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ARTICLE

Mutations in *HPRP3*, a third member of pre-mRNA splicing factor genes, implicated in autosomal dominant retinitis pigmentosa

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Retinitis pigmentosa (RP), the commonest form of inherited retinal dystrophies is a clinically and genetically heterogeneous disorder. It is characterized by progressive degeneration of the peripheral retina leading to night blindness and loss of peripheral visual field. RP is inherited either in an autosomal dominant, autosomal recessive or X-linked mode. A locus (*RP18*) for autosomal dominant RP was previously mapped by linkage analysis in two large pedigrees to chromosome 1p13–q21. The human *HPRP3* gene, the orthologue of the yeast pre-mRNA splicing factor (*PRP3*), localizes within the *RP18* disease interval. The recent identification of mutations in human splicing factors, *PRPF31* and *PRPC8*, led us to screen *HPRP3* as a candidate in three chromosome 1q-linked families. So far, two different missense mutations in two English, a Danish family and in three RP individuals have been identified. Both mutations are clustered within a two-codon stretch in the 11th exon of the *HPRP3* gene. Interestingly, one of the mutations (T494M) is seen repeatedly in apparently unlinked families raising the possibility of a mutation hot spot. This has been confirmed by haplotype analysis using SNPs spanning the *HPRP3* gene region supporting multiple origins of the mutation. The altered *HPRP3* amino acids, which are highly conserved in all known *HPRP3* orthologues, indicate a major function of that domain in the splicing process. The identification of mutations in a third pre-mRNA splicing factor gene further highlights a novel mechanism of photoreceptor degeneration due to defects in the splicing process.

INTRODUCTION

Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous disorder with an incidence of almost 1 in 3500. Individuals affected with RP usually suffer from night blindness early in disease, accompanied or followed by loss of peripheral visual field. The disorders are characterized by bone spicule-like pigmentary deposits and a reduced or absent electroretinogram (1). RP displays all three modes of Mendelian inheritance: autosomal dominant (adRP), autosomal recessive (arRP) and X-linked (xlRP). The autosomal dominant form of RP (adRP) can be caused by mutations in seven genes and a further four loci for which the genes remain to be identified (2–4). Here we report the identification of the gene responsible for adRP localized on chromosome 1q21.1 (*RP18*).

The *RP18* locus was first identified and refined to a less than 2 cM interval between markers D1S442 and D1S2858 in a large four-generation Danish family (5,6). Subsequent haplotype analysis in a pedigree of English origin (RP1188) placed *RP18* within a 1 cM interval between D1S442 and D1S498 (7).

The gene for U4/U6-associated splicing factor (*HPRP3*) has been mapped to the same interval on chromosome 1q21.1 (8). It encodes a protein of 682 amino acids in length with a calculated molecular weight of 77 kDa. The process of intron removal or pre-mRNA splicing occurs on a dynamic RNA protein complex called the spliceosome, which contains a

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pre-mRNA, four essential small nuclear ribonucleoprotein (snRNP) particles (UI, U2, U5 and U4/U6) and many auxiliary proteins. Each snRNP contains one snRNA except U4/U6 snRNP and about a dozen proteins. U4/U6 snRNP contains U4 and U6 snRNA in addition to the protein components. Hprp3p is one of the proteins tightly associated with U4/U6 snRNP, and mutations in its orthologue in yeast (*PRP3*) have been reported to cause U6 snRNA instability and prevent the splicing process (9,10). The recent identification of mutations in splicing factor genes *PRPF31* (3) and *PRPC8* (4) associated with adRP indicates *HPRP3* also to be a strong candidate for the *RP18* locus.

