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Alternative splicing regulates the production of ARD-1 endoribonuclease and NIPP-1, an inhibitor of protein phosphatase-1, as isoforms encoded by the same gene

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Abstract

ARD-1 is an endoribonuclease identified initially as the product of a human cDNA that complements mutations in *rne*, a gene that encodes *Escherichia coli* ribonuclease E. NIPP-1 was identified in bovine nuclear extracts as an inhibitor of protein phosphatase-1. Earlier work has shown that the protein-coding sequence of *ARD-1* is identical to the carboxy-terminal third of NIPP-1. However, whether ARD-1 is present in eukaryotes as a distinct entity has been unclear, as neither *ARD-1*-specific transcripts nor ARD-1 protein were detected in mammalian cells in earlier studies. Here we show that ARD-1 exists in human cells as a discrete protein, and that the ARD-1 and NIPP-1 peptides are isoforms encoded by a single gene and the same alternatively spliced precursor RNA. A retained intron containing multiple translation stop codons that are configured to terminate translation and initiate nonsense-mediated decay, limits the production of cellular ARD-1 protein. Our results establish the process by which functionally disparate ARD-1 and NIPP-1 peptides are generated from the protein-coding sequence of the same gene in human cells.

Keywords: Alternative splicing; ARD-1; Gene expression; NIPP-1; rnase

1. Introduction

RNA processing and degradation are key cellular events whose regulation in both prokaryotic and eukaryotic cells is important for cell survival and propagation in a changing environment (for review see Nierlich and Murakawa, 1996). While considerable progress has been made in recent years toward elucidating the mechanisms of RNA turnover in *Escherichia coli*, little is known about the signals and enzymes that mediate RNA decay in eukaryotic organisms (Ross, 1995). A cDNA expressing a protein coding sequence designated as ARD-1 (for *A*ctivator of *R*NA *D*ecay, Genbank accession No. U14575; Wang and Cohen, 1994) was identified by its ability to reverse the pleiotropic effects of mutations in the *rne* gene of *E. coli*, which encodes an essential multifunctional ribonuclease, RNase E (for a recent review on RNase E, see Coburn and Mackie, 1999). Protein expression from *ARD-1* cDNA in *E. coli* results in RNase-E like cleavages in *rne* mutants in vivo. Affinity-purified ARD-1 protein isolated from bacteria functions as a site-specific Mg^{2+}-dependent endoribonuclease that binds to RNase E substrates, cleaves RNA at the same sites as RNase E, and, like RNase E, generates 5′-phosphate termini at the sites of cleavage

Abbreviations: ARD-1, activator of RNA decay; ASE, ARD-1-specific element; ASF/SF2, alternative splicing factor/splicing factor 2; BLAST, basic local alignment search tool; BSA, bovine serum albumin; cDNA, DNA complementary to RNA; C-terminal, carboxyl terminal; DPBS, Dulbecco's phosphate buffered saline; EBV, Epstein–Barr virus; EDTA, ethylene diamine tetra-acetic acid; MW, molecular weight; NIPP-1, nuclear inhibitor of protein phosphatase-1; NMD, nonsense-mediated decay; NSE, NIPP-1-specific element; nt, nucleotide(s); ORF, open reading frame; PAC, P1 artificial chromosome; PCR, polymerase chain reaction; PEST, penicillin–streptomycin; PP-1, protein phosphatase-1; PTC, premature termination codon; RNase, ribonuclease; *rne*, a gene that encodes *Escherichia coli* ribonuclease E; SDS, sodium dodecyl sulfate; snRNP, small nuclear ribonucleoprotein particle.

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(Wang and Cohen, 1994; Claverie-Martin et al., 1997). However, in contrast to the 118 kDa RNase E protein, the 13.3 kDa ARD-1 protein lacks exoribonucleolytic poly (A) tail-shortening activity (Huang et al., 1998). Sequence comparisons have shown that both ARD-1 and RNase E contain regions that resemble the highly conserved 70 kDa RNA binding component of the U1 small ribonucleoprotein complexes involved in mRNA splicing in eukaryotes, as well as segments of eukaryotic cytoskeletal proteins implicated in regulating the assembly and transport of RNA/protein complexes (Wang and Cohen, 1994).

