RXRβ isoforms in neuroblastoma cells and evidence for a novel 3′-end transcript

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Abstract RXRβ is predominantly involved in retinoid responses in neuroblastoma cells, in particular the N-type SH SY 5Y cells and the S-type SH S EP cells, both derivatives of a mixed phenotype neuroblastoma cell line. The aim of this study was to identify RXRβ isoforms expressed in neuroblastoma cells and to characterise a putative novel RXRβ transcript. RXRβ1 and RXRβ2 were expressed in these neuroblastoma cells. An isoform with an insertion into the ligand binding domain, RXRβ^{SLSR} (referred to in previous studies as RXRβ3), was expressed at a similar level to RXRβ. A novel RXRβ transcript was identified by RNase protection assays and was at least as abundant as the expected RXRβ transcript and expressed in other cell types. Evidence suggests that this novel transcript was transcribed from an internal promoter between exons 5 and 6, contained a retained intron (intron 6) and was alternatively spliced with and without the SLSR insertion. These data show that the pattern of RXRβ expression is complex. The relative abundance of the novel RXRβ transcript suggests that it may be an important aspect of RXRβ function or regulation in a range of cell types.

Key words: RXRβ; Isoform; RNase protection assay; Reverse transcription-polymerase chain reaction; Neuroblastoma

1. Introduction

RXRs are transcription factors that can act as ligand dependent or ligand independent heterodimer partners for retinoic acid receptors (RARs) and other nuclear receptors [1]. Of the three RXRs (α, β, γ) that have been characterised, RXRβ was originally identified as a protein able to bind to the regulatory region II of the murine major histocompatibility complex (MHC) class I promoter and referred to as H2RIIBP [2]. The human *RXRβ* gene has been mapped to the short arm or centromeric region of chromosome 6 (6pter–q13) which also contains the *MHC I* locus [3]. Two major RXRβ isoforms in the mouse, designated RXRβ1 and RXRβ2 (GenBank accession numbers D21830 and D21831), differ in the N-terminal (A) domain with RXRβ1 containing an extra 72 amino acids. These isoform mRNAs are transcribed from two CpG island promoters and the different N-terminal exons are spliced to exons common to both isoforms from the remainder of the gene [4].

Although the two mouse RXRβ isoforms have been characterised in some detail, less information is available about their human counterparts. The human sequence referred to as hRXRβ2 [5] corresponds to the mRXRβ1 isoform [3,6] and is well conserved across species, except that hRXRβ2 contains two additional in-frame ATG initiator codons which may serve as alternative translational start sites [3,6]. Since the human RXRβ2 and mouse RXRβ1 sequences appear to be very similar, a putative human RXRβ1 isoform can be identified by aligning the mouse RXRβ2 sequence to the genomic *hRXRβ* sequence. A third isoform, referred to as hRXRβ3 by its discoverers, contains an in-frame insertion of four amino acids (SLSR) in the ligand binding domain at codon 419 and is generated by use of an alternative 3′-splice acceptor site [7]. The same insertion has been found in mouse RXRβ [8]. It has not yet been established whether the N-terminal end of RXRβ3 is identical to that of the β1 or β2 isoform, or has an alternative sequence. For this reason, we use the superscript SLSR to indicate transcripts encoding the 12 bp insertion isoform as in RXRβ^{SLSR} or RXRβ2^{SLSR}. Since the expression of different RXR isoforms appears to be well conserved between species, each receptor isoform may hold a specific role in retinoid signalling. However, many RXR expression studies have not recognised the importance of establishing which RXR isoforms are expressed in a particular system. RXRβ is predominantly involved in retinoid responses in the N-type SH SY 5Y and the S-type SH S EP neuroblastoma cells [9], both derivatives of a mixed phenotype neuroblastoma cell line. The aim of this study was to identify the RXRβ isoforms expressed in these neuroblastoma cells and to characterise a putative novel RXRβ transcript.

2. Materials and methods

2.1. Cell culture and treatment with retinoic acid isomers

SH SY 5Y and SH S EP neuroblastoma cells [10], SK23 melanoma cells [11], HEP G2 hepatoblastoma cells, A431 epithelial cells (European Collection of Animal Cell Cultures, Porton Down, UK) and human fibroblast cells [12] were grown in Dulbecco's modified Eagle's medium (Hyclone-Europe, Tyne and Wear, UK) supplemented with 10% foetal calf serum (Sera Labs, Crawley, UK) in a humidified atmosphere of 5% CO_2 in air. For some experiments 8×10^6 cells were treated with 1 μM 9-*cis*-retinoic acid or all-*trans*-retinoic acid for 6–24 h, as described previously [13].