RESULTS

Mutation analysis

The genomic organization of the human *HPRP3* gene was ascertained by comparing the cDNA (GenBank accession no. AF_001947) with the genomic sequence of a chromosome 1 bacterial artificial chromosome clone RP5-835F16 (GenBank accession no. AL365403). This showed that the *HPRP3* gene consists of 16 exons and spans ~28 kb of genomic DNA. All coding exons in *HPRP3* (exons 2–16) and on average 50 bp of flanking intronic sequences were screened in the two known *RP18* linked families (RP1188 and the Danish family) by denaturing high performance liquid chromatography (DHPLC) and direct genomic sequencing. This identified the same 1482 C→T transition mutation in exon 11 in both families (Fig. 1A). The mutation resulting in amino acid substitution Thr494Met was shown to segregate perfectly with the disease in each family by virtue of gain of a *Nla*III site on the mutated allele (Fig. 2). A mutation screen of all coding *HPRP3* exons by DHPLC in a sample of 150 adRP and simplex cases (defined as individuals with no previous family history) from the UK and Germany identified three individuals with the same 1482 C→T mutation in exon 11. One was later found to be part of a small British adRP family RP173 whilst the other two were simplex RP cases (SP284 and SP55). The panel screen also yielded a new missense mutation in exon 11 (1478 C→T) causing the non-conservative amino acid change Pro493Ser also within a simplex RP case (SP13) (Fig. 1B). None of the mutations was present in the panel of 100 control subjects thus making it unlikely that the identified nucleotide changes were polymorphisms present in the normal population.

Haplotype analysis

In order to establish whether the three families (RP1188, RP173 and the Danish family) and simplex individuals SP284 and SP55 with the Thr494Met mutation all share a common ancestral haplotype or represent independent mutation events, we typed six markers all located within the *HPRP3* gene. The markers included five SNPs with the respective reference dbSNP numbers (1260408, 1260385, 698918, 698917 and 1136371) and one intragenic $(CA)_n$ located within intron 9 of *HPRP3*. This revealed two different haplotypes segregating with disease among the English and the Danish family (Table 1). However, due to the uninformativeness of the marker set used in the small English family (RP173) and the simplex patients SP284 and SP55, it was not possible to evaluate whether these were related either to the large English family or the Danish family (Table 1). Nevertheless, the data are strongly supportive of T494M (C→T) mutation arising independently in at least the Danish and the large English family (RP1188), raising the possibility of a mutation hot spot at position 1482.

Expression of *HPRP3*

PCR analysis of the cDNAs from a variety of normal tissues including the retina with a primer pair that amplifies exons 5 and 6 of *HPRP3* as a single amplicon produced the expected 305 bp product in all tested tissues (Fig. 3). Control primers for the *PGM1* gene amplified 419 bp fragment in each lane. This result is in agreement with the EST database (http://www.ncbi.nlm.nih.gov/UniGene/), which shows *HPRP3* gene to be expressed in a wide range of tissues including the neural tissues, brain and retina.

Hprp3p protein

Screening of the NCBI non-redundant protein database using the BLAST algorithm revealed highly similar proteins in *Mus musculus* (accession no. AAH06782), *Caenorhabditis elegans* (accession no. T23692), *Drosophila melanogaster* (accession no. AAF49097), *Schizosaccharomyces pombe* (accession no. Q09856) and *Saccharomyces cerevisiae* (accession no. NP_010761). The alignment of amino acid sequences of the human protein and its homologues shows that the C-terminal portion of the sequence is highly conserved throughout evolution whereas the N-terminal portion is not, suggesting a conserved function in the C-terminal end of the protein for pre-mRNA splicing. The residues 493 and 494 mutated in *RP18* show absolute conservation across homologous proteins in *S.cerevisiae*, *S.pombe*, *D.melanogaster* and *C.elegans* (Fig. 4). Hprp3p shows 99% identity with *M.musculus* protein, 50% with *D.melanogaster* protein and 35% with *C.elegans* protein. The overall sequence identities between Hprp3p and its yeast homologues are modest (27% with *S.cerevisiae* Prp3p and 34% with *S.pombe* Prp3) and human Hprp3p is also larger than its yeast homologue, which might reflect the difference in complexity of the splicing systems in yeast and human. Interestingly the mammalian Prp3 proteins (human and mouse) contain a PWI domain at their N-termini. The PWI domain is an 81-residue-long motif with a highly conserved PWI (Pro, Trp and Ile) sequence at its N-terminus, after which the motif has been named (11). It has also been identified in SRm160 protein which functions as a co-activator of constitutive and exon enhancer dependent splicing by mediating interactions between multiple factors bound to pre-mRNA (12,13). The PWI domain is not present in any of the other Prp3 homologues suggesting that it may have evolved to provide a function that is important for the assembly of splicing complexes in higher eukaryotes for mediation of multiple protein–protein interactions.