A sequence virtually identical to human ARD-1 subsequently was found to constitute the C-terminal third of NIPP-1 (Van Eynde et al., 1995), a protein originally identified and isolated from nuclei of bovine thymus as a potent and specific inhibitor of protein phosphatase 1 (PP-1). PP-1, whose phosphatase activity in vivo is modulated by its association to NIPP-1 and other non-catalytic subunits (Jagiello et al., 1995), belongs to a large conserved family of serine/threonine phosphatases that play a key role in the regulation of cellular processes by catalyzing reversible phosphorylation reactions (Wera and Hemmings, 1995). PP-1 is abundant in the nucleus and has been implicated in transcription and mRNA processing (Wera and Hemmings, 1995), and particularly in spliceosome assembly and alternative splice site selection (Cardinali et al., 1994). Full-length NIPP-1 and its C-terminal component, ARD-1, both have RNA binding activity; however, in contrast to ARD-1, NIPP-1 does not cleave RNA (Claverie-Martin et al., 1997; Jagiello et al., 1997). Conversely, ARD-1, which lacks the putative PP-1 binding site identified in the full-length NIPP-1 protein (Vulsteke et al., 1997), does not inhibit PP-1 (P. Cohen and G. Moorhead, personal communication). NIPP-1 has recently been shown to co-localize with pre-mRNA splicing factors (e.g., Sm proteins, U1A, U2AF65 and SR proteins) in nuclei (Trinkle-Mulcahy et al., 1999), and deletion of the ARD-1 domain, which results in the loss of RNA binding, or mutation of the PP-1 binding site does not affect this localization (Trinkle-Mulcahy et al., 1999).

The structural features of *ARD-1* and *NIPP-1* cDNAs are consistent with the notion that the corresponding mRNAs may result from alternative splicing. However, while ARD-1 was identified originally from a cDNA derived from EBV-transformed human B-lymphocytes (Wang and Cohen, 1994), subsequent investigations failed to detect ARD-1-specific transcripts in mammalian cells, and an ARD-1-sized peptide has not been found in bovine or other mammalian tissues by anti-NIPP-1/ARD-1 polyclonal antibody (Van Eynde et al., 1995, Trinkle-Mulcahy et al., 1999). Here we report investigations that resolve the etiological relationship between ARD-1 and NIPP-1. By immunological analysis of cell fractions and by Southern blot, PCR, and sequence analyses of transcripts and genomic DNA, we show that ARD-1 exists in human cells as a discrete protein encoded by the same gene as NIPP-1, and that *ARD-1* and *NIPP-1* transcripts are generated from a common precursor mRNA by alternative splicing.

2. Materials and methods

2.1. Cell lines and growth conditions

Human lung MRC-9 cells were maintained in culture dishes in Dulbecco's modified Eagle's medium (Life Technologies) supplemented with 10% fetal bovine serum (Life Technologies), 100 U Penicillin G and 100 μg streptomycin per ml (PEST; Life Technologies). The Burkitt's lymphoma cell line Raji, the diffuse histiocytic lymphoma cell line DHL-9, the acute lymphoblastic pre-B cell line Nalm-6 and EBV-transformed B-lymphocytes (a generous gift from Dr G. Aversa, DNAX, Palo Alto, CA) were maintained in RPMI 1640 (Life Technologies) supplemented with 10% fetal bovine serum (Life Technologies) and PEST (Life Technologies). All the cell lines were grown at 37°C in a 5% CO_2 atmosphere, except MRC-9 (10% CO_2).

2.2. Protein extracts, antibodies, and Western blotting

For preparation of protein extracts, cells were harvested, washed twice in cold Dulbecco's phosphate buffered saline (DPBS; Life Technologies), and lysed in NP-40 lysis buffer (10 mM Hepes, pH 7.5, 90 mM KCl, 1 mM $Mg(OAc)_2$, 2 mM mercaptoethanol, 2 mM phenylmethylsulfonyl fluoride (PMSF) and 0.5% NP-40). The extracts were cleared by centrifugation at $12\,000 \times g$ for 10 min. Protein concentrations were determined according to Sedmak and Grossberg (1977), using bovine serum albumin as a standard. Fractionation of extracts from the Burkitt lymphoma cell line Raji was performed by SDS–polyacrylamide gel electrophoresis in a Model 491 Prep Cell (Bio-Rad). The sample was centrifuged at $30\,000 \times g$ for 30 min and then filtered through a 0.45 μm filter prior to denaturation and loading onto the column. 130 mg of the protein extract was loaded onto the gel and run at 12 W constant power. Fractions (10 ml each) were collected with a elution buffer flow rate of 1 ml/min, and started when the bromphenol blue dye reached the bottom of the gel. Aliquots of the fractions and from total cell extracts were resolved on 10% tricine–SDS polyacrylamide gels (Schägger and Von Jagow, 1987). For Western blot analysis, aliquots (200 μl) from the fractions were lyophilized and then redissolved in sample cocktail before loading onto the gels. After electrophoresis, the gels were blotted onto 0.1 μm NitroPure membranes

