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Isolation and chromosomal localization of a new human retinoblastoma binding protein 2 homologue 1a (*RBBP2H1A*)

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Using a *NotI* linking clone NR-025 as a probe, we isolated a novel putative member of the RB binding protein family, namely a human retinoblastoma binding protein 2 homologue (*RBBP2H1A*). The maximal open reading frame encodes a protein of 1681 amino acids. Homology analysis indicated that the predicted product has an overall 56% amino acid identity to RBBP2, which plays an important role in RB tumor suppressor regulation. Many extended regions are 100% identical in amino acids sequences. The degree of nucleotide identity is lower. The structure prediction analysis identified three DNA-binding zinc finger domains and two bipartite nuclear localization signals. Northern expression analysis revealed expression in all tissues; however, the level of expression significantly varied between tissues. The highest level of expression was detected in testis and the lowest in skeletal muscle. The mRNA sizes corresponding to two major products are around 6kb and 7kb. Using fluorescence *in situ* hybridization, we mapped the gene to chromosomal band 1q32.1. *European Journal of Human Genetics* (2000) 8, 407–413.

Keywords: *NotI*-linking clone; molecular cloning; human; gene mapping; sequence alignment; fluorescence *in situ* hybridization

Introduction

Several years ago, we proposed an approach to combine physical and gene mapping methods to characterize large regions of human chromosomes^{1,2} and *NotI* linking and jumping clones were proposed as framework markers. We then developed simplified new procedures for jumping and linking library construction and made a number of chromosome 3 specific and total human *NotI* linking libraries.³

One thousand *NotI* linking clones from the chromosome 3 specific grids were partially sequenced and were found to represent 249 unique clones. Among these, we carefully analyzed 150 clones.⁴ The results confirmed previous sugges-

tions from us and others that: (i) *NotI* sites are frequently associated with human genes and (ii) that the sequence data generated can serve as an framework for joining and closing of other types of maps.^{4–7}

We therefore decided to sequence all *NotI* sites in the entire human genome in the search for the new genes and to generate framework markers. The results of a pilot study, consisting of about 10% of the entire clone collection, have been analysed and will be presented elsewhere. Our analysis showed that a surprisingly large number (more than 40%) of *NotI* clones have identity with ESTs. It can be nevertheless being concluded that data from our *NotI* project are complementary to data obtained from various EST projects. We have thus confirmed the opinion that *NotI* sites are located predominantly at the 5' end of genes while the majority of the EST clones have obviously been produced with oligo dT primers resulting in over-representation of the 3' end of genes.^{8,9}

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Our *NotI* project on the entire human genome has already resulted in the discovery of many new genes. Here we report on a novel putative member of the RB binding protein family, namely a human retinoblastoma binding protein 2 homologue *RBBP2H1A*.

Materials and methods

Construction of *NotI* linking libraries was described previously.³

Sequencing was done using ABI377 sequencers according to standard procedures. Homology searches were performed using *blastx* and *blastn* programs at the NCBI server.^{10,11}

Search of protein profiles against the PROSITE-library was done at the ISREC server (<http://www.isrec.isb-sib.ch/software/PFSCAN/form.html>).

Eukaryotic promoters prediction by neural network (NNPP) was done at the BCM server (<http://dot.imgen.bcm.tmc.edu:9331/seq-search/gene-search.html>). The

hybridization with MTN northern filters (Clontech, Palo Alto, CA, USA) was done according to the manufacturer's protocols.

The standard procedure of fluorescence *in situ* hybridization (FISH) analysis with metaphase chromosomes and DNA fibres was performed as described previously.^{12,13}

Results and discussion

To check the validity of similarities between *NotI*-linking clone sequences and genes we selected clone NR-025. We sequenced an area of about 500 bp surrounding this *NotI* site. The sequence displayed 51% identity over 35 amino acids with retinoblastoma binding protein 2 (RBBP2).¹⁴ No significant similarity was found based on nucleic acids (64.8% identity in 108 bp overlap). After extending the sequenced area to 1.2 kb we found two EST clones with overlapping sequences (AA729218 and AA232348). Using these two cDNAs and a combination of different methods, ie cDNA library screening, RT-PCR and *in silico*-cloning, we finally obtained the sequence of 7450 bp which includes 779 bp of 5' genomic sequences and 6671 bp of cDNA sequences (Accession No. AJ243706). This combined sequence encodes a predicted protein of 1681 amino acids (Figure 1, 2). The protein displays a high level of amino acid identity to RBBP2 (see Figure 2). In fact, in many extended regions they are completely identical. This is the reason why we called this gene *RBBP2H1A* (Rb binding protein 2 homologue 1A). Nevertheless, nucleic acid identity is much less profound, in the best cases reaching 79–84%.

