.

IGF2

H19

GAPDH
allele while other promoters could be active from the other parental allele.

**PCR amplification of promoter-specific IGF2 transcripts**

To examine whether or not the P1, P2, P3 and P4 promoters directed expression from different parental alleles in liver specimens, we utilised a reverse transcriptase PCR approach. The IGF2 cDNA was produced by using a primer specific for an exon nine sequence downstream of the (C-A)$_n$ repeat. This was followed by a PCR amplification of promoter-specific transcripts by pairing a 3′-primer in exon 9 (flanking a polymorphic (C-A)$_n$ repeat) with a 5′-primer specific for either of the exons that are found only in transcripts derived from promoters 1, 2, 3 and 4, respectively (Sussenbach et al., 1991) (see strategy outlined in Fig. 3 and map in Fig. 4). Following PCR amplification, DNA products were selected according to size so as to represent properly spliced mRNA products. The size selection also served to minimize the risk of erroneous amplification of the (C-A)$_n$ repeats due to spurious sequence similarities between the 5′ primer and sequences upstream of exon nine in any potential trace of contaminating genomic DNA. Next, we examined the identity of the PCR products by performing a Southern blot hybridization analysis using $^{32}$P-labeled exon-specific probes. Fig. 4 shows that each of the four different PCR reactions generated a specific DNA product representative for transcripts derived from each of the four promoters. The results suggest that the PCR amplification step has faithfully replicated cDNAs, all of which were derived from the expected 5′ exon of each of the four promoters.

**Promoter-specific allele usage of IGF2**

By employing the reverse transcriptase-PCR approach outlined above combined with an RNase protection analysis, we next examined the contribution of parental alleles at the 3′-end of the promoter-specific cDNAs derived from the morphologically normal part of a liver of a 9-month-old infant who suffered from a hepatoblastoma. Fig. 5 shows that promoters P2, P3 and P4 all directed expression preferentially or exclusively from the paternally derived allele (DNA contamination is not, therefore, an issue with our approach) (Fig. 5). In contrast, the P1 promoter directed transcription from both...
parental alleles with approximately the same intensity (Fig. 5). Hence, the P2, P3 and P4 promoters appeared to be silent while the P1 promoter was active on the maternally derived allele. When the allelic usage is determined directly at the RNA level, it is clear that the bulk of the liver mRNA of this patient is expressed from the paternal allele, although a small but noticeable expression from the maternally derived allele can be observed (Fig. 2). We conclude that the activity of the maternal allele in the direct RNase protection assay (Fig. 2) in all likelihood is derived exclusively from the P1 promoter, which is active on both parental alleles as determined by the indirect method of RT-PCR and RNase protection analysis. Similar data has been obtained for both embryonic and neonatal liver specimens (Table 1). In all informative liver specimens investigated (covering a large part of developmental timepoints), the P1 promoter directed expression from both parental alleles (Fig. 5; Table 1).

Although the downstream (P2-P4) promoters directed expression from only one parental allele (in all likelihood the paternally derived) in embryonic, neonatal and younger (9 months) infant liver specimens, the picture becomes more complex when later postnatal samples are included. While the P2 and P4 promoter-derived transcripts were expressed from primarily one parental allele in all cases examined, the P3 promoter-derived transcripts contained sequences from both parental alleles in three of the four adult

Table 1. A summary of allelic contributions in transcripts derived from the four IGF2 promoters in informative human liver specimens

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Promoter</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic liver</td>
<td>a/b</td>
<td>a/−</td>
<td>a/−</td>
<td>a/−</td>
<td>a/−</td>
</tr>
<tr>
<td>Neonatal liver δ</td>
<td>a/b</td>
<td>a/−</td>
<td>a/−</td>
<td>a/−</td>
<td>a/−</td>
</tr>
<tr>
<td>9 month liver δ</td>
<td>a/b</td>
<td>a/−</td>
<td>a/−</td>
<td>a/−</td>
<td>a/−</td>
</tr>
<tr>
<td>18 month liver δ</td>
<td>a/b</td>
<td>a/−</td>
<td>a/−</td>
<td>a/b*</td>
<td>a/−</td>
</tr>
<tr>
<td>Adult liver 21 years</td>
<td>a/d</td>
<td>a/−</td>
<td>a/−</td>
<td>a/d</td>
<td>a/−</td>
</tr>
<tr>
<td>Adult liver no info</td>
<td>a/b</td>
<td>a/−</td>
<td>a/−</td>
<td>a/b*</td>
<td>a/−</td>
</tr>
<tr>
<td>Adult liver 28 years</td>
<td>a/c</td>
<td>a/−</td>
<td>a/−</td>
<td>**</td>
<td>−/c</td>
</tr>
<tr>
<td>Adult liver 62 years</td>
<td>a/b</td>
<td>a/−</td>
<td>a/−</td>
<td>−/b</td>
<td></td>
</tr>
</tbody>
</table>

The allele types are denoted a through d
* = weakly biallelic
** = not detected (probably due to low promoter activity).
†H19 is expressed monoallelically.