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2.2. Extraction of cytoplasmic RNA and RNase protection assays

Total cytoplasmic RNA for RNase protection assays was prepared using a mini-prep method [14]. RNA from mouse dorsal root ganglion cells was provided by Dr. Z. Chrzanowska-Lightowlers. Probes for RNase protection assays were generated using T7 or T3 RNA polymerase and cytidine 5′-[α-^{32}P]triphosphate (specific activity 30 TBq/mmol). The RXRβ probe, giving a 570 nucleotide (nt) protected fragment when hybridised to RXRβ mRNA, was prepared by sub-cloning the full-length hRXRβ2 cDNA from pTL1-RXRβ [5] into Bluescript KS and linearising with *Bam*HI. The hRXRβ plasmid used for these studies, pTL1-RXRβ [5], was found, on re-sequencing, to contain the 12 bp insert in the ligand binding domain described by Mahajna et al. [7]. We therefore refer to this cDNA as RXRβ2^{SLSR}. As a positive control for hRXRβ, sense transcripts were prepared from *Xba*I linearised plasmid to give a 440 nt protected fragment when hybridised to the antisense RXRβ2^{SLSR} probe. PCR fragments cloned into the pCR2.1-TOPO vector were linearised with *Pst*I, *Pme*I or *Not*I and used to make sense (control) and antisense riboprobes from the T7 RNA polymerase promoter. RNase protection assays were performed on 10 μg cytoplasmic RNA using the Ambion RNase protection assay kit (Ambion, AMS Biotechnology, Oxford, UK) according to the manufacturer's instructions [15], and the products separated through denaturing polyacrylamide gels. In all experiments, RNA size markers ranging from 100 to 500 nt were prepared from linearised plasmids (0.5 μg Century™ size markers per assay, Ambion) containing T7 RNA polymerase promoters. These markers were prepared in parallel with the experimental probes and acted as standards with respect to the intensities of protected fragments.

2.3. Preparation of nuclear extracts

Confluent cells were washed once in ice-cold phosphate buffered saline (PBS, Sigma, Poole, UK), scraped off into ice-cold PBS and centrifuged at 13 000×*g* for 5 min at 4°C. Cells were lysed in 100 μl of ice-cold lysis buffer (20 mM HEPES, pH 7.9, 1 mM EDTA, 0.2% NP40, 1 mM dithiothreitol [DTT], 1 mM protease inhibitor E64, 1 mM benzamidine, 1 mM leupeptin and 1 mM freshly added phenylmethylsulphonyl fluoride) and centrifuged at 5000×*g* for 5 min at 4°C. The nuclear pellet was re-suspended in 100 μl of high salt buffer (as for lysis buffer but without 0.2% NP40, and with 20% glycerol and 420 mM NaCl) and incubated for 2 h on ice before centrifugation at 13 000×*g* for 2 min. The resulting supernatant (nuclear extract) was frozen rapidly and stored at −80°C. Protein concentration was determined using the Bio-Rad protein assay (Bio-Rad, Hemel Hempstead, UK).

2.4. Western blotting

For Western blotting 20 μg of nuclear protein was separated by electrophoresis through a 10% sodium dodecyl sulphate–polyacrylamide gel (SDS–PAGE) at 100 V and transferred to nitrocellulose (Bio-Rad) membrane at 4°C and 30 V overnight. Membranes were blocked with blocking solution (5% w/v skimmed powdered milk, in Tris-buffered saline [TBS] with 0.05% Tween 20 pH 8.0), and probed with rabbit antibodies specific for the N-terminal region of human RXRβ1, human RXRβ2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), or a pan-RXR monoclonal antibody (4RX-1D12, a gift from Pierre Chambon), diluted in blocking solution. Membranes were subsequently washed in TBS/Tween for 30 min, and then incubated for 1 h with biotinylated anti-rabbit IgG (Amersham Pharmacia), diluted 1 in 500 in blocking solution. After a final 30 min wash, membranes were agitated for 1 h in a 1 in 500 dilution of streptavidin–biotinylated horseradish peroxidase complex (Amersham Pharmacia) and developed using an enhanced chemiluminescence (ECL) detection kit (Amersham Pharmacia) and Fuji X-ray film.