Search with the amino acid sequences of the gene using ProfileScan at the ISREC server (see Materials and Methods) revealed many potential domains (Figure 1b). Among them the most obvious were three Zn-finger domains (446–496 aa, 1313–1361 aa and 1621–1675 aa) and bipartite nuclear localization signals (1239–1256 aa and 1536–1553 aa). There are also many other motifs, like amidation sites, cAMP- and

cGMP-dependent protein kinase phosphorylation sites, casein kinase II phosphorylation sites, etc. We suggest that two methionine residues could correspond to the start codon: no. 1 in Figure 2 (758 bp) and no. 61 (938 bp). Located upstream are two predicted promoter sites (555–605 bp and 816–866 bp). Specific experiments must be conducted to determine which of them is the correct starting point but here we suggest that the first methionine is indeed the start. The basis for this is that we have found 5' cDNA clone (AA729218) starting at the 779 bp and is thus located upstream of the predicted promoter for the second methionine. Numerous putative transcription factor binding signals can be found throughout the first 500 bp, eg for transcription factors Sp1 (122–129 bp), Pax-5 (209–232 bp), Oct-1 (255–262 bp), PU.1 (262–271 bp), Skn-1 (376–387 bp) etc. Finding the real promoter can be a difficult task since sequences outside the conventional promoter can play a role in this function, for instance pertaining to exon sequences.¹⁵ On the other hand genes can also have multiple promoters. A predicted molecular weight of the longest polypeptide is 185 kDa.

We performed an analysis of the expression with cDNA clone AA232348 as a probe (1080–4510 bp, see AJ243706), using MTN northern filters containing polyA⁺ RNAs from 15 different human tissues (Clontech). Two bands of approximately 6 kb and 7 kb were found (Figure 3). However, testis RNA most probably contains another band, 8.5 kb in size. The gene is expressed in all tested tissues but the level of the expression varies very significantly. The highest level of expression was detected in testis and the lowest in skeletal muscle. Moreover, the ratio between the two main bands also varied between tissues.

We have tested renal cell carcinoma (RCC) biopsies (four cases) and 10 RCC cell lines. No noticeable changes in expression were detected (data not shown).

Using FISH, we assigned the genomic clone NR-025 to chromosomal band 1q32.1 (Figure 4). To test the integrity of the NR-025 linking clone, we performed the fiber FISH. This experiment revealed the absence of any significant rearrangements and confirmed the integrity of the NR-025 (data not shown).

No significant deletions or rearrangements have been reported in the literature to be associated with this area. However, in the region another RB-binding protein, RBQ-3, was mapped by Saijo *et al*.¹⁶

After we started to clone this gene, Ueki *et al*¹⁷ published a paper that described a new method for cloning nuclear-targeted proteins. Among other genes and gene fragments, they isolated a cDNA (AB015348) that contains the 3' end of the *RBBP2H1A*. The homology starts from amino acid 1218 of *RBBP2H1A*. The AB015348 cDNA clone contains a deletion of five amino acids at the extreme 3' end of our gene (1595–1600 aa). Ueki *et al*¹⁷ have also shown that their candidate gene fragment is coding for a nuclear-targeted protein, allowing us to speculate that the complete gene