DISCUSSION

The spliceosome, the complex macromolecular machinery that catalyses pre-mRNA splicing is formed from several RNP subunits, termed uridine-rich small nuclear ribonucleoproteins (UsnRNPs), and numerous non-snRNP splicing factors. Each

Table 1. Genotype data from the families with Thr494Met missense mutation in *RP18*

Nucleotide position[a] of SNPs	Type of polymorphism/variation	Allelles segregating with RP18 mutation				
		RP1188	RP173	Danish family	Simplex case SP284	Simplex case SP55
9868	G/T	G	G/T[b]	T	G/T[b]	G
17584	$(CA)_n$	$(CA)_{11}$	$(CA)_{10}$	$(CA)_{10}$	$(CA)_{10}$	$(CA)_9/(CA)_{11}$[b]
18979	A/G	A	G/A[b]	G	G	A
19286[c]	C/T	T	T	T	T	T
22353	G/A	A	A/G[b]	A	A	–
22475	G/A	G	A/G[b]	G	G	–
22536	A/G	A	A	A	A	–

[a]The A of the initiator codon of *HPRP3* is denoted nucleotide +1. The genomic sequence of HPRP3 is available at GenBank accession no. AL365403.
[b]Marker was uniformative.
[c]The Thr494Met (C→T) missense muatation in exon 11.

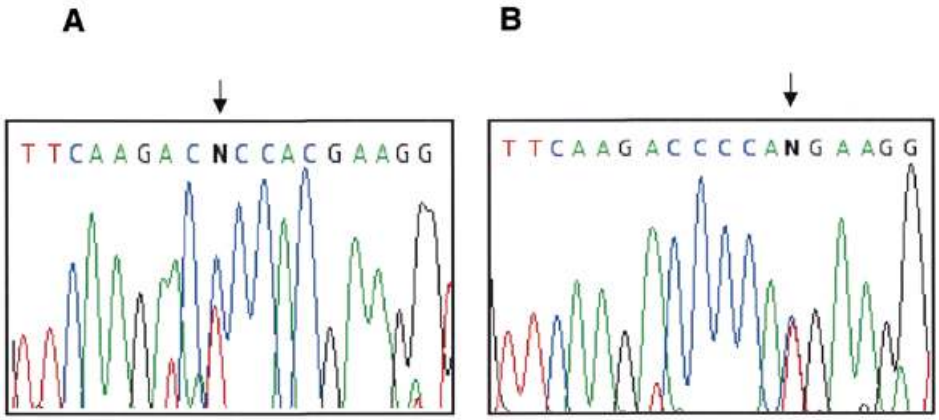

Figure 1. Sequence electropherograms of mutations found in *HPRP3*. (**A**) The Thr494Met (C→T) missense mutation found in the two English and Danish *RP18* families. (**B**) The Pro493Ser (C→T) missense mutation found in a sporadic case from the RP panel. A wild-type sequence is not shown since the sequence shown in (**A**) acts as a control for (**B**) and vice versa.

UsnRNP particle consists of an UsnRNA molecule complexed with a set of seven Sm (B/B′, D3, D2, D1, E, F and G which form the snRNP Sm core structure or so-called subcore complex) or Sm-like proteins (LSm) and several particle-specific proteins. The major spliceosomal snRNPs U1, U2, U4, U5 and U6 are responsible for splicing the vast majority of pre-mRNA introns (so called U2-type introns) (reviewed in 14). In splicing, the U1 snRNA first base pairs with the 5′ splice site to produce a commitment complex. The U2 snRNP then interacts with the branch point sequence in an ATP-dependent fashion to form the pre-spliceosome. The U4/U6 and U5 snRNPs associate with this complex as a single tri-snRNP particle to form the spliceosome. Conformational rearrangements then occur, allowing sequential cleavage at the 5′ and 3′ splice sites followed by ligation of the two exons to form the mature mRNA species.

