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Positional cloning of the gene for X-linked retinitis pigmentosa 3: homology with the guanine-nucleotide-exchange factor RCC1

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The gene for retinitis pigmentosa 3 (RP3), the most frequent form of X-linked RP (XLRP), has been mapped previously to a chromosome interval of less than 1000 kbp between the DXS1110 marker and the OTC locus at Xp21.1-p11.4. Employing a novel technique, 'YAC Representation Hybridization (YRH), we have recently identified a small XLRP associated microdeletion in this interval, as well as several putative exons including the 3' end of a gene that was truncated by the deletion. cDNA library screening and sequencing of a cosmid centromeric to the deletion has now enabled us to identify numerous additional exons and to detect several point mutations in patients with XLRP. The predicted gene product shows homology to RCC1, the guanine-nucleotide-exchange factor (GEF) of the Ras-like GTPase Ran. Our findings suggest that we have cloned the long-sought RP3 gene, and that it may encode the GEF of a retina-specific GTP-binding protein.

INTRODUCTION

Retinitis pigmentosa (RP), a heterogeneous group of disorders characterized by night blindness, progressive constriction of the visual field and 'pigmented' fundus abnormalities, is due to the gradual degeneration of photoreceptor cells which begins in the periphery and progresses to the central region of the retina. In advanced stages, patients present with tunnel vision and may become legally blind. About 80% of the patients have autosomal recessive forms of RP. Autosomal dominant and the more severe X-linked RP (XLRP) account each for approximately 10% of the cases, but in Denmark and the UK, a much higher proportion of XLRP has been reported (1,2).

At least three different gene loci are involved in X-linked retinitis pigmentosa (XLRP), which have been mapped by

linkage studies and heterogeneity testing to Xp11.2-p11.4 (RP2), Xp11.4-p21.1 (RP3) and farther distal on Xp (RP6), respectively (3–5). By molecular characterization of two deletions in patients with RP and other X-linked disorders, the gene for the most frequent form of XLRP, retinitis pigmentosa 3 (RP3), has been assigned previously to an interval of less than 1000 kilobases (kb) between the DXS1110 marker and the ornithine transcarbamylase (OTC) gene (6).

In previous studies by our group that led to the molecular elucidation of two other eye disorders (7,8), intragenic microdeletions of a few kbp had been instrumental for fine mapping and cloning of the respective genes. With conventional (Southern blotting and PCR based probe screening) techniques, such small microdeletions are difficult to find, however, even in genomic intervals of 1000 kb or less. To overcome this difficulty, we have recently developed a novel 'YAC Representation Hybridisation' technique which was employed for microdeletion screening in the chromosomal interval carrying the RP3 gene (9). In one out of 30 patients with XLRP, a 6.4 kb microdeletion was detected. By sequencing of a cosmid spanning this deletion and by cDNA library screening, several exons were found, including the 3' end of a gene that was partially deleted in this patient. None of these exons contained an open reading frame, however, and the search for point mutations in patients with XLRP was unsuccessful.

Therefore, in order to identify additional exons from the 5' end of this gene, we have now isolated and sequenced a neighboring cosmid from a previously established cosmid contig, as well as two *EcoRI* fragments separating this cosmid from the (more distal) one that had been sequenced previously. Computer-assisted analysis of this sequence predicted numerous additional exons which could be confirmed by cDNA cloning and sequencing, and in several patients with XLRP, small mutations were detected which were not observed in healthy controls. Sequence comparisons have revealed that the deduced product of this gene shows strong structural similarities with RCC1, the guanine nucleotide exchange factor of the Ras-like GTPase Ran. These findings suggest that we have identified the long-sought *RP3* gene, and

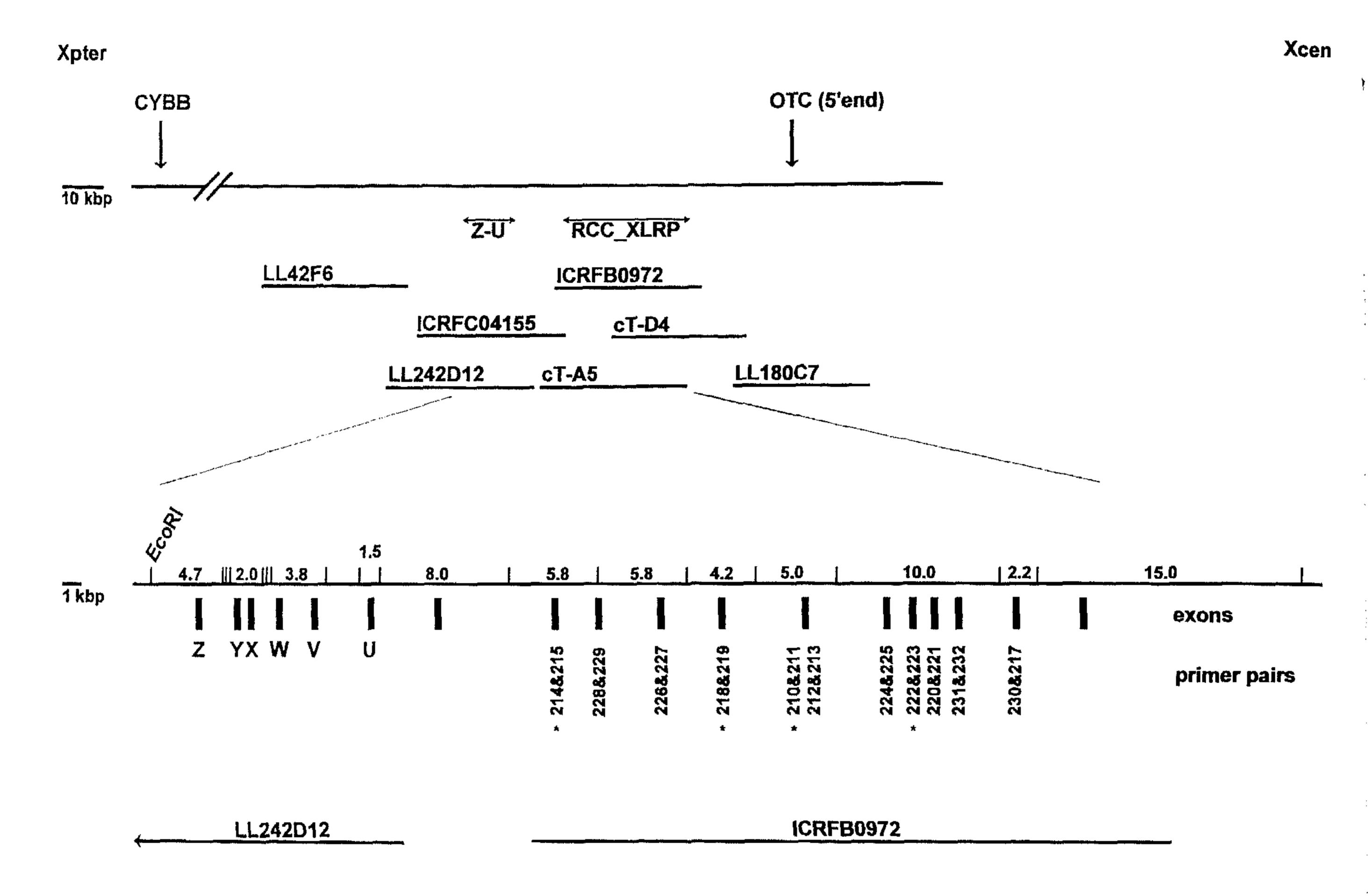


Figure 1. Physical map of the region containing the RP3 gene. The upper part of the figure shows the cosmid contig covering the region between the 3' end of the RP3 gene and the 5' end of the OTC gene. Cosmids derived from the ICRF and Lawrence Livermore (LL) libraries were published previously (9). Clones cT-A5 and -D4 were isolated from a library generated by subcloning a YAC (see Materials and Methods). The lower part of this figure displays the EcoRI restriction map and exon distribution in a 70 kbp region harboring the RP3 gene. Cosmid clones LL242D12 and ICRFB0972 were sequenced. The sequence contig was completed by sequencing the 8.0 and 5.8 kbp fragments between them. Exons were identified by aligning cDNA and genomic sequences. Fragments U-V were described previously (9), and all other exon fragments correspond to cDNAs which were isolated by screening a retina library with cosmid clone cT-A5. Primer numbers given for 10 of the novel exons refer to Table 1. The asterisks indicate fragments containing sequence alterations confined to patients.

they may provide a clue to its role in the pathogenesis of retinitis pigmentosa.