A

1	T	TCA	GTC	TGT	NAA	AGG	ATA	CCC	CCG	TTG	TGA	ATT	TCG	TAC	CTG	TAT	46
47	GAC	CAG	AGA	CAC	CTC	AAA	TTC	AAA	ATG	TCC	AGA	AAT	GAA	CAC	AGT	ATC	94
95	TTC	TTC	CCT	TGA	GTA	ACT	CGC	CTT	CTG	TAT	ATA	ATT	GGG	GTG	AAT	GTT	142
143	CCA	GCA	ATG	CCA	ACG	GGC	TAG	CAT	CTA	CTC	AGG	CAG	GTA	CGC	CAG	ACA	190
191	TCT	GAA	TCT	TAA	TAC	CTC	GTC	GTC	CCC	CCT	CAA	TCT	GTC	ACT	ATG	TCC	238
239	TAA	TTT	TAT	TTC	CTA	CCT	TTG	CAT	CCC	TTC	CTC	ATA	ATT	ATC	AGC	ACC	286
287	ACT	GCC	TTA	ATT	TAG	ATC	CTA	TCA	TTC	CTT	GTC	TGG	ATT	ATT	TCA	AGT	334
335	CTT	TTG	CCC	CTC	TCA	AGT	CTG	ACC	TCG	TCA	TAG	ACT	GTT	TGT	GGA	TGA	382
383	TTA	TTT	TCC	CTG	CAA	CAT	CTC	CAC	CTC	CCA	ATA	GTC	TCC	ATC	ACT	TAT	430
431	TAG	CCA	TGT	GAA	TTA	TGG	CAA	ATT	GCT	TAA	CCT	ACC	TAT	GCC	TCA	CTT	478
479	TTC	CTT	ATT	GTA	AAG	TGG	AAA	TAA	GAG	TAC	CTA	TCT	CAT	AGG	GTT	GTG	526
527	TGA	TTA	ATC	ATG	CGA	TGC	AAT	TAG	AAC	AGT	GCC	TAG	GAT	ATG	AAA	GTG	574
575	CCC	CAA	AAT	GTT	ACG	TTA	TTA	TTA	TTA	TTA	CTA	CTA	TTG	CCT	TCT	CAT	622
623	GAA	CAT	GCT	ATG	TGT	ATG	TTC	AAG	CTG	TTA	TCT	CTA	CTG	GAA	AAT	CCT	670
671	TCT	CCG	TTT	CCA	ATA	CTC	TTT	CCA	AAA	CCC	CCT	CCT	GGG	TTT	TAC	TGC	718
1														M	G	L	3
719	ACC	TAC	CAT	TGT	GTA	CTG	ACG	TGA	TTG	AAT	GAT	TGG	CTT	ATG	GGA	CTG	766
4	I	L	S	T	R	L	N	F	F	R	A	G	T	L	I	H	19
767	ATC	CTT	TCT	ACC	AGA	CTT	AAC	TTC	TTC	AGG	GCA	GGA	ACT	CTG	ATA	CAT	814
20	S	K	W	S	I	K	V	G	S	T	E	T	L	R	I	N	35
815	AGT	AAA	TGG	TCA	ATA	AAA	GTT	GGC	TCA	ACT	GAA	ACC	CTA	AGG	ATA	AAC	862
36	S	I	H	S	G	R	A	R	P	R	P	I	R	G	R	G	51
863	TCC	ATC	CAC	AGC	GGA	AGA	GCG	CGG	CCC	CGC	CCG	ATT	CGG	GGG	CGG	GGC	910
52	G	E	P	R	A	E	G	G	S	M	G	V	G	W	D	S	67
911	GGG	GAG	CCG	CGC	GAG	GGT	GGG	TCG	ATG	GGG	GTG	GGG	TGG	GAC	TCT		958
68	F	S	S	P	W	R	R	R	R	T	A	W	A	C	G	E	83
959	TTT	TCC	TCG	CCG	TGG	CGG	CGG	AGG	CGC	ACG	GCG	TGG	GCT	TGC	GGC	GAG	1006
84	T	S	S	S	E	A	E	K	A	Q	G	A	A	V	A	R	99
1007	ACG	TCG	TCG	TCG	GAG	GCT	GAA	AAA	GCC	CAA	GGT	GCT	GCC	GTT	GCC	CGT	1054
100	T	T	R	T	C	C	C	S	S	R	V	C	T	G	L	G	115
1055	ACA	ACT	CGG	ACT	TGC	TGT	TGC	TCG	AGC	CGC	GTC	TGC	ACG	GGT	CTC	GGA	1102
116	P	S	G	A	R	S	L	G	P	G	A	H	L	R	L	A	131
1103	CCG	AGC	GGA	GCT	CGC	AGC	CTC	GGT	CCC	GGA	GCC	CAC	CTT	CGC	CTC	GCC	1150
132	L	A	Q	P	A	V	M	E	A	A	T	T	L	H	P	G...	147
1151	CTT	GCC	CAG	CCT	GCG	GTG	ATG	GAG	GCG	GCC	ACC	ACA	CTG	CAC	CCA	GGC...	1198