(Osmonics) in cold transfer buffer containing 40 mM Tris, 20 mM sodium acetate, 2 mM EDTA, 20% methanol (pH 8), in a Trans-Blot apparatus with plate electrodes (Bio-Rad) for 2 h with 400 mA. The membranes were blocked in 10% non-fat dried milk (Safeway) prior to probing with ARD-1 antibodies that had been raised in sheep and antigen-purified (Trinkle-Mulcahy et al., 1999), except that the antigen (purified histidine-tagged ARD-1 (Claverie-Martin et al., 1997) was bound to NHS-Sepharose (Pharmacia) instead of CH-Sepharose. Horseradish peroxidase conjugated rabbit anti-sheep IgG (H + L) (Pierce; dilution 1:30 000) was used as the secondary antibody and was detected with the Renaissance Western Blot Chemiluminescence Reagent Plus detection system from NEN.

2.3. Screening of genomic libraries

PAC clones containing *ARD-1* genomic DNA were obtained by PCR screening of a human PAC library (Research Genetics) using oligonucleotide primers 147T and 499B derived from a sequence unique to the *ARD-1* transcript (ASE; Table 1). Using Southern blotting and PCR analyses, two isolated PAC clones were found be congruent in a >20 kb segment encoding the ARD-1 and NIPP-1 peptides. One of these clones (number 13231) was used for subsequent analyses.

2.4. Southern gel blot analysis

Genomic DNA was isolated from EBV-transformed B-lymphocytes and MRC-9 cells as described (Gilbert and Cohen, 1987). The DNA was digested with the restriction enzymes *Nde*I and *Xba*I, separated on a 1% agarose gel, and then transferred to a Hybond-N membrane according to the manufacturer's instructions (Amersham). Probes targeting the ASE, NSE and Z segments were labelled with [α-^{32}P]dATP with a random priming kit from Life Technologies. The probes were hybridized to the Southern blots in Church's buffer (0.5 M sodium phosphate, pH 7.2, 1 mM EDTA, 7% SDS; Church and Gilbert, 1984) for 24 h at 65°C. The blots were washed once in 0.1 × SSC (1 × SSC is 150 mM NaCl and 15 mM sodium citrate, pH 7.0) for 15 min at 45 °C and then once in 0.1 × SSC for 20 min at room temperature. Blots were exposed to X-ray film for 24 h at −70°C.

2.5. PCR amplification of DNA from PAC clones and DNA sequencing

DNA was isolated from PAC clones as described above. Regions between the different exons were amplified by PCR using the primers shown in Fig. 4, line E. The PCR amplifications were carried out with 10–100 ng PAC DNA in 50 μl reactions containing 20 mM Tris–

Table 1
Sequence of primers used in PCR amplification reactions[a]

