ARTICLE

The *IPL* gene on chromosome 11p15.5 is imprinted in humans and mice and is similar to *TDAG51*, implicated in Fas expression and apoptosis

Naifeng Qian¹, Dale Frank¹, Denise O’Keefe¹, Diem Dao¹, Long Zhao¹, Luwa Yuan¹, Qing Wang³, Mark Keating⁴, Colum Walsh² and Benjamin Tycko¹,*

¹Department of Pathology, Divisions of Oncology and Neuropathology and ²Department of Genetics and Development, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA, ³Department of Pediatrics, Section of Cardiology, Baylor University School of Medicine, Houston, TX 77030, USA and ⁴Howard Hughes Medical Institute, Department of Human Genetics, Cardiology Division and Eccles Institute of Human Genetics, University of Utah, Salt Lake City, UT 84112, USA

Received July 24, 1997; Revised and Accepted August 9, 1997

We searched for novel imprinted genes in a region of human chromosome 11p15.5, which contains several known imprinted genes. Here we describe the cloning and characterization of the *IPL* (Imprinted in Placenta and Liver) gene, which shows tissue-specific expression and functional imprinting, with the maternal allele active and the paternal allele relatively inactive, in many human and mouse tissues. Human *IPL* is highly expressed in placenta and shows low but detectable expression in fetal and adult liver and lung. Mouse *Ipl* maps to the region of chromosome 7 which is syntenic with human 11p15.5 and this gene is expressed in placenta and at higher levels in extraembryonic membranes (yolk sac), fetal liver and adult kidney. Mouse and human *IPL* show sequence similarity to *TDAG51*, a gene which was shown to be essential for Fas expression and susceptibility to apoptosis in a T lymphocyte cell line. Like several other imprinted genes, mouse and human *IPL* genes are small and contain small introns. These data expand the repertoire of known imprinted genes and will be helpful in testing the mechanism of genomic imprinting and the role of imprinted genes in growth regulation.

INTRODUCTION

Genomic or parental imprinting is an unusual mode of gene regulation in which the allele inherited from one parent is epigenetically silenced in the offspring (1). Since imprinting is conserved between mice and humans it is thought to have some biological rationale. According to one model, imprinting is a by-product of an epigenetic host defense system, involving cytosine DNA methyltransferase, which has evolved to inactivate retroviruses and retrotransposons (2–5); according to a different model, imprinting has been shaped by paternal–maternal competition for reproductive success (6). These models make predictions regarding the structure and function of imprinted genes. According to the paternal–maternal conflict model, paternally expressed/maternally repressed genes are expected to promote growth of the placenta and fetus while maternally expressed/paternally repressed genes are predicted to have the opposite, growth inhibitory function. According to the host defense model, imprinted genes are expected to resemble retroviruses in that they are small in length and have small, few or absent introns and either contain or are bordered by repeat sequences. More examples of imprinted genes are needed to test these and other theories of imprinting.

On human chromosome 11p15.5 there are several genes with previously documented functional imprinting, defined as a consistent allelic bias in RNA expression in somatic tissues. These are *H19*, *IGF2*, *p57KIP2*, and *KVLQT1*. The syntenic region of mouse chromosome 7 contains most or all of these loci, as well as the imprinted *Mash-2* gene, which is also syntenic and suspected to be imprinted in man based on its lack of expression in androgenetic neoplasms, and the *Ins-2* gene, which shows tissue-specific imprinting (7–18). The size of the region which contains these genes is ~1 Mb, similar to the Prader–Willi syndrome/Angelman syndrome region of chromosome 15, which also contains multiple imprinted genes (19). A strong sex bias in the rates of meiotic recombination in imprinted regions suggested an accessibility model for genomic imprinting in which extended regions of ‘open chromatin’ specific to one type of gamete are selectively modified epigenetically, leading to allele-specific transcription of at least a subset of the genes in these regions in