B

Figure 1 Structure of the *RBBP2H1A* gene **A** Nucleotide and amino acid sequences of the 5' end *RBBP2H1A*. Upstream genomic sequences are in bold italic. The first three methionine residues are in bold. **B** Localization of zink-finger like PHD and bipartite nuclear targeting sequence (NLS BP) motifs in the *RBBP2H1A*.

isolated in the present study is also localized in the cell nucleus. During the preparation of the manuscript a new entry in the EMBL database was revealed. In March 1999 Vogt *et al* (AF087481) reported the sequence of a new RBBP2 homologous protein (*RBBP2H1*) and noted that expression of this gene is differentially regulated during progression of malignant melanomas. The sequence of *RBBP2H1* is very similar to the sequence of *RBBP2H1A* with small deletions/insertions or changes involving ten amino acids. Two

significant differences were revealed, however. Our sequence is missing amino acids 238–273 of *RBBP2H1* and at the same time the starting methionine in their sequence is only the third in our sequence (137 aa). Thus, *RBBP2H1* is 101 amino acids shorter than *RBBP2H1A* described in the present study.

In May 1999 Lu *et al*¹⁸ published the isolation and sequencing of a new gene *PLU-1*. This gene is identical to our gene starting from 1080 bp; thus our gene is 137 aa longer.