RESULTS

Identification of protein coding sequences from the RP3 critical region

In order to extend the cDNA of a previously identified candidate gene for RP3 (9) in the 5' direction, we have sequenced the neighboring cosmid clone ICRFB0972 (see Fig. 1) by a shotgun approach. Sequence data were assembled in 15 contigs, covering 39.776 bp proximal to the cosmid LL242D12 (Fig. 1), which was sequenced recently (9). Moreover, the gap between these two cosmids was closed by sequencing two intervening 8.0 and 5.8 kbp *Eco*RI fragments by primer walking. In total, we have sequenced approximately 75 kbp from the genomic region carrying the RP3 gene. In parallel another cosmid, cT-A5, was employed to screen a cDNA library from adult retina. This cosmid clone which is almost identical to cosmid ICRFB0972 was derived from a cosmid library of YAC ICRFy900E0701 (see Fig. 1). The cDNA screening revealed six positive clones, four of which were characterized in more detail by Southern blot hybridization and sequence analysis. Seven partially overlapping cDNA clones were assembled by using

the program GELMERGE (10). The resulting cDNA consensus sequence was aligned to genomic sequences from cosmids ICRFB0972 and LL242D12 and the 8.0 and 5.8 kbp *EcoRI* fragments. In this way 12 additional exons were identified (Figs 1 and 2). One hundred and one nucleotides at the 3' end of the consensus cDNA sequence did not match genomic sequences which points to a gap in our genomic sequence contig. Since the penultimate exon of the cDNA is present on the 5.8 kbp *EcoRI* fragment, this gap is most likely between this and the adjacent 8.0 kbp *EcoRI* fragment. Five of the exons were also identified by the GRAIL exon prediction program (releases 2.0 and 1.3, see Materials and Methods).

Northern blot hybridization with a probe containing 1177 bp from the 5' end of the cDNA detected a transcript of approximately 3 kbp in heart, brain, placenta, lung, liver, skeletal muscle, and pancreas (Fig. 3). A FASTA search with the consensus cDNA sequence of 1557 bp failed to detect significant homologies with GENEMBL entries. It contains a contiguous reading frame of 518 amino acid residues (Fig. 2), which was used to perform database searches by employing the BLASTP as well as the FASTA programs (10). Significant homologies were detected with RCC1 proteins from several species, including human, hamster, *Xenopus, Drosophila*, and yeast. The most significant homology was

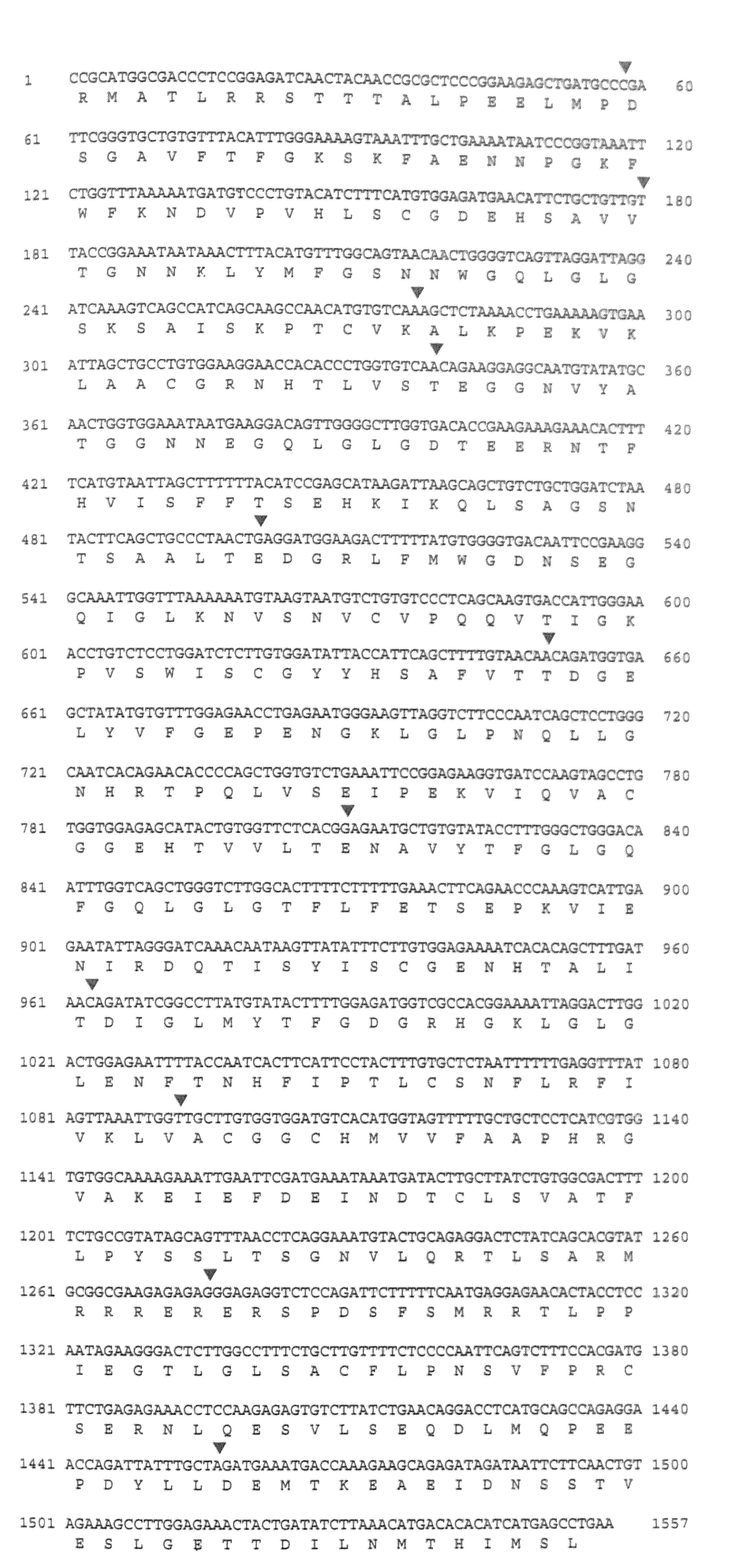


Figure 2. cDNA consensus sequence with the deduced protein translation. The boundaries between the different exons are represented by arrowheads. The sequence contains a reading frame, encoding 518 amino acid residues. A translation start site was not identified, indicating an incomplete 5' end.

observed with human, hamster and *Xenopus* RCC1 proteins, i.e. approximately 30% identity within an overlap of 450 amino acid residues. Amino acid identity with the *Drosophila* and yeast RCC1 peptides was 26 and 20%, respectively.

In a previous study (9), we had identified the 3' end of a gene, which was disrupted in one patient with XLRP by a 6.4 kbp