Name[b]	Location	Sequence
23T	EXON 1	5′-GCG AAC TCC GGC TCT AGC-3′
147T	ASE	5′-TGC TGC GAG CCG AAC GGT-3′
168B	ASE	5′-CTG ACC GTT CGG CTC GCA GCA-3′
322B	ASE	5′-CTA ATC GCT CAA AGG G-3′
477T	ASE	5′-CAT CCC TCT GTG GTT GCT-3′
499B	ASE	5′-GGA TGC AGC AAC CAC AGA GG-3′
589B	EXON 2	5′-TCT CCT TTG ACT ACA TC-3′
645T	EXON 3	5′-ACC CTG ATT TGT GTG ACT-3′
721B	EXON 3	5′-CTC TCT TCA GAT GCT TGT G-3′
281T	EXON 4 (NSE)	5′-CAC ACA GCA GTT TCT TGG-3′
365T	EXON 4 (NSE)	5′-ATC CAC AGG GGC ATA CAC C-3′
386B	EXON 4 (NSE)	5′-AGG TGT ATG CCC CTG TG-3′
501B	EXON 4 (NSE)	5′-ATC AAG CTC AGT TTC CTC-3′
777B	EXON 5	5′-CAG TGT TGA ACT CTG TCA GG-3′
831B	EXON 5	5′-GAA TGT CCA GAT TTC CCT-3′
880T	EXON 5	5′-TGA CAT TCA GTG AGG ATG ATG AGA T-3′
936T	EXON 6	5′-ATG GTG CAA ACT GCA GTG TGC-3′
971B	EXON 6	5′-CTT GAC TGG GAC CAC TGC AG-3′
1408T	EXON 7	5′-TCA GCA GTG AAC ATG AAC CCT-3′
1436B	EXON7	5′-TCA AAT CAG CAA GGA AGG TGT3′
2251B	EXON 7	5′-CAG GTC TAG TCT TGG AGC TCT-3′
pACT2	Vector/cDNA junction	5′-ATG GAT GAT GTA TAT AAC TAT CTA TTC-3′

[a] The specific pairs of primers used in the reactions are shown in Fig. 4, line E.
[b] T and B denote top and bottom strand, respectively.

HCl (pH 9.0), 3.3 mM $MgCl_2$, 16 mM $(NH_4)_2SO_4$, 150 mg/ml BSA, 300 mM each of dATP, dCTP, dGTP and dTTP. The PCR reactions were done in a Perkin-Elmer GeneAmp PCR system 9600 thermocycler for 35–40 cycles at 96°C for 10 s (denaturation), 62°C for 30 s (annealing) and 70°C for 2–15 min (extension). The PCR products were checked by Southern blotting and then purified from agarose gels by Qiaquick gel extraction spin columns (Qiagen). The fragments were subjected to sequence analysis using the primers shown in Fig. 4, Line E. Sequencing reactions were carried out with the Prism dye-terminator cycle sequencing kit (PE Applied Biosystems), and analyzed on an ABI Prism 310 Genetic Analyzer.

2.6. RNA isolation and Northern blot analysis

Total RNA was extracted from EBV-transformed B-lymphocytes using RNA STAT-60 reagent (Tel-Test). Poly(A)$^+$ RNA was isolated using the PolyATtract mRNA isolation system IV (Promega). The Poly(A)$^+$ RNA was separated on an agarose–formaldehyde gel and transferred to a Hybond-N membrane (Amersham). The Northern blots were hybridized with the same probes used for the Southern blots.

3. Results

3.1. Discrete ARD-1 and NIPP-1 peptides are present in human cell extracts

A cDNA segment containing the translational open reading frame of ARD-1 was previously cloned and expressed in *E. coli* as a ribonucleolytically active his–ARD-1 fusion protein (Claverie-Martin et al., 1997). This protein was purified and used to raise sheep antibody that reacted at high affinity with the immunogen (Trinkle-Mulcahy et al., 1999). To further improve the specificity of the antibody, it was subjected to purification by binding to antigen as described in Materials and methods. As seen in Fig. 1, antigen-purified ARD-1 antibody detected two distinct bands corresponding to proteins of 22 and 43 kDa in size in unfractionated extracts from several human cell lines examined and in separate column fractions obtained during fractionation of extracts from the Burkitt's lymphoma cell line, Raji. The protein migrating at 43 kDa corresponds to NIPP-1, which has a calculated molecular mass of 38.5 kDa, but migrates more slowly than expected (Van Eynde et al., 1995). The smaller protein migrates at approximately the position expected for ARD-1 (as indicated by recombinant histidine-tagged ARD-1 isolated from *E. coli*). Both NIPP-1 and ARD-1 contain highly basic regions rich in proline residues (Wang and Cohen, 1994; Van Eynde et al., 1995) which may contribute to their anomalous migration in gels, ARD-1 to a greater degree than NIPP-1. *E. coli* RNase E, which has a molecular mass of 118 kDa, also shows a slower-than-expected migration in SDS–polyacrylamide gels (i.e., at 180 kDa; Coburn and Mackie, 1999) due to its proline-rich regions. Purified histidine-tagged ARD-1 protein containing additional histidine residues at the N-terminus (Claverie-Martin et al., 1997) and synthesized in *E. coli* migrates slightly faster than endogenous mammalian ARD-1, which may reflect possible post-translational modification of mammalian ARD-1.

