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The IPW Gene is Imprinted and is not Expressed in the Prader-Willi Syndrome

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INTRODUCTION

Since the original description of the Prader-Willi syndrome (PWS) in 1956 [1], and the recognition of the involvement of the proximal region of chromosome 15 in this disorder [2], understanding of the molecular basis of the genetic defect in PWS has progressed rapidly. A set of clinical criteria has been defined [3], although the diagnosis on clinical grounds alone remains difficult in the first year of life. Research has focussed both on improving the diagnostic molecular and cytogenetic tests for PWS and on identifying and defining the functions of genes whose expression is altered in this neurobehavioral disorder. Furthermore, this region is known to be subject to genomic imprinting effects, so that expression of genes involved in PWS is expected to be exclusively from the paternal allele.

A critical step in the definition of the region containing such genes was the identification of a subset of unusual patients affected with either PWS or the Angelman syndrome, which also involves a gene or genes in the proximal region of chromosome 15. These unique patients, who have chromosome 15 translocations or deletions, helped to narrow the critical region to an interval containing less than 500 kb of DNA [4-6] (Fig. 1). As will be discussed, below, regulatory elements exist in this 500 kb region which alter the expression of genes located outside this interval [7, 8].

Included in the PWS region is the gene coding for the small nuclear ribonucleoprotein-associated polypeptide N (*SNRPN*), which is silent on the maternal chromosome and thus not expressed in PWS individuals [9-11]. The SNRPN gene product is a developmentally regulated spliceosome component expressed predominantly in neuronal cells and thought to be involved in the splicing of neuron-specific messages [12-14]. Since behavioral abnormalities and retardation in growth and development could be accounted for by abnormal expression of brain-specific mRNAs, it has been proposed that the lack of expression of the SNRPN gene may account for the majority if not all of the features

seen in PWS. If that were the case, one might expect to find a subset of individuals with PWS who have inactivating mutations within the SNRPN gene and no such mutations have been identified in patients with the full or partial PWS phenotype. Therefore, it is likely that the inactivation of additional genes in the deletion region is necessary for the development of PWS.

A new mechanism for control of gene expression has been implicated in PWS. A small number of patients have been found to have an 'imprinting mutation' which causes anomalous methylation patterns in genes in the region [7]. Some of these patients have been shown to have small (<100 kb) deletions of a differentially methylated putative imprinting control element (ICE) in the region just centromeric to *SNRPN* [7, 8] (Fig. 1). In cultured cells from these patients, the methylation status and expression of *SNRPN* and of genes which are hundreds of kilobases away are affected. These findings suggest that limiting the critical region for imprinted genes involved in PWS to only the smallest region of deletion overlap would overlook important genes outside this region. At least three imprinted genes, *SNRPN*, *IPW* (imprinted in PWS) [15] and *ZNF127* [16], a gene encoding a putative zinc finger protein, and two transcribed DNA fragments, PAR1 and PAR7 [8], are not expressed in PWS individuals, including those with ICE deletions. These genes span an approximate distance of 1.8 Mb, and this region, particularly proximal to SNRPN, has not been exhaustively searched for expressed sequences. Therefore, this remains a potentially fruitful region in which to find imprinted genes.

MATERIALS AND METHODS

Analysis of the *IPW* gene for repeat and stemloop structures was performed using the programs STEMLOOP and REPEAT in the GCG sequence analysis package [17]. Methods for yeast artificial chromosomes (YACs), human genomic DNA and human RNA, and cDNA selection were as described elsewhere [15].

RESULTS

Identification and structure of the IPW gene

To search for other imprinted genes in the PWS deletion region, we constructed a YAC contig including approximately 400 kb surrounding the *SNRPN* gene, from the markers D15S13 to D15S174 (Fig. 1). The *IPW* gene was identified using the direct selection method [18, 19] and the 325-kb YAC 457B4 which contains both the *SNRPN* gene and the breakpoint marker D15S 174 [20]. *IPW* lies about 150 kb distal to *SNRPN*, but proximal to PARI [8] and D15S174. Its transcriptional orientation is from centromere to telomere.