2.5. Reverse transcription (RT)-polymerase chain reaction (PCR)

To generate cDNA, 1.5 μg of total cellular RNA, 2 μl of oligo(dT) (0.5 μg/μl) (Amersham Pharmacia) and RNase-free water to a final volume of 10 μl were mixed and incubated at 70°C for 10 min and cooled on ice. To each tube, 2 μl of 0.1 M DTT, 1 μl of 10 mM dNTPs, 1 μl of RNasin and 5 μl of Superscript II reaction buffer (Life Technologies, Paisley, UK) were added, mixed and heated to 37°C for 2 min followed by the addition of 400 U of Superscript II (Life Technologies). The reaction was incubated at 37°C for 1 h and this template cDNA used for PCR. Primers (50 pmol) were added to 5 μl of reaction IV buffer and 5 μl of $MgCl_2$ (both Abgene, Epsom, UK) to a final volume of 50 μl. A wax pellet was added and the tubes heated to 70°C for 1 min and cooled on ice. Template cDNA (5 μl) or genomic DNA with 5 μl of reaction IV buffer, 5 μl of $MgCl_2$ and 1 μl of Red Hot DNA polymerase (Abgene) in a total volume of 50 μl was laid over the wax. The reaction was placed in a PCR machine (Sanyo Gallenkamp, Loughborough, UK) for 30 cycles of 1 min at 95°C, 1 min at 60°C and 1 min at 72°C. PCR products were resolved by electrophoresis through 8% polyacrylamide gels and stained with silver nitrate. Primers used in these reactions are shown in Table 1.

2.6. Cloning of PCR products

PCR products were cloned into pCR 2.1-TOPO cloning vector following instructions provided by the manufacturer (Invitrogen, Groningen, The Netherlands). Colonies of transformed cells were grown up as 10 ml cultures overnight and plasmid DNA prepared using Qiagen kits (Qiagen, Crawley, UK) according to the manufacturer's instructions. Plasmid DNA was sequenced by the Newcastle University Facility for Molecular Biology using an Applied Biosystems (Warrington, UK) sequencer.

2.7. Transfections

RXRβ2^{SLSR} stable transfectants were established using the tetracy-

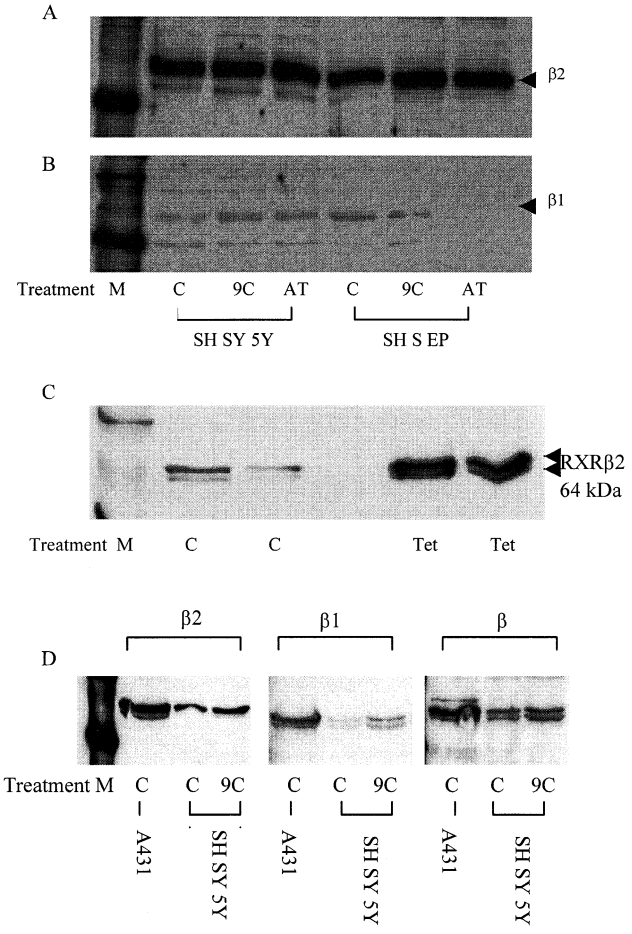

Fig. 1. Western blot analysis of RXRβ1 and -β2 expression in SH SY 5Y and SH S EP cells. A: Expression of RXRβ2 in SH SY 5Y and SH S EP cells detected with a rabbit polyclonal antibody. B: Expression of RXRβ1 in SH SY 5Y and SH S EP cells. C, control; 9C, 1 μM 9-*cis*-retinoic acid; AT, 1 μM all-*trans*-retinoic acid. C: Western blot analysis of nuclear protein from SH SY 5Y cells stably transfected with a tetracycline inducible RXRβ2^{SLSR} construct. C, transfected cells without treatment (vehicle control); Tet, transfected cells treated with 100 μg/ml tetracycline for 24 h to induce expression of the RXRβ2^{SLSR} construct. D: Expression of RXRβ2, RXRβ1 and RXRβ detected with rabbit polyclonal antibodies, and a pan-RXRβ monoclonal antibody, in SH SY 5Y cells and A431 cells. For all panels: M = molecular weight marker.

cline regulated (T-rex) expression system which consisted of the pcDNA6/TR and pcDNA4/TO vectors (Invitrogen). The pcDNA6/TR vector, which constitutively expresses the TetR, had previously been stably transfected into SH SY 5Y cells (unpublished data). The pcDNA4/TO vector was ligated with full-length RXRβ2^{SLSR} cDNA and transfected using Super Transfection reagent (Qiagen) into the pcDNA6/TR transfected SH SY 5Y cells. Transfected colonies/cells were maintained with 5 μg/ml of blastocydin and 500 μg/ml zeocin.