*To whom correspondence should be addressed. Tel: +1 212 305 1287; Fax: +1 212 305 5498; Email: bt12@columbia.edu
Figure 1. The IPL gene. (A) Map positions of some characterized genes in the chromosome 11p15.5 imprinted region. Genes for which there is current evidence of imprinting based on an allelic expression bias in humans and/or mice are in bold (7–17). Genes not in bold have been shown to be expressed biallelically in at least some human or mouse tissues (24,32–34). (B) PCR strategy for assessing functional imprinting of the mouse and human IPL genes. Exons are boxed and the nucleotide polymorphisms are indicated by asterisks. FokI restriction sites yielding the fragments for SSCP analysis are indicated. (C) Sequence of the human IPL cDNA. The exon/intron boundaries are indicated by the lower case dinucleotides in bold; start and stop codons are in bold and the polyadenylation signal is underlined. (D) Amino acid similarity between the predicted mouse and human IPL proteins and TDAG51. In addition to the shared blocks of high similarity, human IPL, and to a lesser extent mouse Ipl, show a proline-rich C-terminal region similar in amino acid composition to that of TDAG51. The alignments with the human P and mouse TDAG51 were almost identical to those with TDAG51 (data not shown). Multiple sequence alignment was performed using the MSA 2.1 algorithm. Identical amino acids are shaded in black when shared by all three predicted proteins and in grey when shared by two proteins.

the offspring (20,21). A prediction is that a positional cloning approach, i.e. chromosome walking in the vicinity of known imprinted genes, should be successful in isolating additional imprinted genes. Here we describe the human and murine versions of a novel imprinted gene, IPL, identified by this approach.
RESULTS

Cloning and sequencing of human IPL

A map of the chromosome 11p15.5 imprinted region is shown in Figure 1A. Exon trapping of a P1 clone (P1-12766; Fig. 1A), which also contained the p57KIP2 gene, yielded a 200 bp putative exon with a region of sequence identity to several human ESTs. The trapped exon probe, which subsequently proved to include part of a true exon as well as flanking sequences, as well as a partial cDNA identified in the EST database, identified a 0.8 kb transcript on Northern blots of fetal and adult human tissues (see below). Screening of a placenta cDNA library yielded a 770 bp cDNA clone which contained an open reading frame of 152 amino acids (Fig. 1C; GenBank accession no. AF001294). A C/G-rich sequence containing a TATA motif was present immediately upstream of the beginning of the cDNA sequence, consistent with a gene promoter (data not shown). The conceptually translated IPL cDNA identified two highly similar proteins (with P values of <e−31) in a BLASTP search of the nr database; murine TDAG51 protein and its human ortholog, the PQ-rich protein (Fig. 1D).

Transfection experiments in T lymphocytes have implicated TDAG51 in inducing Fas expression and susceptibility to apoptosis (22). Genomic sequencing showed that human IPL contains a single 223 bp intron (Fig. 1B and C). When the human IPL cDNA probe was hybridized to Northern blots the pattern of expression was found to be highly tissue specific: very high expression was found in placenta, somewhat lower expression was seen in the chorioamniotic membrane and very low expression was detectable on prolonged exposures of poly(A)+ Northern blots in fetal and adult liver, lung and kidney and in adult pancreas (Fig. 2a and c).

Imprinting of human IPL

To determine whether IPL is imprinted, we searched for nucleotide sequence polymorphisms which would allow the paternal and maternal alleles to be distinguished. Parallel genomic and cDNA PCR (RT-PCR) of a region spanning the intron, followed by single-strand conformation polymorphism (SSCP) analysis in an initial set of nine placentas revealed an exonic sequence polymorphism, with two of the nine samples showing heterozygosity in the genomic PCR products. Comparison of the SSCP band patterns of genomic and cDNA PCR products suggested monoallelic expression of IPL, with one individual showing only the ‘upper’ allele/SSCP conformer in the cDNA lane and the other expressing only the ‘lower’ allele/SSCP conformer. This was confirmed by direct sequencing, which showed heterozygosity in the genomic DNAs and nearly monoallelic representation in the cDNAs (Fig. 3A). The human IPL polymorphism is a T→C substitution at position 93 of the cDNA sequence, which creates an AluI restriction site which is neutral with respect to the predicted amino acid sequence.