rbbp2h1a	1	MGLILSTRLNFFRAGTLIHKSWSIKVGSTETLRINSIHSGRARPRPIRGSGEPRAEGGS
rbbp2h1		-----
rbbp2		-----
rbbp2h1a	61	MGVWDSFSSPWRRTAWACGETSSSEAEKAQGAAVARTTRTCCSSRVCTGLGPSGAR
rbbp2h1		-----
rbbp2		-----
rbbp2h1a	121	SLGPGAHLRLALAQPAVMEAAATTLHPGPRPALPLGGPGPLGEFLPPPECVPFEPWSWEEFA
rbbp2h1	1	-----MEAAATTLHPGPRPALPLGGPGPLGEFLPPPECVPFEPWSWEEFA
rbbp2	1	-----MAGVGPGGYAAEFVPPPECVPFEPWSWEEFT
rbbp2h1a	181	DPFAFIHKIRPIAEQTGICKVRPPDWQPPFACDGDKLHFTPRIQRLNELEAQTRVKLNF
rbbp2h1	44	DPFAFIHKIRPIAEQTGICKVRPPDWQPPFACDVKLHFTPRIQRLNELEAQTRVKLNF
rbbp2	31	DPLSFIGRIRPLAEKTGICKIRPPKDWQPPFACEVKSFRFTPRVQRLNELEAMTRVRLDF
rbbp2h1a	241	LDQIAKYWELQGSTLKIPHVERKILDLFQNLKLVAAEGGFAVVKDRKWTKIATKMGFAP
rbbp2h1	104	LDQIAKYWELQGSTLKIPHVERKILDLFQNLKLVAAEGGFAVVKDRKWTKIATKMGFAP
rbbp2	91	LDQLAKFWELQGSTLKIPVVERKILDLYALSIVASKGGFEMVTEKKWSKVGSRLGYLP
rbbp2h1a	301	GKAVGSHIRGHYERILNPYNLFSGDSLRLCLQKPNLTDTKDKEYKPHDIPQORSVQPSSE
rbbp2h1	164	GKAVGSHIRGHYERILNPYNLFSGDSLRLCLQKPNLTDTKDKEYKPHDIPQORSVQPSSE
rbbp2	151	GKG_TGSLLKSHYERILYPYELFQSGVSLMGV_QMPNLDLKEK---VEPEVLSTDQTQSPE-
rbbp2h1a	361	TCPPARRAKRMRAE-----AMNIKIEPEE
rbbp2h1	224	TCPPARRAKRMRAERQSLAVLPRLECSGAILAHCNRLRLDSSNSSASASQAMNIKIEPEE
rbbp2	207	---PGTRMNLPKR-----TRRVKTQSES
rbbp2h1a	385	TTEARTHNLRRRMGCPTPKCENEKEMKSSIKQEPPIERKDYIVE-NEKEKPKSRSKK---A
rbbp2h1	284	TTEARTHNLRRRMGCPTPKCENEKEMKSSIKQEPPIERKDYIVE-NEKEKPKSRSKK---A
rbbp2	228	GDVSRNTELKKLQIFGAGPKVVGGLAMGT_KDKEDEVTRRRKV_TNRSDAFNM_QMRQKGTLS
rbbp2h1a	441	TNAVDLYVCLLCGSGNDEDRLLLCDGCDDSYHTFCLIPPLHDVPKGDWRCPKCLAQECSSK
rbbp2h1	340	TNAVDLYVCLLCGSGNDEDRLLLCDGCDDSYHTFCLIPPLHDVPKGDWRCPKCLAQECSSK
rbbp2	288	VNFVDLYVCMFCGRGNNEKLLLCDGCDDSYHTFCLIPPLPDVPKGDWRCPKCVAEECSSK
rbbp2h1a	501	PQEAFGFEQAARDYTLRTFGEMADAFKSDYFNMPVHMVPTLVEKEFWRLVSTIEEDVTV
rbbp2h1	400	PQEAFGFEQAARDYTLRTFGEMADAFKSDYFNMPVHMVPTLVEKEFWRLVSTIEEDVTV
rbbp2	348	PREAFGFEQAVREYTL_QSFGEMADNFKSDYFNMPVHMVPTLVEKEFWRLVSSIEEDVIV
rbbp2h1a	561	EYGADIASKEFGSGFPVRDGIKILSPREEEYLDGWNLNMPVMEQSVLAHITADICGMK
rbbp2h1	460	EYGADIASKEFGSGFPVRDGIKILSPREEEYLDGWNLNMPVMEQSVLAHITADICGMK
rbbp2	408	EYGADISSKDFGSGFPVKDGRKILPEEEYALSGWNLNMPVLEQSVLAHINVDISGMK
rbbp2h1a	621	LPWLYVGMCFSSFCWHIEDHWSYSINYLHWGEPKTYGVPGYAAEQLENVMKKLAPELFV
rbbp2h1	520	LPWLYVGMCFSSFCWHIEDHWSYSINYLHWGEPKTYGVPGYAAEQLENVMKKLAPELFV
rbbp2	468	VPWLYVGMCFSSFCWHIEDHWSYSINYLHWGEPKTYGVPSHAAEQLEEVMLRELAPELFE
rbbp2h1a	681	SQPDLLHQLVITIMNPNTLMTHEVPVYRTNQCAGEFVITFPAYHSGFNGQFNFAEAVNFC
rbbp2h1	580	SQPDLLHQLVITIMNPNTLMTHEVPVYRTNQCAGEFVITFPVP-QWFNQGFNFAEAVNFC
rbbp2	528	SQPDLLHQLVITIMNPVLMHGVVYRTNQCAGEFVVTFPRAYHSGFNGQFNFAEAVNFC
rbbp2h1a	741	TVDWLPGRQCVHYRLLHRYCVFSHDEMICKMASKADVLDDVVASTVQKDMAIMIEDEK
rbbp2h1	639	TVDWLPGRQCVHYRLLHRYCVFSHDEMICKMASKADVLDDVVASTVQKDMAIMIEDEK
rbbp2	588	TADWLPGRQCVNHYRRLRRHCVFSHEELIFKMAADPECLDVGLAAMVCKELTLMTEET
rbbp2h1a	801	ALRETVRKLGVIDSERMDFELLPPDERQCVCKTTCFMSAISCCKPGLLVCLHHVKELC
rbbp2h1	699	ALRETVRKLGVIDSERMDFELLPPDERQCVCKTTCFMSAISCCKPGLLVCLHHVKELC
rbbp2	648	RLRESVVMGVLMSEEEVFELVPPDERQCSACRTTCFLSALTCSCNPERLVCLYHPTDLC
rbbp2h1a	861	SCPPYKYKLRYRYTLDDLYPMNALKLRAESYNEWALNVNEALEAKITKKKSLVSFKALI
rbbp2h1	759	SCPPYKYKLRYRYTLDDLYPMNALKLRAESYNEWPLNVNEALEAKITKKKSLVSFKALI
rbbp2	708	PCPMCKKCLRYRYPLEDLPSLLYGVKVRAQSYDTWVSRYTEALSANFNHKKDLIELRVML

Figure 2 Alignment of the deduced amino acid sequence of *RBBP2H1A* with sequences of RBBP2H1 [AF087481] and RBBP2.¹⁴ Grey boxes and underlined letters indicate identical and similar amino acid residues, respectively.