2.8. Data analysis

Genomic sequence data for RXRβ were from GenBank accession number AF065396 and base numbers refer to positions within this sequence. To identify additional splice donor/acceptor and promoter sites within the RXRβ genomic sequence and to confirm alternative splicing sites, MatInspector and the neural network promoter prediction program by Martin Reese [16] were used.

3. Results

3.1. N-terminal RXRβ isoforms in neuroblastoma cells

To identify the N-terminal RXRβ1 and RXRβ2 isoforms, Western blots of nuclear protein from control, 9-*cis*- and all-*trans*-retinoic acid treated SH SY 5Y and SH S EP cells were probed with antibodies specific for RXRβ1 and β2 N-terminal regions. For both cell types, the RXRβ2 antibody gave a strong band at the appropriate molecular weight (Fig. 1A). Conversely, the RXRβ1 antibody produced a faint band, just detectable in both cell types (Fig. 1B). 9-*cis*-Retinoic acid or all-*trans*-retinoic acid had no apparent effect on the level of RXRβ1 or -β2 protein. To verify the specificity of the RXRβ2 antibody, Western blots of nuclear extract from tetracycline induced and non-induced SH SY 5Y cells stably transfected with a tetracycline inducible hRXRβ2^{SLSR} construct were probed with the RXRβ2 antibody. An increase in RXRβ2 signal (detected as two bands) was obtained for the nuclear extract from tetracycline induced cells, compared to the control cells (Fig. 1C). The fact that both RXRβ2 bands were increased in the tetracycline induced cells may have resulted from translation of hRXRβ2 from two translational start sites [6]. A431 cells express both RXRβ1 and RXRβ2 (in-house testing, Santa Cruz Biotechnology), and were used as a positive control for RXRβ1. Relative to A431 cells, SH SY 5Y cells expressed RXRβ protein at a lower level overall, but RXRβ2 was relatively more predominant than RXRβ1 in SH SY 5Y cells (Fig. 1D).

Table 1
Primer sequences used in RT-PCR

Name	Base[a]	Sequence
MHF[b]	5668–5688	ATGTTCGAGATGGCATCCTC
MHR[c]	5892–5911	CCAAAATGCGTGACATGAGG
BDF	4834–4856	AACAGAAGAGTGACCAGGGCGTT
BDR	5715–5738	CAACTCAGCCCATTCAGCAGGAGT
RXRβEx6	5371–5392	CACTTTTCCTCCTTGCCTCTGG

[a]Base sequence as in GenBank accession number AF065396.
[b]Primer hRXRβ1286 of Mahajna et al. [7].
[c]Primer hRXRβ1416 of Mahajna et al. [7].

3.2. Expression of RXRβ^{SLSR} in neuroblastoma cells

To determine whether RXRβ^{SLSR} was expressed in SH SY 5Y and SH S EP cells, RT-PCR experiments were carried out with cytoplasmic RNA using primers MHF and MHR (Table 1) [7]. Products of 128 bp and 140 bp (Figs. 2 and 3), corresponding to the sizes predicted for the normal RXRβ and 12 bp insertion RXRβ^{SLSR} isoforms respectively, were obtained. Three fainter bands of sizes between 298 and 157 bp were also detected; the identity of these additional bands is unknown but the largest of these could have resulted from genomic DNA contamination or the presence of unprocessed transcripts. These results suggest that the RXRβ isoforms in SH SY 5Y and SH S EP cells are expressed with (RXRβ^{SLSR}) and without (RXRβ) the 12 bp insertion.