We next validated the SSCP and AluI RFLP methods for quantitating the allelic expression bias by carrying out mixing experiments with ‘T’ and ‘C’ allele templates (Fig. 3B; see Materials and Methods) and expanded our analysis to include additional human organs/tissues at different developmental stages. Screening of 22 placentas, 15 fetuses and 17 adult autopsies uncovered a total of six individuals heterozygous for the AluI polymorphism (allele frequency 6% in this population). Each of various tissues from these six cases showed a clear allelic
expression bias (Fig. 3C and Table 1) and in one informative case for which parental DNAs were available we were able to assign the active IPL allele as maternal (Fig. 3C). In different cases either the 'T' or the 'C' allele was preferentially expressed and, consistent with imprinting, in cases in which more than one tissue was available all tissues showed the same direction of the allelic expression bias (Table 1). When multiple aliquots of genomic DNAs and cDNAs were amplified the results were quite consistent, producing very small standard errors in the measurements of the strength of the functional imprint (Table 1).

Screening for additional exonic polymorphisms by SSCP and direct sequencing was unproductive; this low level of sequence variability in IPL no doubt reflects the small size of the gene and its moderately high level of sequence conservation (see below). Additional studies will be of interest to address the possibility of inter-individual variation in the strength of IPL imprinting, but because of the low allele frequency of the IPL polymorphism these will require screening of very large numbers of surgical and autopsy cases.

### Table 1. Human heterozygotes analyzed for IPL allelic expression

<table>
<thead>
<tr>
<th>Case</th>
<th>Tissue/organ</th>
<th>Stage</th>
<th>Active allele</th>
<th>Expression from active allele (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>placenta</td>
<td>term</td>
<td>c</td>
<td>83 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>placenta</td>
<td>fetal</td>
<td>t</td>
<td>100 ± 4</td>
</tr>
<tr>
<td></td>
<td>lung</td>
<td></td>
<td></td>
<td>76 ± 2</td>
</tr>
<tr>
<td></td>
<td>kidney</td>
<td></td>
<td></td>
<td>74 ± 2</td>
</tr>
<tr>
<td>3</td>
<td>placenta</td>
<td>term</td>
<td>c</td>
<td>78 ± 1</td>
</tr>
<tr>
<td>4</td>
<td>kidney</td>
<td>fetal</td>
<td>t</td>
<td>77 ± 1</td>
</tr>
<tr>
<td></td>
<td>lung</td>
<td></td>
<td></td>
<td>99 ± 5</td>
</tr>
<tr>
<td></td>
<td>liver</td>
<td></td>
<td></td>
<td>100 ± 4</td>
</tr>
<tr>
<td>5</td>
<td>kidney</td>
<td>juvenile</td>
<td>t</td>
<td>64 ± 2</td>
</tr>
<tr>
<td></td>
<td>lung</td>
<td>(7 months)</td>
<td></td>
<td>61 ± 1</td>
</tr>
<tr>
<td></td>
<td>liver</td>
<td></td>
<td></td>
<td>57 ± 1</td>
</tr>
<tr>
<td>6</td>
<td>kidney</td>
<td>adult</td>
<td>t</td>
<td>77 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>liver</td>
<td></td>
<td></td>
<td>70 ± 1</td>
</tr>
</tbody>
</table>

Values are the mean ± standard error of quantitative SSCP determinations from three aliquots of cDNA. All results were validated qualitatively by parallel Alu RFLP analysis. The active allele was identified as maternal in case 1.

### Identification, mapping, expression and imprinting analysis of mouse Ipl

To confirm the parental origins of the active and repressed allele in a tractable experimental system and to investigate expression patterns, sequence similarities and genomic structure, we searched for the murine homolog of IPL. Screenng of the EST database uncovered several murine cDNAs with high sequence similarity to human IPL. By comparing these sequences and resequencing one of the cDNA clones it was possible to reconstruct a 749 bp cDNA with a 144 amino acid open reading frame following a Kozak consensus start codon. We then sequenced genomic DNA (from a λ phage clone) upstream of the 5'-end of the Ipl cDNA. This showed a TATA box consensus 12 bp upstream of the start of the cDNA sequence, suggesting that the cDNA is essentially full length. The murine cDNA (genomic and cDNA sequences; GenBank accession no. AF002708) showed high nucleotide sequence similarity with the human cDNA and the predicted murine Ipl protein was 64% identical.
(92/144 amino acids) and 71% similar to the human IPL protein and identified the same blocks of similarity to TDAG51 (Fig. 1D). Like human IPL, comparison of genomic and cDNA PCR sequences showed that mouse Ipl contains a single small intron of 239 bp (Fig. 1B). Analysis of Ipl polymorphisms (see below) in DNA samples from a mouse back-cross panel (domesticus / spreitus; Jackson Laboratories) localized this gene to the region of chromosome 7 which contains murine H19 (interval Cyp2e1→tel). Thus, by sequence analysis and conserved synteny, mouse Ipl is likely to be the true ortholog of human IPL.