The *IPW* cDNA is 2.2 kb long and has no significant similarity to known sequences. There are two known polyadenylation sites, approximately 250 bp apart at the 3 end (Fig. 2). The *IPW* cDNA sequence is AT rich (64% AT), and contains a nonpolymorphic stretch of 14 GT dinucleotide repeats within exon 1. Interestingly, the longest open reading frame with an acceptable Kozak consensus surrounding the potential initiator

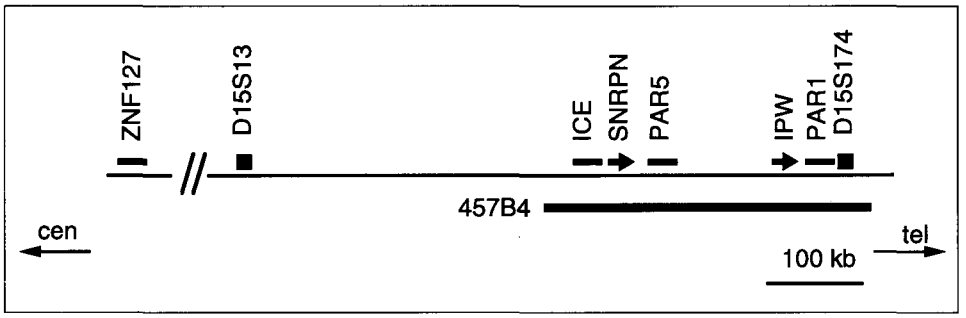

Fig. 1 - Map of the PWS region of 15q11-q13 with the approximate position of markers and genes. The SNRPN and IPW genes are both oriented 5 to 3 centromeric to telomeric, as indicated by the arrows. The position of the yeast artificial chromosome 457B4 is indicated.

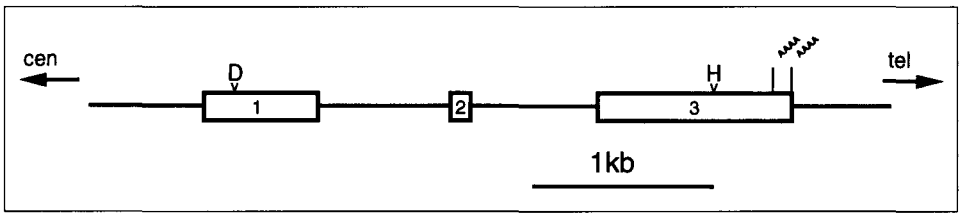

Fig. 2 - Genomic structure of the IPW gene. Boxes indicate exons. The polyadenylation sites are indicated as are the polymorphic *Dde*I (D) and *Hph*I (H) sites.

methionine would encode only 45 amino acids. We therefore propose that the *IPW* gene product functions as an RNA. Inspection of the cDNA sequence revealed a large imperfect inverted repeat of 86 nucleotides starting at position 445. Other short direct repeats were also found. Two other genes that are also postulated to function as RNAs, *H19* [21] and *XIST* [22], also have inverted repeats which could potentially form stemloop type structures [23] (Fig. 3).

The IPW gene contains three exons that span approximately 5 kb of genomic DNA (Fig. 2). Each of the three exons contains stop codons in all three reading frames. The intron-exon junctions were determined and conform to consensus donor and acceptor sites. No evidence for alternative splicing was found by RT-PCR in any of the fifteen adult tissues tested.

IPW is widely expressed exclusively from the paternal allele

By Northern blot analysis, the major approximately 2.2 kb *IPW* mRNA was detected in all tissues tested, while a second message of about 1.5 kb was also seen. Expression was also detected by RT-PCR in a variety of 19-to-20-week-old fetal tissues [15]. Preliminary experiments using slot blot hybridization of cytoplasmic RNA prepared by NP-40 lysis