Most of the proteins associated with snRNP particles have been first characterized in yeast, though much is now known of the human snRNP particles and its associated proteins. For example the U4/U6 snRNP particle contains the extensively base paired U4 and U6 snRNA, two heptameric Sm and LSm complexes and five additional proteins, one of which is Hprp3p. The other proteins include 15.5, 20, 60 and a 61 kDa protein (14). Interestingly the 61 kDa protein is encoded by *PRPF31*, the gene that is mutated in adRP localized to chromosome 19q13.4 (*RP11*). The fact that adRP linked to chromosome 17p (*RP13*) is caused by mutations in *PRPC8* (4), which encodes Prp8, the core component of the U5 snRNP (15), leads one to believe that the U4/U6.U5 tri-snRNP particle may play an important role in the splicing of retinal transcripts. It has now been observed that the 5′ splice site is also recognized by the U4/U6.U5 tri-snRNP, together with U1 at the earliest stages of spliceosome assembly (14). This early interaction appears to represent a functionally important initial recruitment of the tri-snRNP to the pre-mRNA, with subsequent stabilization of tri-snRNP occurring later. The tri-snRNP particle recruitment to the pre-mRNA could be the rate-limiting step in splicing and any deficiency of it is more likely

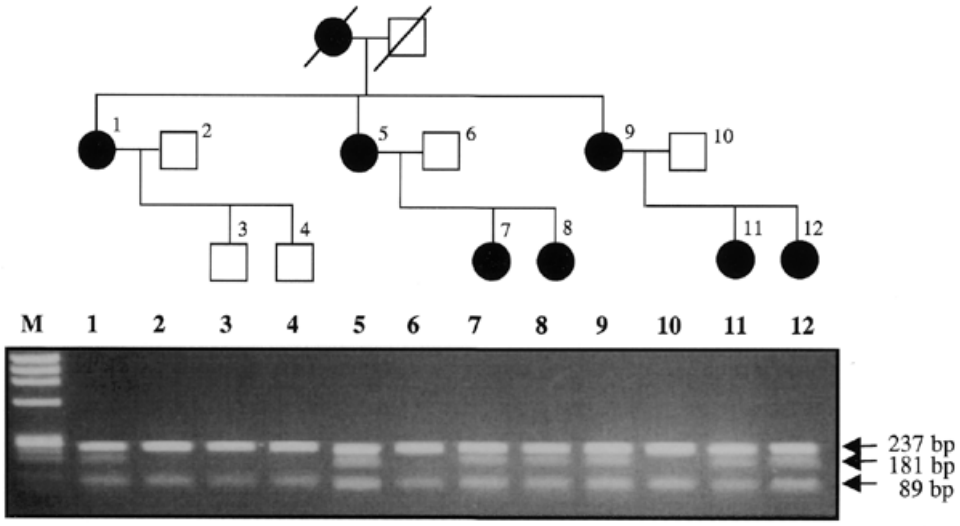

Figure 2. Segregation analysis of the 1482 C→T mutation in the English (RP1188) family by *Nla*III restriction digestion of a PCR fragment containing exon 11. Complete digestion of the PCR product results in a wild-type pattern of bands migrating at 237 and 89 bp due to one *Nla*III site within the fragment. In affected individuals, a heterozygous pattern of four bands with respective length of 237, 181, 89 and 56 bp (which cannot be seen on 2% agarose gel) is observed. This is owing to the extra restriction site for *Nla*III created within the 237 bp fragment due to codon 494 ACG→ATG mutation.