rbbp2h1a 921 EESEMKKFPDNDLLRHLRLVTQDAEKCASVAQQLNGKRQTRYRSGGGKSNQQLTVNELR
rbbp2h1 819 EESEMKKFPDNDLLRHLRLVTQDAEKCASVAQQLNGKRQTRYRSGGGKSNQQLTVNELR
rbbp2 768 EDAEDRKYPENDLFRKLRDAVKEAE TCASVAQ LLLSKKQKHRQSPDSGRTRTKLTVEELK

rbbp2h1a 981 QFVTQLYALPCVLSQTPLLKDLLNRVEDFQOHSQKLLSEETPSAAELQDLLDVSEFDVE
rbbp2h1 879 QFVTQLYALPCVLSQTPLLKDLLNRVEDFQOHSQKLLSEETPSAAELQDLLDVSEFDVE
rbbp2 828 AFVQQLFSLPCVISQARQVKNLLD DVEEFHERAQEAMMDETPDSSKLQMLIDMGSSLIVE

rbbp2h1a1041 LPQLAEMRIRLEQARWLEEVQOACLDPSSLTLLDMRRLIDLGVLAPYSAVEKAMARLQE
rbbp2h1 939 LPQLAEMRIRLEQARWLEEVQOACLDPSSLTLLDMRRLIDLGVLAPYSAVEKAMARLQE
rbbp2 888 LPELPRLKQELQARWLDEVRLTSDPQQVTLDVMKKLIDSGVGLAPHHAVEKAMAELQE

rbbp2h1a1101 LLTVSEHWDDKAKSLKARPRHSLNSLATAVKEIEEIPAYLPNGAALKDSVQARARDWLQD
rbbp2h1 999 LLTVSEHWDDKAKSLKARPRHSLNSLATAVKEIEEIPAYLPNGAALKDSVQARARDWLQD
rbbp2 948 LLTVSERWEEKAKVCLQARPRHVSASLES IVNEAKNIPAFLPNVLSLKEALQKAREWTAK

rbbp2h1a1161 VEGLQAGGRVPVLDTLIELVTRGRSIPVHLNSLPRLETLVAEVQAWKECAVNTFLTENSP
rbbp2h1 1059 VEGLQAGGRVPVLDTLIELVTRGRSIPVHLNSLPRLETLVAEVQAWKECAVNTFLTENSP
rbbp2 1008 VEAIQSGSNYAYLE QLESLSAKGRPIPVRLEALPQVESQVAAARAWRERTGTFLKKNSS

rbbp2h1a1221 YSLEVLCPRCDIGLLGLKRRQQRK-LKEPLPNGKKKSTKLESLSDLERALTESKETASAM
rbbp2h1 1119 YSLEVLCPRCDIGLLGLKRRQQRK-LKEPLPNGKKKSTKLESLSDLERALTESKETASAM
rbbp2 1068 HTLLQVLSPRTDIGVYSGKNRRKKVKELIEKEKEKDLDLEPLSDLEEGLETRDTAMVV

rbbp2h1a1280 ATLGEARLEMEALQSLRLANEGKLLSPLQDVDIKICLCQKAPAAPMIQCELCRDAFHTS
rbbp2h1 1178 ATLGEARLEMEALQSLRLANEGKLLSPLQDVDIKICLCRQKAPAAPMIQCELCRDAFHTS
rbbp2 1128 AVFKEREQKEIEAMHSLRAANLAKMTMVDRIEVEKFCICRKTAGSFMLQCELCCKDWFHNS

rbbp2h1a1340 CVAVPSISQGLR-----TWLCPHCRSEKPPLEKILPLLASIQRIRVRLPEGDALR
rbbp2h1 1238 CVAVPSISQGLR-----TWLCPHCRSEKPPLEKILPLLASIQRIRVRLPEGDALR
rbbp2 1188 CVPLPKSSSQKKGSSWQAKEVKFLCPLCMRSRRPRLE TILSLLVSLQKL PVRLPEGEALQ

rbbp2h1a1391 YMIERTVNWQHRAQQLSS-----
rbbp2h1 1289 YMIERTVNWQHRAQQLSS-----
rbbp2 1248 CLTERAMSWQDRARQALATDELSSALAKLSVLSQRMVEQAAREKTEKIISAELQKAAANP

rbbp2h1a1410 ---GNLKFVD---RVGSGLLYSRWQASAG-----QVSDTNKVS---
rbbp2h1 1308 ---GNLKFVD---RVGSGLLYSRWQPSAG-----QVSDTNKVS---
rbbp2 1308 DLQGHLPSPQQSAFNRVVS SVSSPRQTMDYDEETDSDEDIETYGYDMQDTASVKSSS