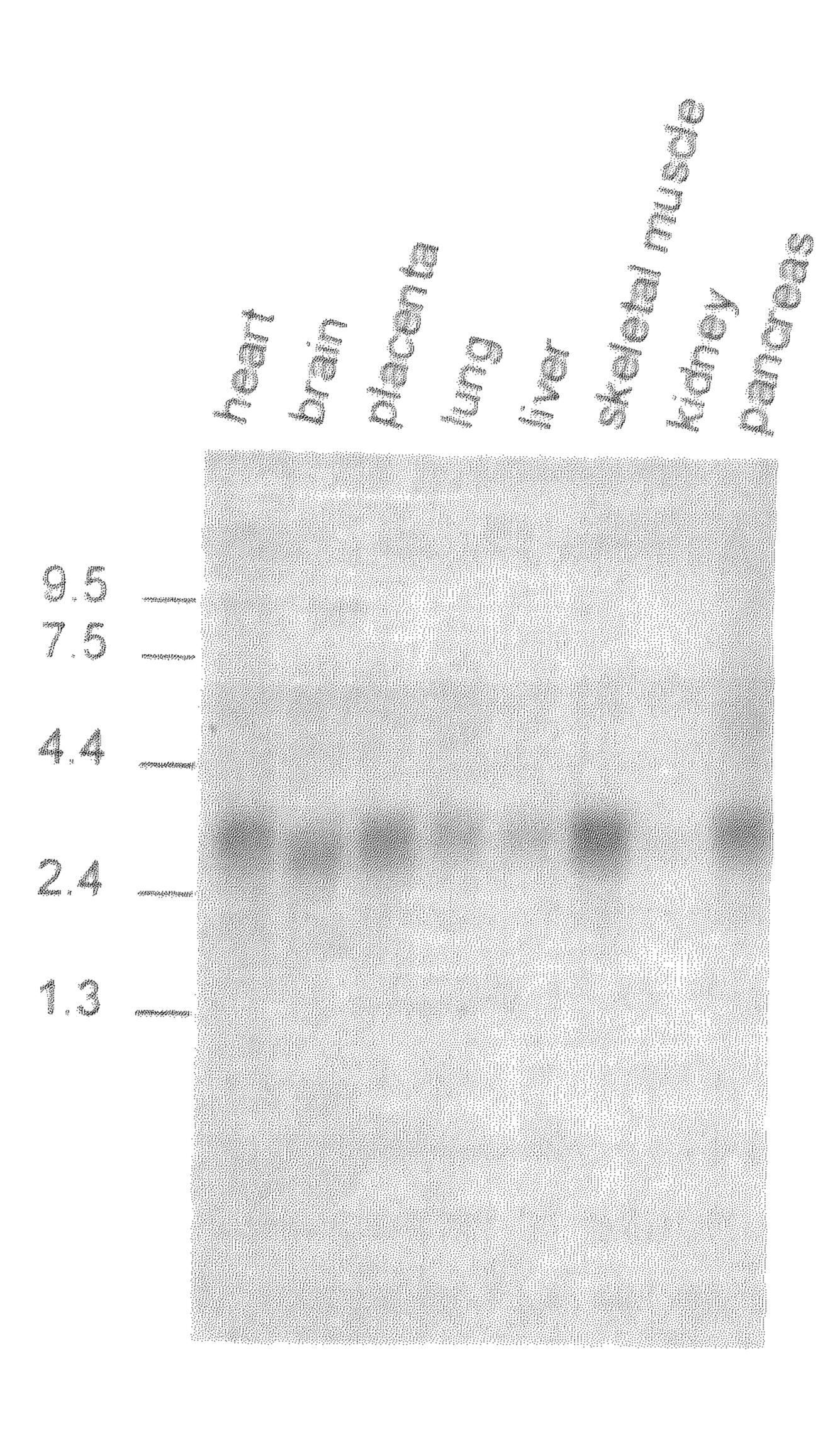


Figure 3. Autoradiograph of a Northern blot containing poly(A)⁺-RNA from various adult tissues after hybridization with a cDNA probe containing 1177 bp from the 5' end of the cDNA presented in Figure 2. Sizes of the marker fragments are given in kbp. The size of the detected transcript was estimated as 3 kbp approximately.

microdeletion. The six exons (fragments U-Z, see Figure 1) showed no disease related sequence alterations but several polymorphisms. RT-PCR experiments have now revealed that these exons belong to the gene presented here. By using an antisense primer from exon W (Fig. 1) in combination with a sense primer from position 472–492 of the consensus cDNA (Fig. 2), a 1.5 kbp fragment was amplified, and sequence analysis from both ends of the PCR product confirmed the identity of the exons. In total, 2.7 kbp of cDNA sequence were identified, representing at least 18 exons of one gene.

Detection of point mutations in patients with X-linked RF

Mutation screening was carried out in 28 XLRP patients by means of SSCP analysis. Intron primers were designed for the PCR amplification of 10 exons (see Table 1). Five bandshifts were observed in patients but not in controls, indicating disease-specific sequence alterations. The corresponding FCR fragments were sequenced which revealed three different nucleotide exchanges and one 4 bp deletion in five patients (see Fig. 4). Patient 4767 carried a T to G transversion at nucleotide position 420, resulting in the substitution of phenylalanine by cysteine. A proline to serine exchange was shown to be the consequence of a C to T transition at position 734 of the cDNA sequence in patient 2884. Another serine residue was generated due to a G to A exchange at postion 854 in patients 2603 and 2555. Strikingly, the two mutations that give rise to a serine residue are located in a highly conserved domain of the protein which shows 40% identity in a 57 amino acid segment of the RCC1 consensus sequence (RCC1_2.PRF, derived from release 10.0 of PROSITE, see underlined residues in Fig. 4). Moreover, the amino acid substitution in patients 2555 and 2603 affect a glycine residue which is highly conserved during evolution (Fig. 4, position 331). A 4 bp deletion was observed in patient 2550 (nucleotides 1433-1436), resulting in a truncated protein with six abnormal C-terminal amino acids, including a conserved glutamic acid residue (see Figs 4 and 5). None of these changes was detected in 84 male controls.

Table 1. Oligonucleotide primers used for PCR amplification, SSCP screening, and sequencing of exon fragments from the RP3 gene

Primer ID	Sequence (5'→3')	Fragment size (bp)	cDNA contained	
210	gta gtt ctc ata gta ttc tta cca	235	716–809	···
211	tta ggt ett eee aat eag ete			
212	tte aga eac eag etg ggg tg	204	651-725	
213	caa atg tat taa gtg atc ctt gtc			
214	caa ett tta gga aag ttt ata tea at	289	1276-1456	
215	aag gea cat tta tee tga gag e			
218	gtg tet get tee ata ate ett g	259	810–963	
219	tgt aat aaa ata tac eca gtt cta ta			
220	etg gaa tga gae ete agt tet e	190	276–338	
221	cat ttg tcc tgg act act gtt ca			
222	gga ete tat agt eta ttg aeg tt	238	339–500	
223	agg gaa tgt gtc cca gac tga a			
224	get tea gag eet gge tae et	256	501–650	
225	aca aca tag aag tgg gag ata ac			
226	ata gat eca tae aag taa eae ttt	250	964-1092	
227	tee atg aaa tae tga aag aet ea			
228	cag tgc tag ata ata cta tta tac	273	1093-1275	
229	tga age caa tgt tga tga gtt t			
217	gtt att tta ata aca ggc aac ata g	266	59–180	
230	aca cag cag cat atc tat aac aa			
231	tga cat taa aga act aca cag tca	229	181-275	
232	tga cet eat ett aca tta tgt gaa			

In addition to these apparently disease-specific changes we have detected two polymorphisms. One was a G to A transition at position 1305 of the cDNA sequence, resulting in a substitution of arginine by leucine. This change was present in two patients with X-linked RP but also in two out of 86 controls and in the sequence of cosmid ICRFB0972. The second polymorphism was detected at position 1322 (A to G, isoleucine to serine), present in one patient and in five out of 86 controls. Both exchanges represent conservative amino acid substitutions and involve a domain of the predicted gene product that is not homologous to RCC1 proteins.

DISCUSSION

RP3, the gene for the most frequent form of XLRP, had long been known to map to an interval of less than 1000 kbp between the DXS1110 marker and the OTC gene at Xp11.4-p21.1 (3-6,11) but numerous efforts to isolate this gene by positional cloning had remained unsuccessful. By making use of a novel 'YAC Representation Hybridization' approach, to screen this interval for microdeletions, we have recently detected a 6.4 kbp deletion in a patient with XLRP (9). Molecular characterization and sequencing of cosmids and cDNAs spanning this deletion

enabled us to define the last six exons of a gene that was expressed in various tissues including retina, and this cDNA was also contained in a larger deletion described by others (12). Here we describe the identification of six novel retina cDNAs and 12 corresponding exons which could be linked to the previously isolated cDNA by RT-PCR and represent the middle and 5' portion of the same gene. This gene encodes a protein of at least 518 amino acids; the outermost sequences of the ORF have not yet been identified. The predicted protein shows significant homology to the guanine nucleotide exchange factor RCC1 of several species. In five out of 28 patients with XLRP, small sequence alterations were found which were not observed in 84 control males and are therefore considered as disease-specific mutations. In two apparently unrelated patients, the same mutation was observed.