The murine Ipl cDNA probe identified an ~800 bp transcript which was highly expressed in the extraembryonic membranes (yolk sac), moderately expressed in placenta, fetal liver and adult kidney and expressed at lower levels in fetal kidney, lung and limb (Fig. 2a and b). Thus, there are both similarities and differences in the expression patterns of the mouse and human IPL genes. In particular, compared with the human gene the mouse gene is more prominently expressed in several of the non-placental tissues. Whether mouse and human IPL mRNAs can be induced by appropriate extracellular stimuli in the 'non-expressing' tissues, as is true for TDAG51 mRNA in mouse lymphocytes (22), remains to be determined.

We next carried out an extensive analysis of Ipl imprinting in staged interspecific mouse crosses. An exonic sequence polymorphism was found: a neutral A (C57BL/6)→C (M.m.molossinus, M.m.castaneus, M.m.spretus) substitution in the first exon, at position 218 of the IPL genomic sequence. As we had done for the human gene, we used more than one method to assess imprinting of mouse Ipl; since the polymorphism did not affect a restriction site, each tissue from each cross was examined by both quantitative SSCP analysis and by direct sequencing of RT-PCR products. As expected from the control experiments with human IPL, mixing experiments showed that SSCP gave a precise measurement of the allelic expression bias and the mixing experiments also validated direct sequencing as a less precise measurement of the allelic expression bias and the mixing experiments also validated direct sequencing as a less precise qualitative assay (Fig. 4a; data not shown). Organs and tissues from a total of nine different crosses were analyzed and several of the crosses were precise reciprocal matings (for example BL/6 female × CAST male and CAST female × BL/6 male). The most important result from this analysis was that in nearly every tissue of every cross there was a consistent bias towards higher expression of the maternal Ipl allele (Fig. 4a and b and Table 2). These data are in agreement with our assignment of the direction of imprinting of the human IPL gene and indicate that the imprinting of Ipl is evolutionarily conserved.

Specific quantitative findings shown in Table 2 are also of interest: when analyzed across various matings and strain combinations, the functional imprint was consistently strongest in the extraembryonic membranes (yolk sac), placenta, fetal liver and lung and adult spleen (Table 2). Brain, a site of very low expression, consistently showed weak functional imprinting. While adult kidney expressed Ipl mRNA at high levels, there was only a weak allelic expression bias in this organ and while adult spleen showed low absolute levels of Ipl mRNA, there was a strong and reproducible allelic expression bias in this organ. Thus, the strength of the functional imprint was tissue and stage specific and there was no simple correlation between the levels of mRNA expression and the strength of the functional imprint (compare Fig. 2b and Table 2). This suggests that the ‘leakiness’ of imprinting of mouse Ipl had done for the human gene, we used more than one method to the first exon, at position 218 of the human RT-PCR products. As expected from the control experiments with affect a restriction site, each tissue from each cross was examined qualitative assay (Fig. 4a; data not shown). Organs and tissues experiments also validated direct sequencing as a less precise precise measurement of the allelic expression bias and the mixing experiments also validated direct sequencing as a less precise qualitative assay (Fig. 4a; data not shown). Organs and tissues from a total of nine different crosses were analyzed and several of the crosses were precise reciprocal matings (for example BL/6 female × CAST male and CAST female × BL/6 male). The most important result from this analysis was that in nearly every tissue of every cross there was a consistent bias towards higher expression of the maternal Ipl allele (Fig. 4a and b and Table 2). These data are in agreement with our assignment of the direction of imprinting of the human IPL gene and indicate that the imprinting of Ipl is evolutionarily conserved.