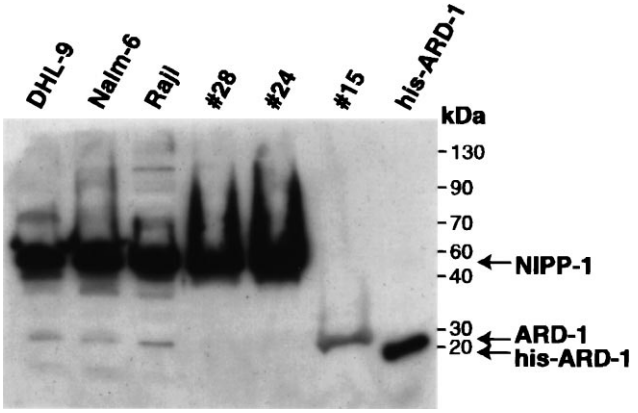

Fig. 1. Identification of NIPP-1 and ARD-1 peptides in human cell lines and in fractionated human cell extract by Western blot analysis. Protein extracts from human B-cells (approx. 100 μg/lane), aliquots (200 μl each) from Raji cell extracts fractionated on a Model 491 Prep Cell (Bio-Rad) and from *E. coli* purified histidine-tagged ARD-1 were separated by 10% tricine–SDS–PAGE and blotted onto a nitrocellulose membrane. The membrane was probed with purified antibody raised against his–ARD-1. The numbers on the top of the panel designate fractions obtained from the Raji extract fractionation. The arrows to the right of the panel indicate the positions of NIPP-1, ARD-1 and recombinant his–ARD-1, respectively. The positions of MW standards are shown at the right.

3.2. Alternatively-spliced transcripts encode ARD-1 and NIPP-1

Comparison of previously cloned cDNAs for the human ARD-1 and NIPP-1 proteins indicates that the 5′-untranslated region of *ARD-1* cDNA contains a 478 nt sequence that is absent from *NIPP-1* [the *ARD-1*-specific element (ASE)]. Conversely, the coding region segment of *NIPP-1* cDNA contains a 221 nt insert lacking in *ARD-1* cDNA [the *NIPP-1*-specific element (NSE)]. These two segments, plus a third region corresponding to sequences present at the carboxyl termini of both ARD-1 and NIPP-1, and thus common to both mRNAs (probe Z, Fig. 2) were used to analyze mRNA from EBV transformed B-cells by Northern blotting. As seen in Fig. 2A and B, discrete transcript species 2600 nt and 2370 nt in length were detected by the ASE and