So far, mutations in the *RP3* gene have only been detected in 18% of the patients with XLRP, which is at variance with linkage studies suggesting that *RP3* accounts for at least two-thirds of the familial cases. There are several possible explanations for this discrepancy. First, systematic mutation screening has only been done so far in 16 out of 18 exons identified. Secondly, we have not yet found all exons of the *RP3* gene; at the 5' end, at least one exon is still missing, and the promotor region of this gene, which has not yet been cloned,

	1				50
RCC_HUMAN RCC_MESAU	MSPKRIA	KRRSPPADAI	PK.SKK	v	SHRSHSTEPG
RCC_XENLA	MKGKKTL	KRRSPPBDAI KRTIAABESN	GT.SDVKK.	VK,V	SERSHKTEPG
RCC_DROMB	MPRRKAL	TNNNNAGRAE	QQPPKAKR	ARTAPHL	ELPKRRTVIA
RCC_YEAST	MVKRTV	ATNGDASGAH	RAKKMSKTHA	SHIINAORDY	KHMYLSVOPI.
RCC_XLRP3	RRIMPUSGAV	FTFGKSKFAE	NNPGKPWPKN	DVPVHLSCGD	EESAVVTGNN
	51				100
RCC_HUMAN	LVLTLGQGDV	GQLGLGEN	VMERKKPALV	SIPEDV	VONEAGGMET
RCC_MESAU RCC_XENLA	LVLTLGQGDV	GQLGLGES	VLERKKPALV	PLLQDV	VQABAGGMHT
RCC_XENIA RCC_DROMB	NVLVCGNGDV	GQLGLGED	ILERARLSDV	TLTRDI	VQAAAGGMHT
RCC_YEAST	DIFCWGTGSM	CELGLGPLAK	NKEVKRPRLN	PPLPRDEAKI	ISFAVGGMHT
RCC_XLRP3	KLYMPGSNNW	GQLGLGSKSA	ISKPTCVK	ALKPEKV	KLAACGRNHT
	101				100
RCC_HUMAN		SPGCMDEGAL	GRDTS	VEGS	150 EMVPGKVRLO
RCC_MESAU	VCLNQSGQVY	SFGCMDEGAL	GRDTS	VEGS	EMVPGKVELO
RCC_XENLA	VCLGASGSIY	TFGCMDEGAL	GRDTS	REGS	EM QPGKVELA
RCC_DROME RCC_YEAST	LALDEESNVW	SPGCMDEGAL SWGCMDVGAL	GROTSNAKRO	LKDMDannee	ESKPDLIDLP
RCC_XLRP3	LVSTEGGNVY				
mut 4767					C
	151				the co
RCC_HUMAN	E	К	VVQVSAGDSH	TAALTDDGRV	200 PLNGSFRDNN
RCC_MBSAU	E		VVQVSAGDSH	TAALTEDGRV	FLNGSFRDNN
RCC_XENLA		K			
RCC_DROME RCC_YEAST		SFPPLAEGHK			
					