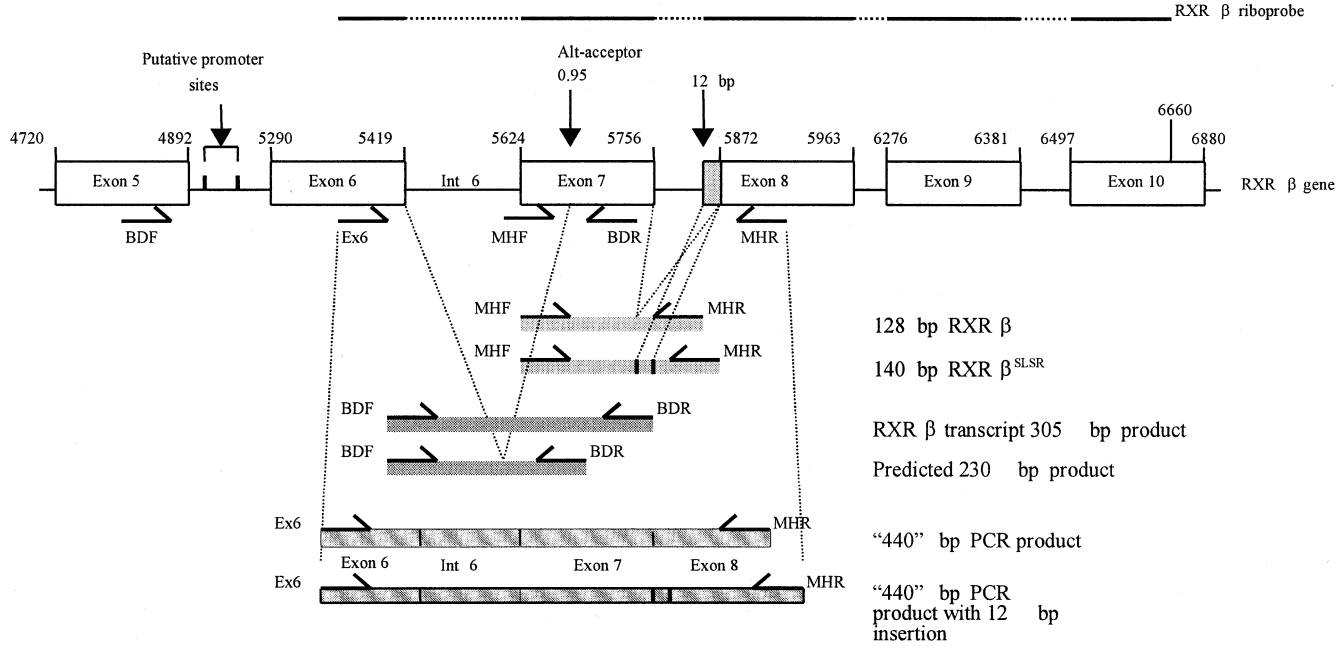

Fig. 2. Schematic diagram of hRXRβ genomic sequence to indicate the identity of the bands arising from RT-PCR using the various primer pairs specified in the text. The RXRβ riboprobe and the exons it encompasses are indicated at the top of the diagram. The start and end of each exon are indicated as numbered in the sequence of GenBank accession number AF065396.

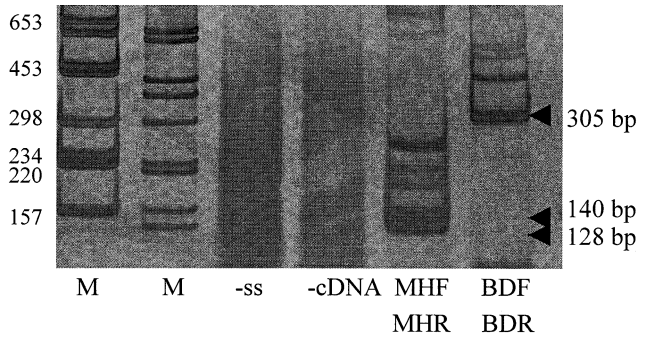

Fig. 3. Silver stained polyacrylamide gels showing RT-PCR products using the BDF, BDR and MHF, MHR primer sets to identify the presence of the RXRβ^{SLSR} isoform and a potential alternatively spliced RXRβ transcript. The first two lanes (from the left, M) are different molecular weight markers, and sizes (bp, left side of figure) are given for the markers in the first lane only. Components of the PCR reactions loaded into each lane are indicated on the figure; controls were without Superscript reverse transcriptase (−ss), and without cDNA (−cDNA). Sizes of relevant PCR products are indicated on the right of the figure.

3.3. RNase protection assays using a 3′-RXRβ2^{SLSR} probe

In RNase protection assays, two prominent bands resulted from the use of a RXRβ2^{SLSR} 3′-end probe: the predicted 570 nt band complementary to the probe and corresponding to RXRβ^{SLSR} mRNA, and a more intense band of approximately 455 nt (Fig. 4). Since hybridisation of the RXRβ2^{SLSR} 3′-end probe to RXRβ mRNA would result in a smaller fragment of approximately 355 nt, the 455 nt fragment could not be accounted for by the 12 bp insertion. Furthermore, the 570 nt and 455 nt protected fragments were also observed when fibroblast, melanoma cell and mouse dorsal root ganglion cell RNA was used in RNase protection assays, implying that the novel 455 nt fragment was not unique to neuroblastoma cells (Fig. 4B). A small (1.9-fold) but consistent induction of the 570 and 455 nt fragment was observed in SH SY 5Y cells treated with retinoic acid (Fig. 4). To investigate the possibility that the 455 nt band resulted from alternative splicing, the genomic RXRβ sequence was screened for splice donor/acceptor and promoter sites involving the 3′- or 5′-end of the mRNA complementary to the riboprobe sequence. In addition to the intron/exon boundaries consistent with the hRXRβ cDNA sequence (Fig. 2), there was a high probability (0.95) alternative acceptor site present in the genomic sequence corresponding to the 5′-end of the mRNA complementary to the RXRβ2^{SLSR} riboprobe (Fig. 2). Alternative splicing at this site would splice exon 6 to within exon 7, missing 75 bases at the 5′-end of exon 7, but retaining the correct reading frame. To test this idea, primers (BDF and BDR, Table 1) were designed to amplify the putative novel RXRβ transcript by RT-PCR. However, RT-PCR experiments with these primers using cytoplasmic RNA from SH SY 5Y cells produced the 305 bp band (Fig. 3) predicted for RXRβ or RXRβ^{SLSR} but not a 230 bp band predicted for the putative novel transcript.