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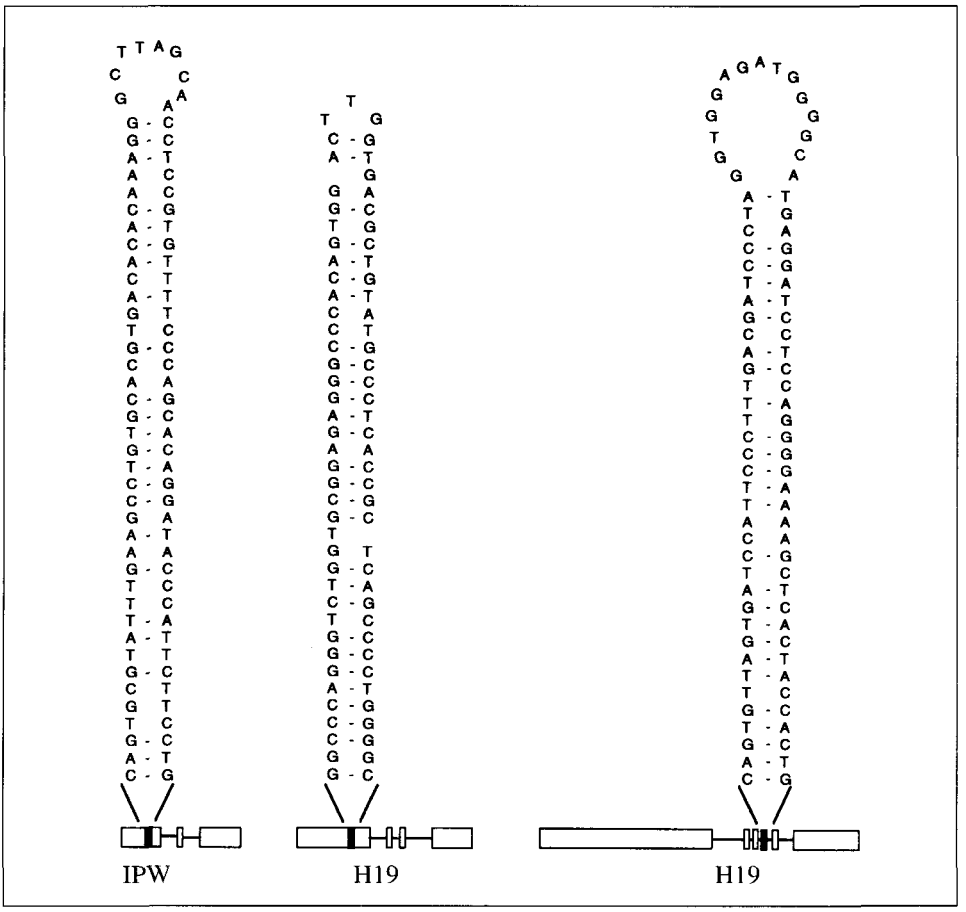

Fig. 3 - Sequence and location of potential stemloop structures in the IPW, H19 XIST genes. Exons are marked with boxes and are not drawn to scale.

of lymphoblasts have shown that the RNA is found in the cytoplasmic fraction. Thus, the *IPW* transcript resembles the *H19* transcript in its cytoplasmic localization [21], but differs from the *XIST* transcript which is localized in the nucleus [22].

Uniparental expression was demonstrated by two different experimental approaches. On Northern blots prepared from total lymphoblast RNA from individuals with PWS who have paternal deletions of 15q11-q13, no expression of *IPW* was seen whereas lymphoblasts from normal controls and Angelman syndrome patients with maternal 15q11-q13 deletions express normal amounts of *IPW* message [15]. RT-PCR analysis confirmed this result, with faint or no amplification derived from the *IPW* mRNA. This analysis has now been extended to include fresh lymphocyte RNA samples from three additional *PWS* individuals. Expression was also not seen by RT-PCR in lymphoblasts from three unusual PWS individuals (from families E and O [8]) who have deletions of the imprinting control element upstream of *SNRPN* (data not shown).

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This suggests that the regulation of *IPW* is under the control of this imprinting control element.

Monoallelic expression was established in normal individuals by studying the expression of alleles at two common intragenic sequence polyporphisms that alter either a *Dde*I or an *Hph*I site (Fig. 2). In heterozygotes for whom the parental origin of each allele could be determined, the expression of IPW was restricted to the paternal allele [15]. When the expression pattern of *IPW* was also examined in tissues from heterozygous adults and 20-week-old fetuses, monoallelic expression was detected in all tissues examined [15].