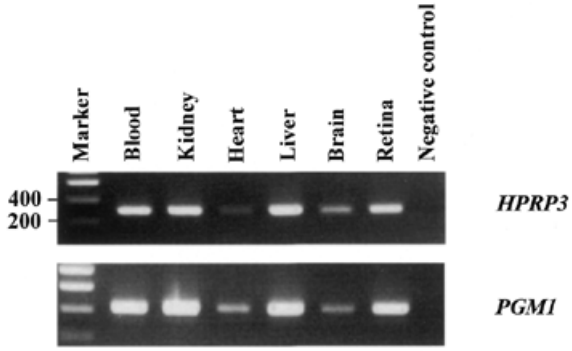

Figure 3. Expression of *HPRP3* in human tissues. PCR was carried out with primers selected from exons 5 and 6. A specific band of 305 bp was observed in all tested Quick-Clone™ human cDNAs (Clontech) from retina, brain, heart, kidney, liver and peripheral blood leukocytes. A ubiquitously expressed gene, *PGM1* was used as a control. Its 419 bp PCR fragment was also observed in all tissues. Please note that due to the quality of RNA from heart and brain tissues, a weak signal is observed for both *PGM1* and *HPRP3*.

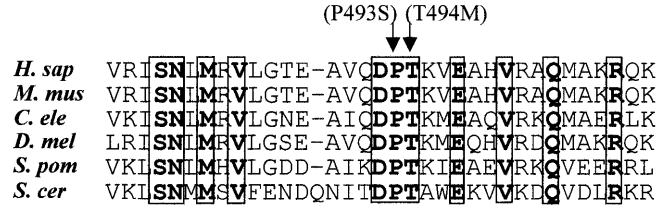

Figure 4. The amino acid alignment of human *HPRP3* exon 11 with that of *M.musculus* (M.mus), *C.elegans* (C.ele), *D.melanogaster* (D.mel), *S.cerevisiae* (S.cer) and *S.pombe* (S.pom). Amino acids, which exhibit complete identity across species, are boxed. Both missense mutations Thr494Met (C→T) and Pro493Ser (C→T) are marked by arrows.

to be detrimental in a highly metabolically active tissue such as the retina. The high rate of opsin turnover prior to disc shedding (16,17) in the rod photoreceptors is likely to place a considerable demand on the spliceosome machinery and may require a highly efficient splicing mechanism to be in place at such times. Therefore, even though *HPRP3* is widely expressed similar to *PRPF31* and *PRPC8*, mutations in *HPRP3* only show a phenotype in rod photoreceptors as the high rate of rod opsin production makes the rod photoreceptors particularly susceptible to the sub-normal activity of the spliceosome machinery (3). Another interesting possibility is the existence of a retina-specific splicing element that may interact with all three proteins that would explain the tissue specificity of mutations for all three of these ubiquitously expressed genes.

The effect of the *HPRP3* mutations on the function of the protein is as yet unknown. However, according to ScanProsite (http://ca.expasy.org/tools/scnpsit1.html) codons 494–497 of Hprp3p form a recognition motif for phosphorylation by casein kinase II (CK2), which is lost due to the T494M mutation. Whether this mutation actually affects this potential phosphorylation of Hprp3p remains to be tested. Hprp3 has been shown to interact with Hprp4 (60 kDa protein in U4/U6 snRNP) and in yeast the formation of the yPrp3/Prp4 heterodimer is a prerequisite prior to their association with the U4/U6 snRNP (18). The human Hprp3p with its first 100 amino acids deleted was still shown to interact with Hprp4p, thus indicating that this portion of the N-terminus including the PWI domain is not essential for interaction with Hprp4p (10). It is possible that the T494M and P493S mutations, which are in the highly conserved C-terminal end of the protein, may

have an effect on the binding of Hprp3p with Hprp4p. The fact that both the mutations are of the missense type suggests a dominant negative mechanism for disease causation.

In conclusion, we have shown that mutations in *HPRP3*, a putative human orthologue of yeast pre-mRNA splicing factor, are responsible for dominant retinitis pigmentosa (*RP18*) on 1q21.1. The finding in a third pre-mRNA splicing factor gene highlights the novel mechanism of photoreceptor degeneration owing to defects in the splicing process.

MATERIALS AND METHODS

Patients' sample collection

Patients with RP have been examined in the different university eye hospitals and gave informed consent for genetic studies. The study protocol was approved by the local ethics committee. The subjects also included individuals from families that have previously been shown to be linked to the *RP18* locus for dominant RP on chromosome 1. Blood samples were collected and DNA was extracted using standard protocols.