RCC_HUMAN	GVIGLLER	. MKKSMVPVQ	V OLDOMINATO	A COMPANY 1941	250
RCC_MBSAU		.MKKSMVPVQ			
RCC_XENLA		.MKKSMVPVQ			
RCC_DROME		.GNK.RTPID			
RCC_YBAST RCC_XLRP3	GILGFYQDKI	.VSNVCVPQQ	TFSKYNIVQL VTIGKPVSWT		
	- g				1450551770
	251		***		300
RCC_HUMAN RCC_MBSAU		PELFANRGGR PELFANRGGR			-
RCC_XENLA		PERFINEGGE			-
RCC_DROMB		SERSISGEGR			
RCC_YRAST					
RCC_XLRP3 mut 2884	RARMGKTGUA	NOLLGNHRTP	OLASKI	- PRKATOAY	CGGEHTVVLT
		S			
		2			
DCC UMMXX	301		CHOLONVIAL	a abampea	350
RCC_HUMAN RCC MESAU	DAFCGAYFTF	A. ISHEGHVY			FIPQNLTSFK
RCC_HUMAN RCC_MESAU RCC_XENLA	DAFCGAYFTF DAFCGAYLTF		GFGLSNYHQL	GTPGTASC	FIPONLTSFK FIPONLTSFK
RCC_MESAU RCC_XENLA RCC_DROME	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF	A.ISHEGHVY A.ISREGHVY A.VSQEGHVY MRESQTQVIW	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL	GTPGTASC GTKNTQAC AHETKGKE	FIPONLTSFK FIPONLTSFK YAPONLTSFK PALTPIK
RCC_MESAU RCC_XENLA RCC_DROME RCC_YRAST	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL	A.ISHEGHVY A.ISREGHVY A.VSQEGHVY MRESQTQVIWTKDNKLV	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC	GTPGTASC GTKNTQAC AHETKGKE GVSEDVEDGA	FIPONLTSFK FIPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL
RCC_MESAU RCC_XENLA RCC_DROME	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL	A.ISHEGHVY A.ISREGHVY A.VSQEGHVY MRESQTQVIW	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC	GTPGTASC GTKNTQAC AHETKGKE GVSEDVEDGA	FIPONLTSFK FIPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL	A.ISHEGHVY A.ISREGHVY A.VSQEGHVY MRESQTQVIWTKDNKLV	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC	GTPGTASC GTKNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS	FIPONLTSFK FIPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLV	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGQL	GTPGTASC GTKNTQAC AHETKGKE GVSEDVEDGA GLGTPLFETS S	FIPONLTSFK FIPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETICMD	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR	GTPGTASC GTKNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS S	FIPONLTSFK FIPONLTSFK YAPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL LVTKPKRLAL EPKVIENIR 400AEYGRLGAEYGRLG
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCMD GGQHETVCVD	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGQL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR	GTPGTASC GTKNTQAC AHETKGKE GVSEDVEDGA GLGTPLFETS S	FIPONLTSFK FIPONLTSFK YAPONLTSFK YAPONLTSFK FALTPIK LVTKPKRLAL LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHHTVCMD GGQHHTVCMD GGQHHTVCVD GGQHHTVCVD	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR	GTPGTASC GTKNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS S	FIPONLTSFK FIPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLGAEYGRLG
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCMD GGQHETVCVD	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS S	FIPONLTSFK FIPONLTSFK YAPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLGPEYGRLG DNLPEYTYKD
RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA DQTISYIS	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVCVD GGQHETVLT AGEHESLILS	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS S	FIPONLTSFK FIPONLTSFK YAPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_YEAST RCC_XLRP3	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA DQTISYIS	A.ISHEGHVY A.ISREGHVY A.VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHHTVCMD GGQHHTVCVD GGQHHTVCVD GGQHHTVLT AGEHHSLILS CGENHTALIT	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR DIGLMYTFGD	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTPLFETS S	FIPONLTSFK FIPONLTSFK YAPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL LVTKPKRLAL LEPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG 450
RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST	DAFCGAYETF DAFCGAYETF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS PDNVVIRSIA PDNVVIRSIA PDNVVIRSIA DQTISYIS	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVCVD GGQHETVLT AGEHESLILS	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .VVSSVACGA	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS S	FIPONLTSFK PIPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG A50
RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_XLRP3	DAFCGAYFTF DAFCGAYLTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS DAFCGAEEKS LG.EGAEEKS LG.EGAEEKS LG.EGAEEKS	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHHTVCMD GGQHHTVCVD GGQHHTVCVD GGQHHTVLT AGEHHSLILS CGENHTALIT IPTLISR.LP IPTLISR.LP EPTPIPD.LP	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR GDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .VVSSVACGA .KINSVASGA	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTPLFETS S	FIPONLTSFK FIPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG A50
RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_YEAST RCC_XLRP3	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWIGFS TELKDIRHIA PDNVVIRSIA PDNVVIRSIA DQTISYIS 401 LG.EGAEEKS LG.EGAEEKS LG.EGAEEKS LG.ENAEEQS LG.DVKDVVE	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCMD GGQHETVCVD GGQHETVLT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGQL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR ODGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .VVSSVACGA .KINSVASGA EKIVSVGCGE	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS S	FIPONLTSFK FIPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG A50
RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_XLRP3	DAFCGAYFTF DAFCGAYLTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS ASTKSWVGFS ASTKSWVGFS NSTKSWVGFS N	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHHTVCMD GGQHHTVCVD GGQHHTVCVD GGQHHTVLT AGEHHSLILS CGENHTALIT IPTLISR.LP IPTLISR.LP EPTPIPD.LP	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .VVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAGS	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTPLFETS S	FIPQNLTSFK FIPQNLTSFK YAPQNLTSFK YAPQNLTSFK FALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG 450
RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_XLRP3 RCC_XLRP3	DAFCGAYETF DAFCGAYETF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA PDNVVIRSIA DQTISYIS 401 LG.EGAEEKS	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVLT AGEHESLILS CGENETALIT IPTLISR.LP IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .VVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAGS	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTPLFETS S LDMFEVGIPK S	FIPONLTSFK FIPONLTSFK YAPONLTSFK FALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGPEYGRLG DNLPEYTYKDGRHGKLG 450 A50 RGVAKEIEFD
RCC_MESAU RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_YEAST RCC_XLRP3 RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS ASTKSWVGFS NSTKSWVGFS N	A.ISHEGHVY A.ISREGHVY A.VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVLT AGEHESLILS CGENETALIT IPTLISR.LP IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGQL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR GDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .VVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTPLFETS S S	FIPQNLTSFK PIPQNLTSFK YAPQNLTSFK YAPQNLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG A50 A50 RGVAKEIEFD
RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_XLRP3 RCC_XLRP3	DAFCGAYFTF DAFCGAYLTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS APDNVVIRSIA PDNVVIRSIA	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVLT AGEHESLILS CGENETALIT IPTLISR.LP IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR GDGDLYSCGR QDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .VVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK	FIPQNLTSFK FIPQNLTSFK YAPQNLTSFK YAPQNLTSFK FALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG A50 A50 RGVAKEIEFD 500 EDAWS
RCC_MESAU RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XENLA RCC_LENLA RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_XLRP3	DAFCGAYFTF DAFCGAYFTF DAFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA PDNVVIRSIA DQTISYIS 401 LG.EGAEEKS LG.EGA	A.ISHEGHVY A.ISREGHVY A.VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCMD GGQHETVCVD GGQHETVLLT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNELRVGYAVIKDVGYAVIKDVGYAVSKDVSYAVSTD	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR QDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .VVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GCVFAWGMGT	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK S S V H CHMVVFAAPH NYQLGTGQ.D NYQLGTGQ.D NLQLGTGE.E	FIPQNLTSFK FIPQNLTSFK YAPQNLTSFK YAPQNLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG 450 A50 EDAWS EDAWS EDAWS
RCC_MESAU RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_LENLA	DAFCGAYFTF DAFCGAYFTF DAFCGAYFTF AVECTORY ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA PDNVVIRSIA DQTISYIS 401 LG.EGAEEKS LG.EGA	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVENAVY GGQHETVCMD GGQHETVCVD GGQHETVCVD GGQHETVLT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR VGYAVTKDVGYAVTKDVGYAVSKDVSYAVSTDCSYAVTID	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR QDGDLYSCGR QDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GCVFAWGMGT GCVFAWGMGT	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK S	FIPQNLTSFK FIPQNLTSFK YAPQNLTSFK YAPQNLTSFK FALTPIK LVTKPKRLAL LVTKPKRLAL LEPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG A50 A50 EDAWS EDAWS EDAWS EDVWS DDELE
RCC_MESAU RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XENLA RCC_LENLA RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_XLRP3	DAFCGAYFTF DAFCGAYFTF DAFCGAYFTF AVECTORY AVECTORY ASGENHOTAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA DQTISYIS 401 LG.EGAEEKS LG.EGAEEK	A.ISHEGHVY A.ISREGHVY A.VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCMD GGQHETVCVD GGQHETVLLT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNELRVGYAVIKDVGYAVIKDVGYAVSKDVSYAVSTD	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR QDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GCVFAWGMGT GKLYSWGFGE	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK LDMFEVGIPK S	FIPQNLTSFK FIPQNLTSFK YAPQNLTSFK YAPQNLTSFK FALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG 450 A50 EDAWS EDAWS DDELE DDTEV
RCC_MESAU RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_MESAU RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3 RCC_YEAST RCC_XLRP3 RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWIGFS TELKDIRHIA PDNVVIRSIA PDNVVIRSIA DQTISYIS 401 LG.EGAEEKS LG.EGA	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVCVD GGQHETVLLT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR VGYAVIKDVGYAVIKDVGYAVSKDVSYAVSTDCSYAVIIDHSVAVAQN	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR QDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GCVFAWGMGT GKLYSWGFGE	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK LDMFEVGIPK S	FIPONLTSFK FIPONLTSFK YAPONLTSFK YAPONLTSFK FALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG 450GRHGKLG A50 RGVAKEIEFD 500 EDAWS EDAWS DDELE DDTEV PDSFSMRRTL
RCC_MESAU RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROMB RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_YEAST RCC_XLRP3 RCC_YEAST RCC_XLRP3	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA PDNVVIRSIA DQTISYIS 401 LG.EGAREKS LG.EGAREKS LG.EGAREKS LG.EGAREKS LG.ENAERQS LG.DVKDVVE VH.GKARAVP LGLENFTNHF 451 EINDTCLSVA	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCMD GGQHETVCVD GGQHETVLT AGEHESLILS CGENETALIT IPTLISR.LP IPTLISR.LP IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNELR VGYAVIKDVGYAVIKDVGYAVSKDVSYAVSTDCSYAVTIDHSVAVAQN TFLPYSSLTS	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGQL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR QDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GCVFAWGMGT GKLYSWGSGV GIAYSWGFGE GNVLQRTLSA	GTRNTOAC AHETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK S	FIPONLTSFK FIPONLTSFK YAPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL LVTKPKRLAL LEPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG 450 450 CONTRESSED 500 EDAWS EDAWS DDELE DDTEV PDSFSMRRTL