Possible internal promoters were identified between exons 5 and 6 (probability score 0.90 and 0.99 for bases 4946–4996 and 5190–5240 respectively, Fig. 2). If an RXRβ transcript were generated from one of these internal promoters, the BDF (exon 5) and BDR (exon 7) primer pair would not amplify the cDNA of a transcript in which the donor site of exon 6 was spliced to the alternative acceptor in exon 7. Therefore, RT-PCR was repeated using a primer (Ex 6) in exon 6, downstream of these internal promoters, together with the MHR primer (Table 1). RT-PCR of cytoplasmic RNA from SH SY 5Y cells produced six bands ranging from approximately 517 bp to 228 bp in size (Fig. 5A). The uppermost band (approximately 517 bp) was due to genomic DNA contamination and the lower two bands of sizes 240 bp and 228 bp corresponded

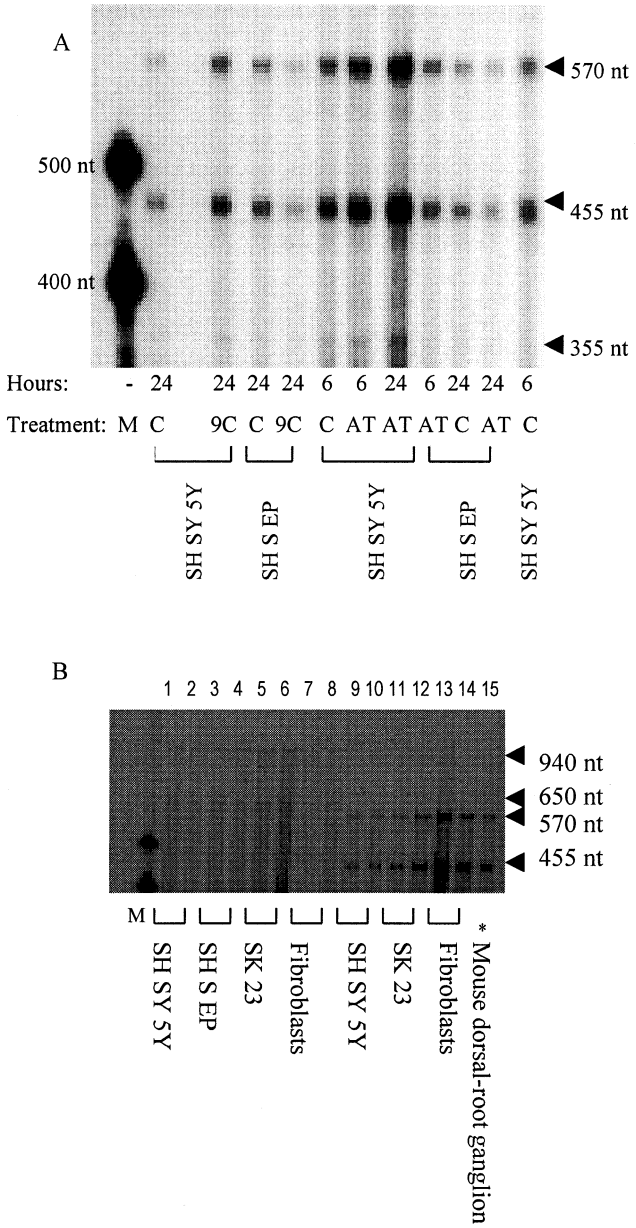

Fig. 4. RNase protection assay for RXRβ. A: RNase protection assay in SH SY 5Y and SH S EP neuroblastoma cells showing the 570 nt, 455 nt and 355 nt protected fragments (probe length 616 nt). The cells were treated (Treatment) with control ethanol (C), 1 μM 9-*cis*-retinoic acid (9C) or 1 μM all-*trans*-retinoic acid (AT) for 6 h or 24 h (Hours). B: RNase protection assay, using a cloned human genomic DNA fragment as a riboprobe template (lanes 1–8), in neuroblastoma cells, human melanoma cells (SK23), and human fibroblasts (fibroblasts). A protected fragment of approximately 650 nt was detected in these cells, and a fragment of approximately 940 nt representing undigested probe. Lanes 9–15, RNase protection assay using RXRβ2^{SLSR} 3′-end (as in A) riboprobe using neuroblastoma cells, human melanoma cells (SK23), human fibroblasts (fibroblasts) and mouse dorsal root ganglion RNA. M, marker RNA.