Fig. 4. Amplification of promoter-specific IGF2 transcripts. The gene map shows the exon organization of the human IGF2 gene and the splicing pattern for the primary transcripts generated by the four different promoters. Reverse transcription was followed by thermocyclic amplification using exon-specific primers (see Materials and methods). Southern blot hybridization analysis of size-selected products was performed in four sets with the four promoter-specific amplification products in each set, using exon 1-, 4-, 5-, and 6-specific probes respectively. The figure verifies the identity of the 5′-end for each of the four PCR-amplified cDNA products.
specimens, as well as in the 18-month-old liver sample (Table 1). Hence, it appears that there is a loss of imprinting during liver development as far as the P3 promoter is concerned. Since the biallelically active P3 promoter is flanked by the monoallelically active P2 and P4 promoters, it is conceivable that there are promoter-specific imprinting states which can be independently maintained. The lack of common and coordinated patterns of promoter-specific allele activity is highlighted, however, by the two cases in which the parental allele transcribed via the P2 and P3 promoters, respectively, was opposite to the one used by the P4 promoter (Fig. 5; Table 1). All of these data have been repeated several times with identical results. Hence, the PCR amplification of the polymorphic region within exon nine does not appear to produce a bias for any particular allele irrespective of whether the template is cDNA or genomic DNA (see also Ohlsson et al., 1993).

**H19 is monoallelically expressed in postnatal human livers**

In conjunction, the close physical proximity between the IGF2 and H19 loci, their expression from opposite parental alleles during prenatal development and their close overlap in expression patterns during human prenatal development (Ohlsson et al., 1994) are indicative of some form of common control of expressivity and allele usage (Bartolomei et al., 1993; Li et al., 1993; Surani, 1993). It was of interest, therefore, to examine whether or not the complex allele usage of IGF2 in postnatal liver also applied to H19. Six different liver specimens, informative for the Rsal polymorphism within exon 5, were subjected to RT-PCR followed by Rsal restriction analysis. Fig. 6 shows that all six samples contain H19

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**Fig. 5.** Promoter-specific allele usage in the human IGF2 gene. The amplified IGF2 cDNA products originating from each of the four promoters (indicated on the top of each lane) were analysed for the presence of the polymorphic (C-A)n repeat region in exon nine (at the 3′-end of the amplified cDNAs) as described in the legend of Fig. 1. As can be seen from the figure the a allele, or minor variants of it, is present in both the 9-month liver and the adult liver specimen. The sequence differences between the 32P-labeled RNA probe (which originates from the pPA1 plasmid; Ohlsson et al., 1993) and the b and c alleles, however, differ more extensively. The figure verifies the identity of the 3′-end for each of the four PCR amplified cDNA products.

**Fig. 6.** H19 is monoallelically expressed during postnatal liver development. Genomic DNAs and cDNAs produced from total cellular RNA extracted from informative liver specimens were PCR-amplified with nested primers specific for human H19 (Zhang and Tycko, 1992). The PCR products were then digested with Rsal and analysed on agarose gels. Panel A shows that PCR products generated from genomic DNA digested with Rsal display primarily two bands typical of the Rsal− and Rsal+ alleles. Panel B shows that the PCR products generated from cDNA appear as either Rsal− or Rsal+ alleles. Note that the RT-PCR products are smaller than the genomic PCR products due to the absence of introns. The size marker was a 123 bp DNA ladder (Gibco BRL).
transcripts derived from only one of the parental alleles, in all likelihood the maternally derived allele. Since at least four of these samples also display promoter-dependent IGF2 allele usage (see Table 1), we conclude that the complex allele usage of IGF2 appears to be either partly or completely independent of the functional imprinting of H19.

**DISCUSSION**

The results reported here document the existence of a promoter-specific imprinting in humans. While the P2, P3 and P4 IGF2 promoters can direct expression exclusively from the paternally derived allele during prenatal and early postnatal development, the P1 promoter is, in all informative liver specimens examined, expressed biallelically. Although P1 promoter activity was undetectable in an embryonic liver specimen by RNase protection analysis (Li and Ekström, unpublished data), RT-PCR analysis detected a biallelic activity of the P1 promoter during embryogenesis as well. These results may reflect some sort of a P1/P2 imprinting boundary involving a regional heterochromatinisation of the maternal allele. Such a boundary would be particularly interesting if it turns out that the human insulin gene, which is immediately upstream of the P1 promoter of the IGF2 gene, is parentally imprinted as has been shown for the mouse insulin 1 and 2 genes (Giddings et al., 1994). The P1 promoter is equally active on both parental alleles, however, and its primary transcript encompasses the entire IGF2 transcriptional unit (including the P2-P4 promoter sequences). Hence, a regional heterochromatinisation of the maternal IGF2 allele is not likely. This notion is reinforced by the demonstration of the open chromatin structure of both parental Igf2 alleles of the mouse as shown by the accessibility to nucleases (Sasaki et al., 1992).