DISCUSSION

In attempting to elucidate the molecular genetic basis of PWS, we have cloned a new gene (*IPW*) which is a candidate for involvement in PWS for three reasons. The localization of the IPW gene to the critical region and its apparent regulation by the imprinting control element in this region argue that it may be part of what is effectively a contiguous deletion syndrome with modification by imprinting. *IPW* is widely expressed both in fetal and adult tissues, and finally, *IPW* is only expressed from the paternal allele, and is not expressed in PWS individuals.

It has now become clear that PWS involves the lack of expression of at least two genes in the proximal region of chromosome 15. Barring the discovery of a typical PWS patient with a mutation affecting the expression of only one of the genes in this region, a search of this region for other imprinted genes continues to be worthwhile. Currently, *SNRPN* and *IPW* remain the only two characterized imprinted genes in the deletion region, while *ZNF127*, PAR5 and PAR1 are candidate imprinted genes which are currently under investigation [8, 10, 16].

The first imprinted gene discovered in this region, *SNRPN*, is expressed in neurons during development and in the adult brain and has a putative role in the splicing of neuron-specific messages. It is conceivable that faulty splicing of neuron-specific messages is the primary factor in the pathogenesis of the various manifestations of PWS.

Unlike *SNRPN*, *IPW* does not appear to encode a polypeptide. The IPW transcript, distributed over three exons, is spliced and polyadenylated to generate a moderately abundant cytoplasmic mRNA of 2.2 kb, with a less abundant cross-hybridizing 1.5 kb mRNA of unknown origin.

There are only a few examples of mammalian genes which code for spliced, polyadenylated RNA which is not translated. The RNA product of the imprinted *H19* gene does not appear to code for a protein and is thought to be associated with a cytoplasmic particle, based on cellular fractionation experiments [21]. One proposed function for *H19* is that of a tumor suppressor gene, given that many Wilms'tumors that show loss of heterozygosity at 11p15 or loss of imprinting of the adjacent *IGF2* gene also show loss of expression of *H19* [24, 25]. Additionally, transfection of *H19* expression constructs into tumor cell lines abrogates clonogenicity in soft agar, and tumorigenicity [26].

In contrast, the *XIST* gene codes for a large, nuclear RNA which may play a direct role in initiation and spreading of X inactivation. Interestingly, *XIST* expression is limited to the paternal allele in extraembryonic tissues, whereas its expression in the embryo and adult is exclusively from the randomly inactivated X chromosome. The ubiquitous

expression pattern of *IPW* gives no clue as to its role in the cell. The presence of stem-loop structures in the *IPW*, *H19* and XIST RNAs suggests that these RNAs may act at a structural level, perhaps as part of a RNA-protein complex similar to a ribosome or spliceosome, but with an as yet unidentified function. Parallels between the *IPW* RNA and other mammalian RNAs with similar features may become apparent as we learn more about the frequency of nontranslated genes in the mammalian genome and whether they share a common role.

What is the function of the *IPW* RNA? Its sequence does not reveal many clues. We can consider the possibility that *IPW* may itself have a direct role in the imprinting process of genes in this region of human chromosome 15. In this scenario, the transcriptionally active state of the *IPW* gene or the RNA product of IPW may act in cis to activate or maintain the active status of genes in this imprinted region. This model is similar to that proposed for the *H19/IGF2* genes whereby these two adjacent genes share enhancer elements and compete for regulatory elements, but differs in that *H19* and *IGF2* are oppositely expressed [27]. The transcription of the *XIST*RNA has been proposed to have a cis-acting role in chromatin structure or gene expression at the X-inactivation center by maintaining adjacent genes in an inactive state. This may arise through the speading of a local conformational change induced by *XIST* expression or through the action of the *XIST RNA* product [28]. Again, XIST is oppositely expressed from the genes around it, in contrast to the situation that exists with *IPW* and *SNRPN*. The second possibility is that the *IPW* RNA has a completely separate role in the cell which is independent of the imprinting process, perhaps as a structural RNA. What that role is and what part its lack of expression plays in the PWS phenotype will be the focus of our future experiments.

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