Figure 4. Imprinting of mouse Ipl. (a) Assessment of imprinting by direct sequencing of RT-PCR products. The mixing experiment (top) validates the usefulness of direct sequencing as a qualitative measure of allelic expression bias. The region of the sequence containing the polymorphism is boxed and the arrow shows the A→C polymorphism (loading is AGCT). The lower panels show nearly monoallelic expression of the maternal allele in the placenta and yolk sac in reciprocal crosses. In the fetus (embryo proper) there is leakier functional imprinting, also with a maternal expression bias (compare with the allelic representation in the F1 genomic sequence). A similar mixing experiment using SSCP showed that this method was as accurate for mouse Ipl as for human IPL (data not shown). (b) Assessment of imprinting by SSCP analysis. Patterns obtained with parental and F1 genomic DNAs and with cDNAs from various tissues and organs are shown. There is selective expression of the maternal allele in all of the fetal tissues and in adult spleen and rib. Note the reciprocal allele usage in the reciprocal crosses (top). Allelic bands are indicated by dashes; there is also a lower constant band in every lane. Only the relevant portion of the gels are shown; additional intron-derived bands were present in the genomic lanes at other positions. BL/6, BL/6 genomic; CA, castaneus genomic; MO, molossinus genomic; FIG, F1 genomic. cDNA lanes are designated: fe, fetus (embryo proper); pl, placenta; ys, yolk sac (extraembryonic membranes); li, liver; lu, lung; ri, rib; ki, kidney; br, brain; ht, heart; spl, spleen; in, intestine.
of Ipl imprinting does not simply reflect a background of non-imprinted basal expression. Leaky functional imprinting of Ipl and other genes such as human p57Kip2 (16) might reflect mosaicism for imprinting at the tissue or organ level.

**IPL in the context of other loci in the imprinted domain**

A KVLQT1 exon 14 probe hybridized to P1-12767 but not P1-12766 and the full-length IPL cDNA hybridized to P1-12766 but not to P1-12767. This and additional mapping using the previously published KVLQT1 contig position IPL centromeric to both p57Kip2 and KVLQT1, with gene order and approximate distances as shown in Figure 1a. This assignment is consistent with a recently published map of the genomic KVLQT1 locus and its relationship to the p57Kip2 gene (17), as well as with the recently deposited genomic sequence of the region centromeric to p57Kip2 (human 155 kb contig from chromosome 11p15.5, HTGS phase 3, complete sequence; GenBank accession no. U90582). Based on these data, together with previous pulsed-field gel mapping data (23) and the estimated sizes of additional clones in a continuous contig spanning this region (the entire distance between TH and Ipl is covered by six overlapping P1 clones; B. Tycko, D. O’Keefe and L. Yuan, unpublished data), the currently mapped chromosome 11p15.5 imprinted domain therefore extends over at least ~1 Mb, from H19 (telomeric) to IPL (centromeric). The NAP2 gene, which is biallelically expressed (i.e. not subject to functional imprinting) in many human tissues (24), is centromeric of IPL and is transcribed over an interval of 48 kb to within 14 kb of the IPL transcriptional start site (both genes are transcribed towards the telomere), but the imprinted domain might extend centromeric of NAP2. There are also several additional genes between IPL and the TH/INS/IGF2 locus, within the already defined imprinted domain, which are under study for imprinting.