Mutation screening and co-segregation analysis

For mutation analysis of the *HPRP3* gene we amplified coding exons from patient genomic DNA using primers located in flanking intron and UTR sequences (primer sequences are available on request). PCR reactions were carried out in 25 µl reaction volumes containing 10 mM Tris–HCl pH 8.9, 50 mM KCl, 1.5 mM $MgCl_2$, 12.5 pmol of each primer, 200 µM each dNTP, 50–100 ng of patient genomic DNA and 0.25 U BioTaq™ thermostable DNA polymerase (Bioline). Cycling parameters were 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at the melting temperature (T_m) of the primers (54–65°C), and 30 s at 72°C with a final 5 min extension at 72°C. PCR products were purified using Qiaquick columns (Qiagen). DHPLC and direct sequencing was used for mutation detection. For DHPLC, PCR products were analysed using the WAVE® nucleic acid fragment analysis system (Transgenomic, Inc., San Jose, CA). The buffers used for DHPLC consist of buffer A, 0.1 M triethylammonium acetate (TEAA); and buffer B, 0.1 M TEAA with 25% acetonitrile. DNA fragment elution profiles were captured online and visually displayed using the Transgenomic WAVEMAKER™ software. Chromatograms were compared with those of normal controls to detect samples with altered elution profiles. Sequence variations were identified by automated bi-directional sequencing using an ABI 377 (Perkin Elmer) and BigDye terminator chemistries (Perkin Elmer). Co-segregation analysis of the mutation T494M in the English *RP18* (RP1188) family was tested by digestion of the 326 bp exon 11 PCR fragment generated by forward 5′-GGAAGTGAGTTTAGAGCAGAC-3′ and reverse primer 5′-AGAGCAACGGAGAACTCTCC-3′. In each case 10 µl of a 25 µl PCR reaction was incubated with 5 U *Nla*III in a 20 µl reaction volume at 37°C for 12 h. The product was analysed by electrophoresis on a 2% agarose gel.

Haplotype analysis

Five SNPs with the respective reference dbSNP numbers (1260408, 1260409, 698918, 698917 and 1136371) and one $(CA)_{10}$ repeat was selected in the region of introns upstream and downstream of the T494M mutation site. PCR and sequencing analysis were performed to show the segregation of the polymorphisms in the three *RP18* families (RP173, RP1188 and the Danish family).

Expression studies

Expression of *HPRP3* was assessed by PCR amplification of Quick-Clone™ human cDNAs (Clontech) from retina, brain, heart, kidney, liver and peripheral blood leukocytes, using exonic primers from exons 5 and 6, respectively (forward 5′-ATCAAACAGATGATGGAGGCAG-3′ and reverse 5′-GCAATGCCCATGGCATGGAG-3′) which amplify a 305 bp product. PCR products were then electrophoresed on a 2% agarose gel and visualized by ethidium bromide staining. The ubiquitously expressed *PGM1* gene was used as an amplification control.