RCC_MESAU RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_MESAU RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3 RCC_YEAST RCC_XLRP3 RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST	DAFCGAYFTF DAFCGAYFTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA PDNVVIRSIA DQTISYIS 401 LG.EGAREKS LG.EGAREKS LG.EGAREKS LG.EGAREKS LG.EGAREKS LG.ENAEROS LG.DVKDVVE VH.GKARAVP LGLENFTNHF 451	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVCVD GGQHETVLLT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR VGYAVIKDVGYAVIKDVGYAVSKDVSYAVSTDCSYAVIIDHSVAVAQN	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR QDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .KINSVASGA EKIVSVGCGE .KPKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GCVFAWGMGT GKLYSWGSGV GIAYSWGFGE GNVLQRTLSA	GTRATOAC AHETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK S	FIPONLTSFK FIPONLTSFK YAPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG A50 EDAWS EDAWS DDELE DDTEV PDSFSMRRTL
RCC_MESAU RCC_LYRAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROMB RCC_XENLA RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_LYRAST RCC_XLRP3 RCC_LYRAST RCC_XLRP3 RCC_LYRAST RCC_XLRP3 RCC_LYRAST RCC_XLRP3 RCC_LYRAST RCC_XLRP3 RCC_LYRAST RCC_XLRP3	DAFCGAYFTF DAFCGAYLTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWIGFS TELKDIRHIA PDNVVIRSIA PDNVVIRSIA DQTISYIS 401 LG.EGABEKS LG.EGAB	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVCVD GGQHETVLIT AGENETALIT IPTLISR.LP IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR VGYAVTKDVGYAVTKDVGYAVSKDVSYAVSTDCSYAVTIDHSVAVAQN TFLPYSSLTS KQLENRVVLT KHLEDREVLS	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GRVFAWGMGT GCVFAWGMGT GCVFAWGMGT GKLYSWGSGV GIAYSWGFGE GNVLQRTLSA VSSGGQHTVL VSSGGQHTVL VSSGGQHTVL	G. TRATCAC G. TKNTOAC A. HETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK S	FIPQNLTSFK PIPQNLTSFK YAPQNLTSFK YAPQNLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGPEYGRLG DNLPEYTYKDGRHGKLG 450 RGVAKEIEFD 500 EDAWS EDAWS EDAWS DDELE DDTEV PDSFSMRRTL
RCC_MESAU RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3	DAFCGAYFTF DAFCGAYLTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS N	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVLIT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR VGYAVTKDVGYAVTKDVGYAVSKDVSYAVSTDLPYSSLTS KQLENRVVLS KQLENRVVLS KQLENRVVLT KHLEDREVLS KNTQGKHMLL	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .VVSSVACGA .KINSVASGA EKIVSVGCGE .KPKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GRVFAWGMGT GCVFAWGMGT GKLYSWGSGV GIAYSWGFGE GNVLQRTLSA VSSGGQHTVL VSSGGQHTVL VSSGGQHTVL ASGGGQHAIF	G. TRNTQAC A. HETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK S	FIPQNLTSFK FIPQNLTSFK YAPQNLTSFK YAPQNLTSFK PALTPIK LVTKPKRLAL LEPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLFEYTYKDGRHGKLG 450 EDAWS
RCC_MESAU RCC_YEAST RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST	DAFCGAYPTF DAFCGAYLTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA PDNVVIRSIA DQTISYIS 401 LG.EGABEKS LG.EGABEKS LG.ENAEBQS LG.DVKDVVB VH.GKARAVP LGLENFTNHF 451 PVEMMGPVEMMGPVEMMGPVEMMGPIVVVSPTRIKN	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVLIT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR VGYAVTKDVGYAVTKDVGYAVSKDVSYAVSTDCSYAVTIDHSVAVAQN TFLPYSSLTS KQLENRVVLS KQLENRVVLS KNTQGKHMLL TATQDHNIIL	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .VVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRGAWGT GRGA	GTRNTQAC AHETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK LDMFEVGIPK S	FIPONLTSFK FIPONLTSFK YAPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG A50 EDAWS EDAWS DDELE DDTEV PDSFSMRRTL 550 ENVPVKVSGS EKRADEMDD.
RCC_MESAU RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XLRP3	DAFCGAYPTF DAFCGAYLTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA PDNVVIRSIA DQTISYIS 401 LG.EGABEKS LG.EGABEKS LG.ENAEBQS LG.DVKDVVB VH.GKARAVP LGLENFTNHF 451 PVEMMGPVEMMGPVEMMGPVEMMGPIVVVSPTRIKN	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVLIT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR VGYAVTKDVGYAVTKDVGYAVSKDVSYAVSTDLPYSSLTS KQLENRVVLS KQLENRVVLS KQLENRVVLT KHLEDREVLS KNTQGKHMLL	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .VVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRGAWGT GRGA	G. TRNTQAC A. HETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK LDMFEVGIPK S	FIPONLTSFK FIPONLTSFK YAPONLTSFK YAPONLTSFK PALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGAEYGRLG DNLPEYTYKDGRHGKLG A50 EDAWS EDAWS DDELE DDTEV PDSFSMRRTL 550 ENVPVKVSGS EKRADEMDD.
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XLRP3 RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3	DAFCGAYPTF DAFCGAYLTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA DQTISYIS 401 LG.EGAEEKS LG.EGAEEKS LG.ENAEEQS LG.DVKDVVE VH.GKARAVP LGLENFTNHF 451 EINDTCLSVA 501PVEMMGPVEMMGPVEMTG	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVLIT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR VGYAVTKDVGYAVTKDVGYAVSKDVSYAVSTDCSYAVTIDHSVAVAQN TFLPYSSLTS KQLENRVVLS KQLENRVVLS KNTQGKHMLL TATQDHNIIL	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .VVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRGAWGT GRGA	G. TRNTQAC A. HETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK LDMFEVGIPK S	FIPONLTSFK FIPONLTSFK YAPONLTSFK YAPONLTSFK FALTPIK LVTKPKRLAL .EPKVIENIR 400AEYGRLGAEYGRLGPEYGRLG DNLPEYTYKDGRHGKLG A50 EDAWS BDAWS DDELE DDTEV PDSFSMRRTL 550 ENVPVKVSGS EKRADEMDD. EEPDYLLDEM
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XLRP3 RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3	DAFCGAYPTF DAFCGAYLTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA DQTISYIS 401 LG.EGAEEKS LG.ENAEEQS LG.DVKDVVE VH.GKARAVP LGLENFTNHF 451 EINDTCLSVA 501PVEMMGPVEMMGPVEMTG	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCVD GGQHETVCVD GGQHETVCVD GGQHETVLIT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR VGYAVTKDVGYAVTKDVGYAVSKDVSYAVSTDCSYAVTIDHSVAVAQN TFLPYSSLTS KQLENRVVLS KQLENRVVLS KQLENRVVLT KHLEDREVLS KNTQGKHMLL TATQDHNIIL ACFLPNSVFP	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GRVFAWGMGT GRVFAWGMGT GKLYSWGSGV GIAYSWGFGE GNVLQRTLSA VSSGGQHTVL VSSGGQHTVL VSSGGQHTVL ASGGGOFSVS RCSBRNLQES	G. TRNTQAC G. TKNTQAC A. HETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK S	FIPQNLTSFK FIPQNLTSFK YAPQNLTSFK FA. LTPIK LVTKPKRLAL LEPKVIENIR 400AEYGRLGAEYGRLGPEYGRLG DNLPEYTYKDGRHGKLG A50 RGVAKEIEFD 500 EDAWS EDAWS DDELE DDTEV PDSFSMRRTL 550 ENVPVKVSGS EKRADEMDD. ENVPVKVSGS EKRADEMDD. MEPDYLLDEM NQIIC* 600
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST	DAFCGAYPTF DAFCGAYLTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA DQTISYIS 401 LG.EGAEEKS LG.EGAEEKS LG.ENAEEQS LG.DVKDVVE VH.GKARAVP LGLENFTNHF 451 BINDTCLSVA 501PVEMMGPVEMMGPVEMTGPVEMTGPVEMTGPVEMTGPVEMTGPIVVVSPTRIKN PPIEGTLGLS	A. ISHEGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVLIT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR VGYAVTKDVGYAVSKDVSYAVSTDCSYAVTIDHSVAVAQN TFLPYSSLTS KQLENRVVLS KQLENRVVLS KQLENRVVLT KHLEDREVLS KNTQGKHMLL TATQDHNILL ACFLPNSVFP	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GRVFAWGMGT GCVFAWGMGT GCVFAWGMGT GCVFAWGMGT GCVFAWGMGT GCVFAWGMGT GCVFAWGMGT CCVFAWGMGT GCVFAWGMGT GCVFAWGMGT CCVFAWGMGT GCVFAWGMGT CCVFAWGMGT CCVFAWGMG	G. TRNTOAC G. TKNTOAC A. HETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK S S V H CHMVVFAAPH NYQLGTGQ.D NYQLGTGQ.D NYQLGTGQ.D NYQLGTGG.E NNQLGVGD.G TYAVGLGPFE RMRRRERERS LVKDKEQS LVKDKEQS LVKDKEQS LVKDKEQS LVKDKEQS LVKDKEQS RWRRS RGGVKLSDEDA VLSEQDLMQP R	FIPQNLTSFK FIPQNLTSFK YAPQNLTSFK PA. LTPIK LVTKPKRLAL LEPKVIENIR 400 .AEYGRLG .AEYGRLG .AEYGRLG DNLPEYTYKD .GRHGKLG A50 AS0 EDAWS PDSFSMRRTL 500 EDAWS PDSFSMRRTL 550 ENVPVKVSGS EKRADEMDD. EPPDYLLDEM NQIIC* 600
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_XLRP3 mut 2550 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XLRP3 mut 2550	DAFCGAYPTF DAFCGAYLTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS NSTKSWVGFS TELKDIRHIA PDNVVIRSIA DQTISYIS 401 LG.EGAEEKS LG.ENAEEQS LG.DVKDVVE VH.GKARAVP LGLENFTNHF 451 BINDTCLSVA 501PVEMMGPVEMMGPVEMT	A.ISHEGHVY A.ISREGHVY A.VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVLIT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR .VGYAVTKD .VGYAVTKD .VGYAVSKD .VSYAVSTD .CSYAVTID .HSVAVAQN TFLPYSSLTS KQLENRVVLS KQLENRVVLS KQLENRVVLT KHLEDREVLS KNTQGKHMLL TATQDHNILL TATQDHNILL ACFLPNSVFP	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GRVFAWGMGT GRVFAWGMGT GKLYSWGSGV GIAYSWGFGE GNVLQRTLSA VSSGGQHTVL VSSGGQHTVL VSSGGQHTVL ASGGGQFSVS RCSBRNLQES	G. TRNTQAC G. TKNTQAC A. HETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK S	FIPQNLTSFK FIPQNLTSFK FIPQNLTSFK YAPQNLTSFK YAPQNLTSFK FA. LTPIK LVTKPKRLAL .EPKVIENIR 400 .AEYGRLG .AEYGRLG .AEYGRLG DNLPEYTYKD .GRHGKLG 450 EDAWS EDAWS DDELE DDTEV PDSFSMRRTL 550 ENVPVKVSGS EKRADEMDD. ENVPVKVSGS EKRADEMDD. MQIIC* 600
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XLRP3 mut 2550 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XLRP3 mut 2550	DAFCGAYFTF DAFCGAYLTF DVFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS N	A. ISHEGHVY A. ISREGHVY A. ISREGHVY A. VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVCVD GGQHETVLIT AGEHESLILS CGENETALIT IPTLISR.LP IPTLISR.LP IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR VGYAVTKDVGYAVTKDVGYAVSKDVSYAVSTDCSYAVTIDHSVAVAQN TFLPYSSLTS KQLENRVVLS KQLENRVVLS KQLENRVVLT KHLEDREVLS KNTQGKHMLL TATQDHNILL ACFLPNSVFP	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR GDGDLYSCGR QDGDLYSCGR QDGDLYSCGR OIGLMYTFGD .AVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GRVFAWGMGT GCVFAWGMGT GCVFAWGMG	G.TPGTASC G.TKNTQAC A.HETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK S S V H CHMVVFAAPH NYQLGTGQ.D NLQLGTGQ.D NLQLGTGE.E NNQLGVGD.G TYAVGLGPFE RMRRERERS LVKDKEQS	FIPQNLTSFK FIPQNLTSFK FIPQNLTSFK YAPQNLTSFK YAPQNLTSFK FA. LTPIK LVTKPKRLAL .EPKVIENIR 400 .AEYGRLG .AEYGRLG .AEYGRLG DNLPEYTYKD .GRHGKLG 450 EDAWS BOO EDAWS BODTEV PDSFSMRRTL 550 ENVPVKVSGS EKRADEMDD. EPPDYLLDEM NQIIC* 600 KNKTPPQDNA
RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 mut 2555/2 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_XENLA RCC_DROME RCC_YEAST RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XLRP3 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_YEAST RCC_XLRP3 mut 2550 RCC_HUMAN RCC_MESAU RCC_YEAST RCC_XLRP3 mut 2550	DAFCGAYFTF DAFCGAYLTF DAFCGAYLTF DAFCGAYLTF DAFCGAYFTF AIWATNYCTF ASGENHCFAL 603 351 NSTKSWVGFS	A.ISHEGHVY A.ISREGHVY A.VSQEGHVY MRESQTQVIWTKDNKLVKNAVY GGQHETVCMD GGQHETVCVD GGQHETVLIT AGEHESLILS CGENETALIT IPTLISR.LP EPTPIPD.LP KPTIVKK.LT LPTKLNN.VP IPTLCSNFLR .VGYAVTKD .VGYAVTKD .VGYAVSKD .VSYAVSTD .CSYAVTID .HSVAVAQN TFLPYSSLTS KQLENRVVLS KQLENRVVLS KQLENRVVLT KHLEDREVLS KNTQGKHMLL TATQDHNILL TATQDHNILL ACFLPNSVFP	GFGLSNYHQL GFGLSNYHQL ATGLNNFKQL SWGLNQFGQC TFGLGOFGOL SEGKAYSLGR SEGKAYSLGR SEGKAYSLGR TDLKCSVVGR QDGDLYSCGR DIGLMYTFGD .AVSSVACGA .KINSVASGA EKIVSVGCGE .KFKSVAAGS FIVKLVACGG GRVFAWGMGT GRVFAWGMGT GRVFAWGMGT GCVFAWGMGT GCVFAWGMG	G. TRNTQAC G. TKNTQAC A. HETKGKE GVSEDVEDGA GLGTFLFETS S LDMFEVGIPK S S V H CHMVVFAAPH NYQLGTGQ.D NYQLGTGQ.D NYQLGTGQ.D NYQLGTGE.E NNQLGVGD.G TYAVGLGPFE RMRRRERERS LVKDKEQS	FIPQNLTSFK FIPQNLTSFK FIPQNLTSFK YAPQNLTSFK PA. LTPIK LVTKPKRLAL .EPKVIENIR 400 .AEYGRLG .AEYGRLG .AEYGRLG DNLPEYTYKD .GRHGKLG A50 EDAWS BDAWS DDELE DDTEV PDSFSMRRTL 550 ENVPVKVSGS EKRADEMDD. EPPDYLLDEM NQIIC* 600 KNKTPPQDNA