**DISCUSSION**

**IPL and the biological rationale for imprinting**

Features of the mouse and human IPL genes described here are of interest in terms of the evolution, mechanism and biological consequences of imprinting. First, based on their similarity to TDA5G1, the mouse and human IPL genes establish the existence of an interesting new gene family. Previous findings concerning TDA5G1 suggest that IPL might play a role in negatively regulating tissue growth by participating in programmed cell death or a related negative growth pathway. TDA5G1 was identified as the gene encoding a cDNA which restored FAS expression and susceptibility to apoptosis in a T lymphocyte cell line which had first been mutagenized and selected for resistance to apoptosis on cross-linking of the T cell antigen receptor (22). In this cell line the endogenous TDA5G1 gene was in fact found to be mutated, suggesting that the TDA5G1 cDNA was indeed restoring a physiological function in the transfected cells. The TDA5G1 protein was reported to be nuclear, but the precise biochemical function of TDA5G1 is not yet known. A number of imprinted genes, including the KVLQT1 gene on chromosome 11p15.5, cannot yet be assigned an effect on cell or tissue growth, but for all of the previously defined imprinted genes which can be assigned such an effect, paternal repression and maternal expression has correlated with negative growth regulation (H19, p57Kip2, Mash-2, Igf2r) and the opposite has been true for the maternally imprinted genes (FAS, KIP2, p57Kip2, Igf2, the insulin genes, Mas (25), Grf-1 (26)). If the predicted negative effect on net tissue growth of mouse and human IPL genes is borne out by functional studies, this will provide incremental support for paternal–maternal conflict as the evolutionary rationale of imprinting (5). However, functional experiments could hold some surprises, as Ipl protein could function outside of an apoptotic pathway and even structurally related molecules involved in apoptosis pathways can have opposite functional effects (anti-apoptotic Bcl-2 versus pro-apoptotic Bax, for example).

**Table 2. Functional imprinting of the Ipl gene in tissues of F1 mice from interspecific crosses**

<table>
<thead>
<tr>
<th>Organ/tissue</th>
<th>BL/6 × CAST 12.5 d.p.c.</th>
<th>CAST × BL/6 12.5 d.p.c.</th>
<th>BL/6 × MOLD 14.5 d.p.c.</th>
<th>CAST × BL/6 14.5 d.p.c.</th>
<th>BL/6 × CAST adult</th>
<th>BL/6 × MOLD adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placenta</td>
<td>76</td>
<td>85</td>
<td>73</td>
<td>77</td>
<td>69</td>
<td>72</td>
</tr>
<tr>
<td>Yolk sac</td>
<td>79</td>
<td>86</td>
<td>75</td>
<td>84</td>
<td>85</td>
<td>83</td>
</tr>
<tr>
<td>Whole fetus</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetus (–liver)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>87</td>
<td>81</td>
<td>65</td>
<td>86</td>
<td>65</td>
<td>76</td>
</tr>
<tr>
<td>Lung</td>
<td>70</td>
<td>72</td>
<td>79</td>
<td>70</td>
<td>71</td>
<td>56</td>
</tr>
<tr>
<td>Kidney</td>
<td>59</td>
<td>73</td>
<td>55</td>
<td>73</td>
<td>50</td>
<td>54</td>
</tr>
<tr>
<td>Brain</td>
<td>61</td>
<td>65</td>
<td>63</td>
<td>60</td>
<td>53</td>
<td>56</td>
</tr>
<tr>
<td>Limbs</td>
<td>66</td>
<td>56</td>
<td></td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>53</td>
<td>73</td>
<td>55</td>
<td></td>
<td>47</td>
<td>50</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>85</td>
<td>82</td>
</tr>
<tr>
<td>Ribs</td>
<td>77</td>
<td>65</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are the percentage of total mRNA expression derived from the maternal allele, determined by quantitative SSCP analysis. Data are from single determinations; a second experiment using independent cDNA aliquots gave similar results for all samples. All results were validated qualitatively by parallel direct sequencing.
**IPL gene structure and the host defense model for imprinting**

In the host defense model for imprinting, epigenetic inactivation systems such as CpG DNA methylation have evolved to transcriptionally silence retroviruses and retrotransposons and imprinted genes are targeted for inactivation in one of the parental genomes by these same mechanisms (2–5). This predicts that imprinted genes should resemble retroelements by being small and containing few and small introns, as well as by containing or being flanked by direct repeats. The mouse and human IPL genes conform to the first prediction since they are small and have a single small intron interrupting the coding sequences. Clearly, not all imprinted genes are small and the model has been supported by statistical rather than qualitative data (4). Other predictions of this theory, such as the presence of direct repeat sequences in and around imprinted genes and/or the proximity of imprinted genes to bona fide retrotransposons can now be tested since an extensive human genomic sequence has been obtained for the region containing IPL and p57KIP2 (human 155 kb contig from chromosome 11p15.5, HTGS phase 3, complete sequence; GenBank accession no. U90582). In fact, this sequence does contain several clusters of direct repeats near the IPL gene (see Materials and Methods) and not unexpectedly numerous SINE retroelements, but interpretation of the significance of these features for imprinting will require comparison with the corresponding region of mouse chromosome 7. Our results provide further evidence for conservation of the entire human 11p15.5/mouse distal 7 imprinted chromosomal domain and also show that the imprinting of mouse Ipl is robust and not strain specific. These findings suggest that cross-species comparisons for conserved cis-acting DNA sequences regulating domain-wide imprinting should be informative.