We also observe here what superficially appeared to be a temporally controlled loss of the imprinting status of IGF2 during liver development (a similar observation has been reported by Davis (1994)). The activation of the silent (probably maternal) allele, seems to be initiated during the first year following birth. Part of this pattern could be explained by a postnatal increase in the P1 promoter activity and in part by the complex allele usage dictated by the activity of the P2, P3 and P4 promoters. In particular, we present two different liver specimens in which the opposite parental alleles are used by the P2/P4 and P3/P4 promoters, respectively. The apparent lack of consistency in the allele-specific activities of the P2, P3 and P4 promoters has some bearing on the theory that the imprinting of IGF2 and H19 is linked. A number of investigators have suggested some form of coordination between these loci based not only upon their close physical proximity but also on the striking overlap in the expression patterns between these loci and their opposite allele usage (Bartolomei et al., 1993; Li et al., 1993; Surani, 1993). According to this model, an overriding control exists to prevent these genes from being expressed simultaneously from the same chromosome. The complex and apparently local character of the promoter-specific allele usage in the late postnatal human liver specimens (in particular the opposite allele usage of the P2/P4 and P3/P4 promoters in different samples) is not, however, readily compatible with an imprinting mechanism common to the IGF2 and H19 loci. This deduction is underscored by our observation that the H19 gene is monoallelically expressed in these liver samples. Each promoter must either be imprinted independently from the others (and hence IGF2 and H19 are independently imprinted) or there exists an imprinting hierarchy. We are currently examining the CpG methylation status for each of the IGF2 promoters.

The observation of distinct imprinting patterns for transcripts derived from the different promoters of IGF2 raises a number of potentially important issues. Although this phenomenon has so far been referred to (and thought of) in terms of ‘imprinted genes’, the case of IGF2 in the liver suggests that it may be prudent to think more in terms of ‘imprinted gene regulatory elements’. Since imprinting involves the parental allele-specific transcription of genes, it would be expected that DNA sequences involved in transcriptional regulation would represent the targets for the imprinting mechanism. It may well be, therefore, that genes that are associated with several cell type/developmental stage-specific promoters and enhancers will not exhibit the same imprinted status in all situations. This notion is certainly in line with our observation reported here that the P1 promoter of IGF2 is not functionally imprinted in the liver.

Can the plasticity in imprinting of IGF2 be explained in terms of a change in the epigenetic imprint or a change in the response to the transcriptional mechanisms to this imprint? Whereas it is not easy to imagine how a new allele-specific imprint could be established in postnatal somatic cells, the complexity and diversity of the eucaryotic transcription process perhaps provide the basis for a more plausible explanation. Changes in the availability of general and auxillary transcription factors as well as transactivators (whether in a cell type-specific, developmentally regulated or disease-related situation) could be envisaged to produce new transcriptional response by ‘imprinted’ alleles. In this scenario, the targeting of an ‘imprint’ to a particular protein-DNA binding DNA sequence would make this redundant in response to a new repertoire of factors. This is all the more plausible in the light of the ‘modular’ nature of transcriptional control elements (Dyan, 1989), i.e. that promoters and enhancers are known to be made up of multiple modules that represent discrete protein binding sites. These can interact to produce a different overall effect of the element, depending on which of the potential element-binding factors (and indeed the element itself) is available (Tijan and Maniatis, 1994). 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 be limited to genes that are regulated by multiple promoters and/or enhancers.

In summary, we show here the existence of a promoter-specific imprinting status in humans. Special care should, therefore, be taken in the evaluation of any potentially imprinted gene which may be driven by multiple promoters and/or enhancers. Hence, plausible explanations can be offered to a number of recent observations without invoking modifications in the primary or secondary imprints. The tissue-specific imprinting status of the Ins-1, Ins-2 (Giddings et al., 1994) and WT-1 (Jinno et al., 1994) genes, for example, could reflect the presence of sets of tissue-specific transactivating factors that are attenuated by epigenetic imprints or making these redundant. Similarly, the loss of imprinting of IGF2 in a subset of Wilms tumors and Beckwith-Wiedemann syndrome.
cases may depend on the abberant activation of sets of factors ignoring or reinterpreting the epigenetic imprints.

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