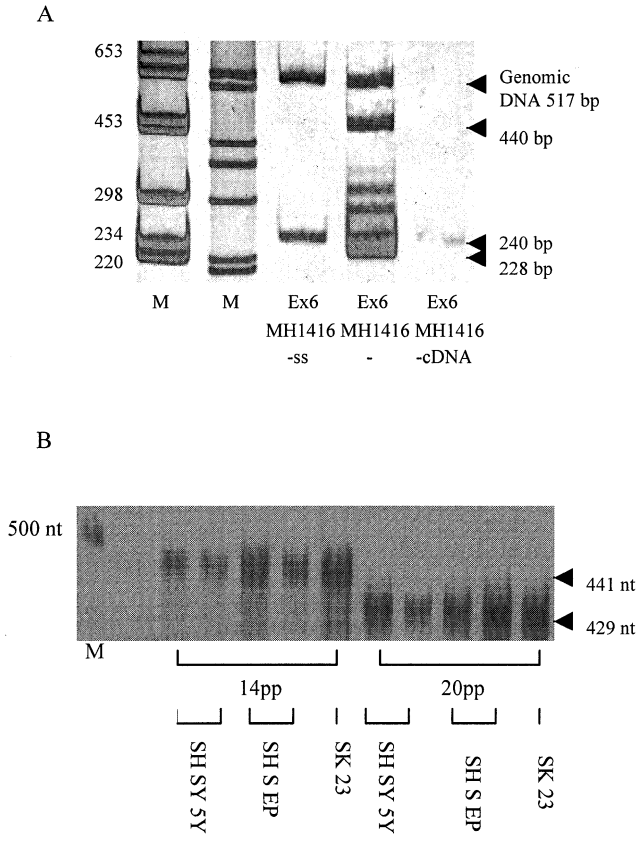

Fig. 5. A: Silver stained polyacrylamide gel showing RT-PCR products using the Ex 6 and MHR primer pair. The first two lanes (from the left, M) are different molecular weight markers; sizes (bp) are shown for the markers in the first lane only. Components of the PCR reaction loaded into each lane are indicated on the figure: controls were without Superscript reverse transcriptase (−ss), and without cDNA (−cDNA). The bands present in the −ss control lane are genomic DNA and a product from the pTL1 (RXRβ2^{SLSR}) plasmid DNA (lower band) present as contaminants. B: RNase protection assays to demonstrate the presence of a novel RXRβ transcript containing an intronic region (RXRβint6), with and without the 12 bp insertion, in SH SY 5Y, SH S EP neuroblastoma cells and SK 23 melanoma cells. Probes were prepared from the 14pp (probe length 520 nt) and 20pp (probe length 508 nt) clones, with and without the 12 bp insertion, respectively. M, marker track showing the 500 nt marker.

to RXRβ^{SLSR} and RXRβ respectively. Of the three remaining bands, a PCR product of approximately 440 bp was particularly prominent and was therefore cloned. Four clones, with inserts of the appropriate size were sequenced and alignment of the 440 bp PCR fragment sequence to the genomic RXRβ sequence indicated that the PCR product included the intron between exon 6 and exon 7 (Fig. 2) and that two of the clones had the 12 bp (SLSR) insertion (441 bp) and two did not (429 bp). A transcript with this structure would give a predicted 455 nt protected fragment on RNase protection assays with the hRXRβ2^{SLSR} 3′-end probe.

To verify that transcripts with this structure were expressed, RNase protection assays were carried out in parallel using riboprobes prepared from two clones of the 440 bp PCR product (clones 14pp and 20pp with and without 12 bp insertion) to probe cytoplasmic RNA from SH SY 5Y, SH S EP neuroblastoma cells, and SK23 melanoma cells. Protected fragments of appropriate sizes were observed (Fig. 5B), confirming the presence of RXRβ transcripts with an intron sequence with and without the SLSR insertion (termed RXRβint6^{SLSR} and RXRβint6 respectively) in the sample RNA. Since the 14pp and 20pp probes were prepared and used in parallel, these results also suggested that both transcripts were expressed at similar levels. Furthermore, the fact that the protected fragments were of similar or slightly greater intensity than the 500 nt marker suggest that the complementary transcripts were roughly comparable in abundance to transcripts detected with the 3′-RXRβ2^{SLSR} probe (Fig. 4).