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REFERENCES

1. Sullivan,L.S., Heckenlively,J.R., Bowne,S.J., Hide,W.A., Gal,A., Denton,M., Inglehearn,C.F., Blanton,S.H. and Daiger,S.P. (1999) Mutations in a novel retina-specific gene cause autosomal dominant retinitis pigmentosa. *Nat. Genet.*, **22**, 255–259.
2. Bessant,D.A., Ali,R.R. and Bhattacharya,S.S. (2001) Molecular genetics and prospects for therapy of the inherited retinal dystrophies. *Curr. Opin. Genet. Dev.*, **11**, 307–316.
3. Vithana,E.N., Abu-Safienh,L., Allen,M.J., Carey,A., Papaioannou,M., Chakarova,C., Al-Maghtheh,M., Ebenezer,N.D., Willis,C., Moore,A.T. *et al.* (2001) A human homolog of yeast pre-mrna splicing gene, prp31, underlies autosomal dominant retinitis pigmentosa on chromosome 19q13.4 (rp11). *Mol. Cell*, **8**, 375–381.
4. McKie,A.B., McHale,J.C., Keen,T.J., Tarttelin,E.E., Goliath,R., van Lith-Verhoeven,J.J.C., Greenberg,J., Ramesar,R.S., Hoyng,C.B., Cremers,F. *et al.* (2001) Mutations in the pre-mRNA splicing factor gene PRPC8 in autosomal dominant retinitis pigmentosa (RP13). *Hum. Mol. Genet.*, **10**, 1555–1562.
5. Xu,S., Rosenberg,T. and Gal,A. (1998) Refined genetic mapping of autosomal dominant retinitis pigmentosa locus *RP18* reduces the critical region to 2 cM between D1S442 and D1S2858 on chromosome 1q. *Hum. Genet.*, **102**, 493–494.
6. Xu,S.J., Schhwartz,M., Rosenberg,T. and Gal,A. (1996) A ninth locus (*RP18*) for autosomal dominant retinitis pigmentosa maps in the pericentromeric region of chromosome 1. *Hum. Mol. Genet.*, **5**, 1193–1197.
7. Inglehearn,C.F., Tarttelin,E.E., Keen,T.J., Bhattacharya,S.S., Moore,A., Taylor,R. and Bird,A.C. (1998) A new dominant retinitis pigmentosa family mapping to the *RP18* locus on chromosome 1q11–21. *J. Med. Genet.*, **35**, 788–792.
8. Heng,H.H.Q., Wang,A. and Hu,J. (1997) Mapping of the human HPRP3 and HPRP4 genes encoding U4/U6-associated splicing factors to chromosomes 1q21.1 and 9q31–q33. *Genomics*, **48**, 273–275.
9. Anthony,J.G., Weidenhammer,E.M. and Woolford,J.L.,Jr (1997) The yeast Prp3 protein is a U4/U6 snRNP protein necessary for integrity of the U4/U6 snRNP and the U4/U6.U5 tri-snRNP. *RNA*, **10**, 1143–1152.
10. Wang,F.A.J., Luo,Y., Chow,Y., Plumb,J., Friesen,J.D., Tsui,L., Heng,H.H.Q., Woolford, J.L. and Hu, J. (1997) Identification and

characterisation of human genes encoding Hprp3p and Hprp4p, interacting components of the spliceosome. *Hum. Mol. Genet.*, **6**, 2117–2126.
11. Blencowe,B.J. and Ouzounis,C.A. (1999) The PWI motif: a new protein domain in splicing factors. *Trends Biochem. Sci.*, **24**, 179–180.
12. Blencowe,B.J., Issner,R., Nickerson,J.A. and Sharp,P.A. (1998) A coactivator of pre-mRNA splicing. *Genes Dev.*, **12**, 996–1009.
13. Eldridge,A., Li,Y., Sharp,P.A. and Blencowe,B.J. (1999) The SRm160/300 splicing coactivator is required for exon-enhancer function. *Proc. Natl Acad. Sci. USA*, **96**, 6125–6130.
14. Will,L.C. and Lührmann,R. (2001) Spliceosomal UsnRNP biogenesis, structure and function. *Curr. Opin. Cell Biol.*, **13**, 290–301.
15. Brown,J.D. and Beggs,J.D. (1992) Roles of PRP8 protein in the assembly of splicing complex. *EMBO J.*, **11**, 3721–3729.
16. Korenbrot,J.I. and Fernanald,R.D. (1989) Circadian rhythm and light regulate opsin mRNA in rod photoreceptors. *Nature*, **337**, 454–457.
17. von Schantz,M., Lucas,R.J. and Foster,R.G. (1999) Circadian oscillation of photopigment transcript levels in the mouse retina. *Brain Res. Mol. Brain Res.*, **72**, 108–114.
18. Ayadi,L., Callebaut,I., Saguez,C., Villa,T., Mornon,J. and Banroques,J. (1998) Functional and structural characterization of the Prp3 binding domain of the yeast Prp4 splicing factor. *J. Mol. Biol.*, **284**, 673–687.