**IPL and the chromosome 11p15.5 imprinted domain**

That the IPL gene was isolated from the immediate neighborhood of known imprinted genes supports the regionality of imprinting and, by extension, the chromatin accessibility model, in which an observed sex bias in meiotic recombination frequencies is taken as evidence for differential accessibility of the chromatin in imprinted domains to epigenetic modification in the two parental germlines (20,21). With the current data, a majority of genes in the chromosome 11p15.5 domain are paternally silenced (IPL, H19, p57KIP2, Mash-2 and KVLQT1) and since the maternal silencing of Igf-2 and Ins-2 is at least in part a secondary effect based on enhancer competition with H19 in the mouse (27) and most likely also in humans (28–30), it is likely that the primary effect of the chromosome 11p15.5 regional imprint is paternal silencing. This is consistent with the observed higher rate of recombination of markers in this region in male compared with female meiosis, i.e. greater accessibility for epigenetic modification in the male germline (21).

What might be the telomeric and centromeric limits of the chromosome 11p15.5 imprinted domain? The answer to this question will be important for understanding the mechanism of imprinting, but it is also pertinent to the study of Wilms’ tumor (WT) and the other embryonal tumors which show recurrent chromosome 11p15.5 loss of heterozygosity (LOH). WT tumor progression in both LOH and non-LOH cases frequently involves a shift to a bipaternal epigenotype over multiple imprinted 11p15.5 genes, so far including three growth-related imprinted genes, H19, IGF2 and p57KIP2. At this point the IPL gene is not a strong candidate for involvement in WT pathogenesis since human fetal and adult kidney are not major sites of its expression. However, since our results concerning IPL indicate very dense clustering of imprinted genes in the 11p15.5 domain and since the known WT-related genes H19, IGF2 and p57KIP2 were all initially ascertained independently of studies on WT, it is reasonable to suspect that additional genes in the imprinted domain may be found to be involved in the disregulated cell growth and differentiation characteristic of these tumors. We previously characterized three genes consecutively downstream of H19 and all are biallelically expressed in multiple human tissues (31,32). This suggests that a telomeric boundary of the imprinted domain may be immediately downstream of H19. In terms of mapping the centromeric boundary, additional gene isolation and imprinting analysis, such as that which has been reported for the biallelically expressed NAP2 gene (24), and DNA replication timing studies (33) will be useful and it will be important to correlate the results with 11p15.5 LOH mapping in WTs.

**MATERIALS AND METHODS**

**Genomic clones and exon trapping**

The PI clones shown in Figure 1a were obtained from Genome Systems (St Louis, MO) by screening with a p57/KIP2 probe. Clone addresses were DMPC-HFF#1-1035-(G5)/12766 and DMPC-HFF#1-1260-(F10)/12767. Mapping of gene order and approximate distances was carried out by Southern blotting of the clones using IPL, p57KIP2 and KVLQT1 probes, as well as by comparison with the previously published KVLQT1 contig (23), and the results were consistent with the subsequently deposited extended genomic sequence of this region (human 155 kb contig from chromosome 11p15.5, HTGS phase 3, complete sequence; GenBank accession no. U90582). BamHI and BamHI–BglII restriction fragments of P1 clone 12766 were exon trapped using the pSPL3 vector (Exon Trapping System, Life Technologies, Gaithersburg, MD). Mouse Ipl genomic sequences were obtained from λ phage clones isolated from a strain 129/Sv library by probing with the mouse cDNA.