3.4. Human genomic DNA RNase protection assays

To obtain further evidence that the RXRβint6 and RXRβint6^{SLSR} transcripts were transcribed from an internal promoter between exons 5 and 6, a genomic DNA fragment corresponding to bases 4843–5747 (GenBank accession number AF065396) was amplified by PCR from a human blood sample using BDF and BDR primers (Table 1) and cloned into pCR2.1 TOPO. After sequence verification, the PCR product insert was used as a riboprobe in RNase protection assays. In addition to undigested probe (940 nt), a protected fragment of approximately 650 nt was detected (Fig. 4B). As a positive control, sense RNA from this clone was also prepared and this hybridised with the riboprobe to give a predicted fragment size of approximately 900 nt (data not shown). These results are consistent with a transcriptional start site between exons 5 and 6. A search of the human genome database with the RXRβ intronic region gave only a single match to chromosome 6, suggesting that this novel transcript was specific to RXRβ and not a product of a pseudogene.

4. Discussion

Multiple isoforms have been reported for all members of the nuclear hormone receptor superfamily, including the RARs and RXRs. We have shown that RXRβ is the main RXR type expressed in SH SY 5Y and SH S EP neuroblastoma cells [9] and the results of the present experiments suggest that RXRβ and RXRβ^{SLSR} transcripts are expressed in SH SY 5Y and SH S EP neuroblastoma cells. RXRβ^{SLSR} is expressed in a variety of mouse tissues and human tumour cell lines; Mahajna et al. [7] reported that the level of RXRβ^{SLSR} compared to RXRβ varied widely from one RNA source to another but the significance of these differences remains unknown. Although an accurate quantitative comparison of RXRβ isoforms was not made in this study, both RXRβ and RXRβ^{SLSR} appeared to be expressed at comparable levels in SH SY 5Y and SH S EP neuroblastoma cells. It is not known if the SLSR insertion is present in both RXRβ1 and RXRβ2 transcripts.

Conflicting results for the functional role of RXRβ^{SLSR} with respect to DNA binding capacity and heterodimerisation ability have been reported [7,8]. Mahajna et al. [7] concluded that RXRα/β^{SLSR} chimeras behaved in a dominant negative fashion and failed to bind ligand or DNA, and was not active in forming heterodimers with RARα, although was capable of forming homodimers with RXRα. Thus the SLSR insertion apparently affected ligand binding properties as well as heterodimerisation functions. Fujita and Mitsuashi [8] reported that mouse RXRβ^{SLSR} also failed to bind ligand with high affinity, or to bind to RXREs in the presence or absence of 9-*cis*-retinoic acid, but did form heterodimers with TRα and

RARα. Clearly the RXRβ^{SLSR} isoform has a potential regulatory role in the cell, either as a dominant negative receptor or as a protein that represses the action of other receptors by preventing their binding to DNA.

The pattern of expression of RXRβ is clearly more complex than has been realised. The experiments reported here gave evidence that the 455 bp RXRβ transcript observed with RNase protection assays corresponded to the 3′-end of RXRβ but was initiated from a promoter between exons 5 and 6, included a retained intron and was alternatively spliced to give isoforms with and without the SLSR insertion. Although these RXRβint6 transcripts were clearly abundant, perhaps more so than RXRβ^{SLSR} and RXRβ transcripts, and were major RNA species in melanoma cells, fibroblast cells and mouse ganglion cells, it is unclear whether these are translated into functional proteins. Translation of the RXRβint6 and RXRβint6^{SLSR} transcripts in all three reading frames gave the carboxy-terminal end of RXRβ, incorporating only part of the ligand binding domain with a predicted molecular weight of 15 kDa, as the largest open reading frame. If such a protein is expressed in neuroblastoma cells it may act in a dominant negative manner and could be of biological significance.

A number of studies have identified alternative transcripts from various genes, but few have reported evidence that these transcripts are translated into functional proteins. Lu et al. [17], have identified a number of splice forms of the tumour suppressor gene BRAC-1 in both malignant and non-malignant breast epithelial cells. These splice variants are predicted to encode 80–85 kDa BRAC-1 derived proteins lacking approximately 60% of the internal amino acids of full length BRAC-1. Furthermore, alternative transcripts of the gene for human thrombospondin 2, an extracellular glycoprotein, have been shown to undergo exon skipping and/or retain introns [18]. A similar event occurs with the glycine receptor subunit α-3 gene whereby splice events allow a large intronic sequence to remain in the 3′-untranslated region [19]. Thus, the expression of alternative transcripts that contain intronic sequences is not unusual. Further work is needed to establish the functional role, if any, of the novel RXRβ transcript, although its relative abundance at the message level suggests that it may be important in regulating RXRβ function in a range of cell types.

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