might also harbour several of the 'missing' mutations. Mutations in the RP2 gene, located in the Xp11.2-p11.4 region proximal to RP3, is thought to be involved in almost one-third of the cases—but then, the contribution of RP2 and RP3 mutations in XLRP is not well defined and may vary in different populations. Finally, the possibility cannot be ruled out that there is yet another XLRP gene in the vicinity of RP3.

The predicted amino acid sequence of the RP3 gene identified in this study shows the highest homology (31.5%) with the human RCC1 protein (13), which acts as a guanine-nucleotide-exchange factor (GEF) of Ran, a GTPase which recently was shown to be involved in nuclear protein import (14). GEFs associate with GDP-bound forms of GTPases and accelerate GDP dissociation and GTP binding, thereby activating the GTPase. A large number of GEFs have been identified, most of which are involved in Ras signalling (15). RCC1 is composed of seven repeats of about 60 residues each. The RP3 gene thus far is the only gene showing homology to RCC1. In another X-linked eye disorder, choroideremia, the defective gene plays a role in the geranylgeranylation of several different Rab proteins, another family of Ras-related GTPases (7,16). In view of the clinical similarities between choroideremia and RP3, it is tempting to speculate that the RP3 protein might function as a GEF for retina specific Rab proteins. Thus far, two different rabGEFs, i.e. Mss4 (mammalian suppressor of sec4) and Dss4-1 (dominant suppressor of sec4) have been cloned (17,18). These proteins do not show any sequence similarity to RCC1 or other GEFs, nor to the RP3 protein described here. Given the large number of GEF molecules involved in ras signalling, the RP3 protein might represent the first member of a new family of GEFs regulating the activity of rabs or related GTPases.

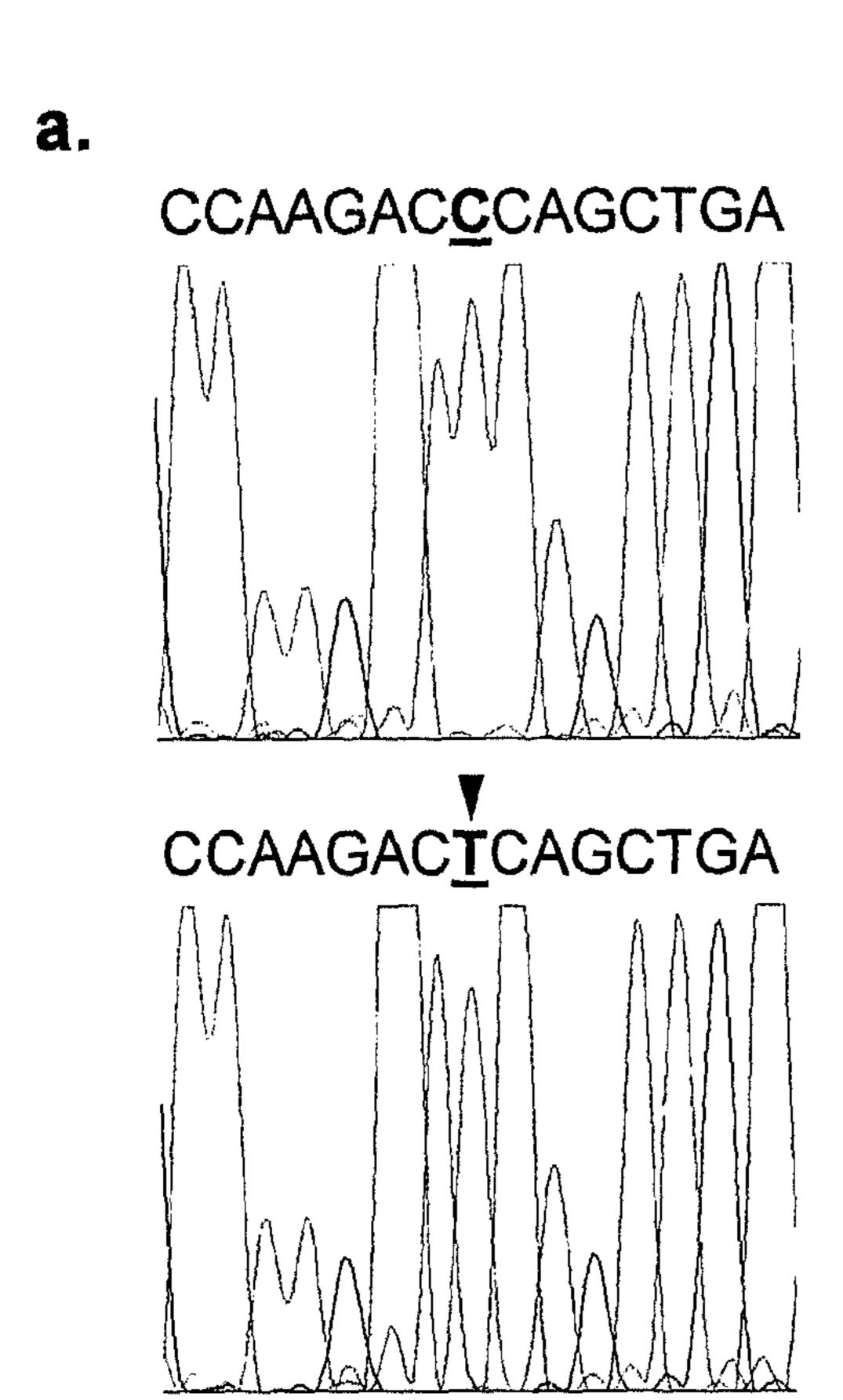
Previously, the presymptomatic diagnosis and carrier detection in XLRP families has been severely hampered by the genetic heterogeneity of this disorder and particularly by the fact that neither clinically nor genetically, RP3 and RP2 could be reliably distinguished. Therefore, the direct detection of *RP3* mutations in presymptomatic affected males and female carriers will also facilitate the diagnosis in other forms of XLRP, and the molecular elucidation of the fundamental defect underlying RP3 may be a clue to the etiology and pathogenesis of RP2, too.