**cDNAs and sequences**

A combined strategy of EST database searches and direct screening of a placental cDNA Agt11 library (Clontech, Palo Alto, CA) was used to obtain the human IPL cDNA sequence. The mouse Ipl cDNA sequence was obtained from EST database searches and resequencing of two Image Consortium cDNA clones (clone accession nos W74902 and AA014727). The human and mouse Ipl intron sequences were obtained from genomic PCR products using primers based on the cDNA sequences and the 5'-end of the mouse sequence was obtained from the mouse genomic clones. GenBank accession nos of the sequences described in this paper are: human IPL cDNA, AF001294; mouse Ipl genomic and cDNA, AF002708.

**Sequence analysis for direct repeats**

For screening the extended genomic sequence (human 155 kb contig from chromosome 11p15.5; HTGS phase 3, complete sequence; GenBank accession no. U90582) we initially used a standard matrix algorithm (DNA Strider; matrix settings, stringency = 9, window = 11) which detects direct repeats with >9 bp repeating motifs in large...
PCR, SSCP and direct sequencing

Reverse transcription of total RNA was done with the Superscript Preamplification System (Gibco-BRL Life Technologies, Gaithersburg, MD), direct sequencing of PCR products and cDNA and genomic clones was done with the fmol Cycle Sequencing System (Promega, Madison, WI) and SSCP analyses were carried out as previously described (34), but the gels were run at room temperature (500 V, 10–16 h) and the gels for analysis of mouse Ipl included 6% glycerol. The radiolabeled PCR products were digested with FokI prior to SSCP analysis and the 160 bp (human) or 200 bp (mouse) FokI fragments contained the exonic polymorphisms. PCR amplification of genomic DNA and cDNAs was with Expand Taq polymerase (Boehringer-Mannheim) using buffer 2, supplied by the manufacturer, with addition of DMSO to 5%. Primers were: human IPL 5′, acaaatgccagaggg, Ipl 3′-1 (used with IPL 5′-primer for initial polymorphism screening), tgcagatgcagctg, IPL 3′-2 (used with IPL 5′-primer for subsequent imprinting analysis), gtgcagctgctcccggg, murine IPL 5′, atgaaatcccccgacgtgac, IPL 3′-2 (used with IPL 5′-primer for subsequent imprinting analysis), ggtccgactcgtcactgctcccggg, Ipl 3′-repeat 1) repeated 15 times in a continuous array located between 2 and 2.5 kb downstream of Ipl and a mixed 15/20 bp motif (aatacgttagtggatgatcgtggttggtt, Ipl-repeat 2) repeated ~100 times in a continuous array spanning the interval 7–9 kb upstream of the Ipl transcription start site. Rescreening of the region spanning 10 kb on either side of Ipl for smaller blocks of repeats identified a 43 bp sequence (gtgcttgctgctgcagctgctcgcagctgctcccggg, Ipl-repeat 3) repeated five times located 6.5 kb downstream of Ipl.

Quantification of allelic expression

Mixing experiments used defined amounts of gel-isolated cDNA PCR products as templates. For quantification of the allelic expression bias by SSCP, RFLP and direct sequencing, genomic DNA PCR products were included in each experiment. The densitometric ratio from the genomic bands obtained in the heterozygotes was used as the 50:50 allelic representation value and the cDNA data were normalized to this value. For human Ipl, the C allele band on SSCP gels of the genomic PCR products was reproducibly slightly more intense (3–6%) than the T allele band (attributable to minor alternative conformers of the C allele PCR product migrating at other positions in the gel) and the results for the cDNAs were therefore corrected by 3–6% based on the mean value of the genomic results, which were assayed in triplicate on the same gel as the cDNAs and which were highly reproducible (standard error ≤2%) in a given experiment. All SSCP results were validated qualitatively by AliI RFLP analysis of the same radiolabeled products. For mouse Ipl the two SSCP bands which were chosen for analysis were consistently of equal intensity in the genomic lanes and all SSCP results were validated qualitatively by direct sequencing of the same templates. Densitometry of lightly exposed films was with a flatbed scanner (SuperVISTA S-12, UMAX) using NIH Image v.1.49 software.

ACKNOWLEDGEMENTS

We thank Tim Bestor for interspecific F1 mice and for comments on the manuscript and Xuliang Jiang for sequence analysis. This work was supported by grant RO1CA60765 from the NIH to BT.

REFERENCES