rbbp2h1a1443 -----QPPGTTSFSLPDDWDNRTSYLHSPFS-TGRSCIPLHG-----
rbbp2h1 1341 -----QPPGTTSFSLPDDWDNRTSYLHSPFSTTGRSCIPLHG-----
rbbp2 1368 SLEPNLFCDEEIP IKSEEVVTHMTAPS FCAEHAYSSASKSCSQVFFGKGSSTPRKQPRK

rbbp2h1a1479 -----VSP---EVNELLMEAQLLQVSLPEIQELYQTLLAKPSPAQQTDRS
rbbp2h1 1378 -----VSP---EVNELLMEAQLLQVSLPEIQELYQTLLAKPSPAQQTDRS
rbbp2 1428 SPLVPRSLPEPPVLELSPGAKAQLEELMMVGDLLEVSLDETQHWRILQATHPSE--DRF

rbbp2h1a1521 SPVRPSS---EKNDCCRGKRDGINSLEKRLKRLEREGLSSERWERVKMRTPKKKIKIL
rbbp2h1 1420 SPVRPSS---EKNDCCRGKRDGINSLEKRLKRLEREGLSSERWERVKMRTPKKKIKIL
rbbp2 1486 LHIMEDDSMEEKPLKVKGKDSSEKRRKKLEKVEQLFGEGKQKSKELKKMDKPRKKIKIL

rbbp2h1a1578 SHPKDMNFKLER-----ER-----SYELVRSATHSLSPTSYSYEQ
rbbp2h1 1477 SHPKDMNFKLER-----ER-----SYELVRSATHSLSPTSYSYEQ
rbbp2 1546 GADKSKKLNLAKKLAKKEERKKKKKEAAAAKVELVKES TEKKREKVLDIPSKYDWSGA

rbbp2h1a1615 EDSEDEDAICPAVSCLOPEGDE-----VDWVQCDGSCN
rbbp2h1 1514 EDSEDEDAICPAVSCLOPEGDE-----VDWVQCDGSCN
rbbp2 1606 EESDDENAVCAEPDQRPCKDKGVVFVTKKREIKNISFKSVLCDCFSKKVDWVQCDGGCD

rbbp2h1a1648 QWFHQVCVGVSPEMAEKEDI CVRCTVKDAPSRK
rbbp2h1 1547 QWFHQVCVGVSPEMAEKEDI CVRCTVKDAPSRK
rbbp2 1666 EWHRVVCVGVSPEMA-----

Figure 2 Continued.

Figure 3 Hybridization of the *RBBP2H1A* to two Clontech northern blots **a** Human 12-Line MTN Blot I; **b** Human MTN Blot IV. The lower panels show hybridization of the β -actin to the same filter.

Figure 4 Localization of *RBBP2H1A* by FISH. Arrows indicate the position of *RBBP2H1A*.

Northern expression data are strikingly different for *RBBP2H1A* and *PLU-1*. Lu *et al*¹⁸ reported highly restricted expression of *PLU-1* in adult tissues. *PLU-1* showed high expression in testis and low levels of expression in placenta, ovary and tonsil. We detected two RNA bands (6 kb and 7 kb) in all 15 tested normal adult tissues (in testis even 3 RNA species were detected, additional 8.5 kb band is also seen). However, the level of expression varied very significantly in these tissues. In skeletal muscles and small intestine expression is hardly detected and in testis it is very strong. It is important to mention that we used as a probe a cDNA clone containing a majority of the *RBBP2H1A* coding sequences including 5' part absent in the *PLU-1* probe (1080–4510 bp). Lu *et al* used the *PLU-1* probe containing mainly the 3' untranslated region of the *PLU-1* gene (4712–6636 bp of

RBBP2H1A). Furthermore we used northern blots with poly A⁺ RNA and Lu *et al* used total RNA for the northern blots.

The authors reported elevated expression of *PLU-1* in breast cancer. As was mentioned above, we found no elevated expression in RCC biopsies and cell lines.

One explanation of all the results from different groups is that these genes represent alternatively spliced mRNAs of the same gene. Alternatively, these genes may represent closely related members of the same gene family. As the *RBBP2H1A* and *PLU-1* were mapped to the same chromosomal band it is more likely that they represent splice variants of the same gene but further experiments are needed to resolve this question.

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