MATERIALS AND METHODS

Construction of a cosmid library

In order to construct a complete cosmid contig from the RP3 critical region we have subcloned the YAC ICRFy900E0701 into the sCOGH2, an improved version of the recently described cosmid vector sCOGH1 (19). The cosmid library was prepared as described previously (8). Briefly, YAC DNA was partially digested with the restriction enzyme Sau3A, separated on a

Figure 4. Sequence alignment of RCC1 proteins from different species by using the PILEUP program. Peptide sequences for the human, hamster, *Xenopus, Drosophila* and yeast RCC1 molecules are derived from the Swiss.prot database (accession numbers P18754, P23800, P25183, P25171, P21827). Amino acid residues conserved between the RCC1 polypeptides and the predicted *RP3* gene product are depicted in bold. The RCC1-profile (RCC1_2.PRF, derived from release 10.0 of PROSITE) is underlined. Changes in the amino acid sequence, detected in patients only, are indicated by: mut 4767 (position 145), mut 2884 (position 270), mut 2555/2603 (position 331) and mut 2550 (positions 540–546)



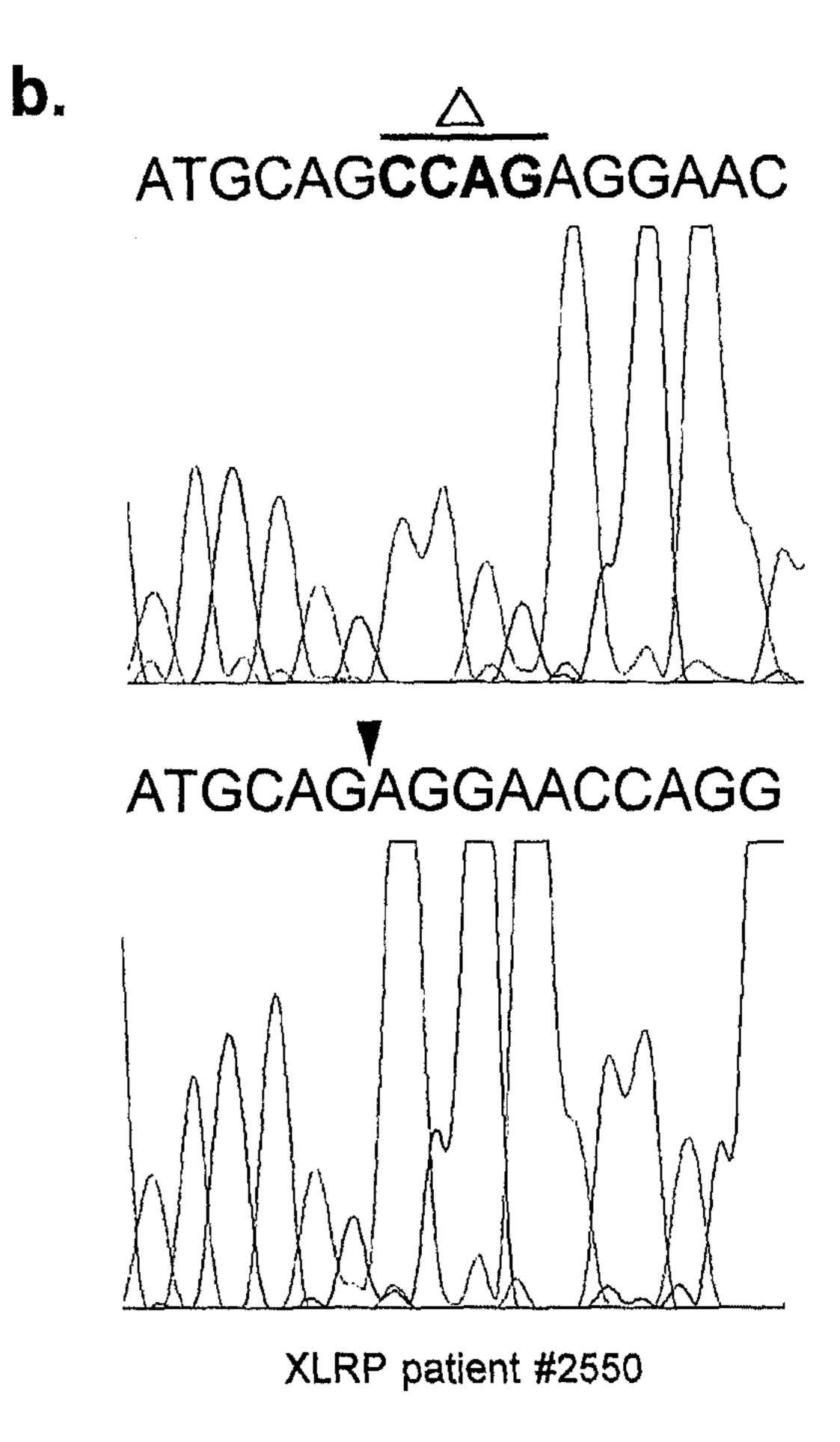


Figure 5. Sequencing patterns from PCR products of controls (top) and patients (bottom). The PCR amplification was performed with primer pairs (a) 218 & 219 and (b) 214 & 215, and sequencing reactions were carried out with primers 218 and 214, respectively.

sucrose gradient, and the fraction between 30–40 kbp was cloned into the *Bam*HI digested sCOGH2 vector. The ligation mix was packaged by using Stratagene packaging extract (Gold), and 4200 resulting colonies were screened with human placenta DNA. One hundred and forty-four clones containing human DNA inserts were picked and gridded on Nylon membrane (GeneScreenPlus). The contig between the previously identified *RP3* candidate gene (9) and OTC was completed by hybridizing these grids with cosmids LL242D12 and ICRFB0972 as well as the OTC cDNA probe.

XLRP patient #2555

Screening of cDNA libraries

The *Not*I insert of cosmid cT-A5 was used to screen a cDNA library, established from adult retina (courtesy of J. Nathans). Screening of 2 million p.f.u. of each library, plated on *E.coli* LE392, was performed as described previously (8). Positive plaques were purified and phage DNA was prepared by using the Qiagen Lambda kit. Inserts were isolated preparatively and subcloned into the pGEM3 vector.

DNA sequencing and data processing

Shotgun cosmid sequencing was performed by dye-primer chemistry on an ABI377 automated sequencer as described previously (9). Assembly of the data was achieved by using the Staden package. Sequencing of the two genomic *Eco*RI fragments (8.0 and 5.8 kbp) and the cDNA clones was carried out by primer

walking on subcloned fragments using the DyeDeoxy terminator cycle sequencing kit (ABI). The data were assembled by the GELMERGE program. Exon-intron boundaries were determined by aligning the cDNA sequences to the genomic sequence contig by using BESTFIT and FASTA programs. Prediction of putative exon sequences was done by GRAIL, using releases 2.0 and 1.3 from the GRAIL e-mail server (grail@ornl.gov).

mRNA isolation and RT-PCR

Total RNA was isolated from Hela cells as described elsewhere (20) and poly A⁺ mRNA was isolated using the oligotex mRNA kit (QIAgen). RT-PCR was performed mainly as described elsewhere (20), using the sense primer 57 (5'-TGGATCTAAT-ACTTCAGCTGC) and the antisense primer 1136 (5'-GTTTCA-GCTGAGCTATCATCA) for PCR amplification of the cDNA products. 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 2 min and extension at 72°C for 3 min were carried out with an initial denaturation step of 5 min and a final extension step of 6 min. The PCR products were analysed on a 1.5% agarose gel, purified using the QIAquick gel extraction kit (QIAgen) and sequenced as described above.

Single strand conformation polymorphism (SSCP) analysis

Exon fragments from patients and controls were amplified with intron primers as described (9). The primer sequences, the

corresponding positions within the cDNA and sizes of the fragments are summarized in Table 1. SSCP-PCR was carried out in the presence of α[³²P]dCTP, and the denatured radiolabeled fragments were separated in a non denaturing polyacrylamide gel as described (21). Sequencing of aberrant PCR fragments was performed by dyedeoxy termination cycle sequencing (ABI) on an ABI370A automated sequencer.

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