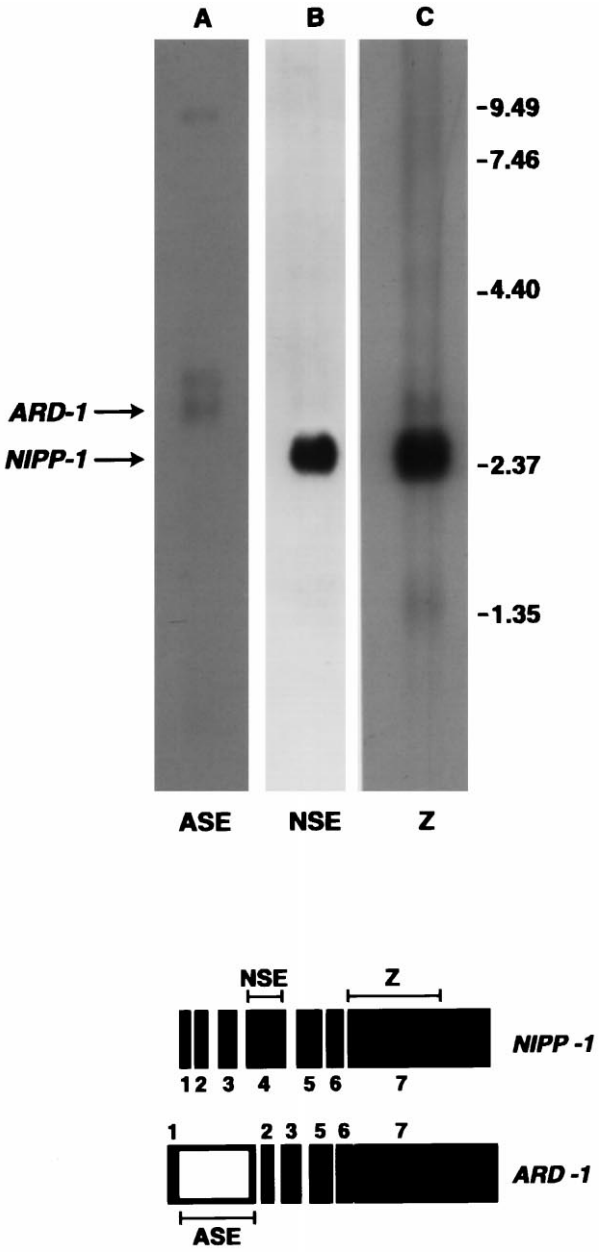

Fig. 2. Northern blot analysis. (A), (B) and (C) Northern blot of RNA isolated from a EBV-transformed B-cell line that was hybridized with the probes ASE, NSE and exon Z, respectively. The lower boxes show schematic representations of the *NIPP-1* and *ARD-1* transcripts; the size and location of the sequences used for the probes are shown by lines above and below the *NIPP-1* and *ARD-1* transcript boxes, respectively.

NSE probes, respectively, and two mRNA species that correspond to the transcripts individually detected by ASE and NSE probes hybridized to the *ARD-1*/*NIPP-1* common region probe (probe Z). Two larger transcripts, approx. 3100 nt and 9400 nt in length, also hybridized with the ASE probe, which consists entirely of an intron (see below) (Fig. 2A); however, no hybridization signal was detected using the NSE or Z probes, indicating that these transcripts contain sequences that cross hybridize with the ASE intron and are not related to *ARD-1* or *NIPP-1*. The 2370 nt human cell transcript detected by the NSE and common region probes in human cells is about 100 nt longer than *NIPP-1* mRNA identified previously in bovine cells (Van Eynde et al., 1995). The separate transcripts hybridizing to the ASE probe, which were present at less than 5% of the steady state level of *NIPP-1* transcripts, were not detected earlier in bovine and human tissues (Van Eynde et al., 1995).

In order to analyze the coding regions of human *ARD-1* and *NIPP-1*, cDNA was made from total RNA isolated from an EBV-transformed human B-lymphocyte cell line. The RNA was treated with DNase I and reverse transcribed with oligo-dT and Superscript I (Life Technologies). PCR amplification of the cDNA performed with primers 23T and 1436B (sequences derived from the common region of the 5′- and 3′-translational termination sites of *ARD-1* and *NIPP-1* cDNAs) resulted in two products of 1250 bp and 1050 bp in length. The 1050 bp cDNA fragment hybridized to the NSE probe in Southern blot analyses. The PCR product was then cloned and sequenced. The nucleotide sequence and amino acid sequence of the putative protein-coding region (Fig. 3) of this cDNA indicate that the human and bovine NIPP-1 peptides are identical except for one amino acid at position 245, where valine in bovine NIPP-1 is replaced by glycine in human NIPP-1 (Fig. 3, line 16) as a result of a single base change of GTC to GGC in the human counterpart. In addition to the codon change resulting in this amino acid substitution, there are 50 genetically silent differences in nucleotide sequence in the coding regions of the bovine and human *NIPP-1* genes as shown in Fig. 3.

To determine the sequence for the 5′-non-coding regions of both *ARD-1* and *NIPP-1* transcripts, PCR amplification reactions were carried out on cDNA libraries derived from EBV-transformed human B-lymphocytes and from adult human and human fetal brain (the cDNA libraries were generous gifts from Drs R. Stokowski and D. Cox, Stanford University). One of the primers used for amplification was derived from the junction of the cDNA insert with the vector, whereas the other was derived entirely from either the ASE segment (168B, 322B or 499B) or the NSE segment (386B or 501B). After two rounds of PCR, the products were then cloned. 12 clones containing 5′-sequences of *NIPP-1,* and six clones with 5′-sequences of *ARD-1* were obtained. Both *ARD-1* and *NIPP-1* were found to have identical sequences including ATG, the translational initiation sequence for NIPP-1 (Van Eynde et al., 1995). The 5′-non-coding sequence we determined for *ARD-1* differed from the one obtained for an *ARD-1* clone examined earlier (Wang and Cohen, 1994), perhaps as

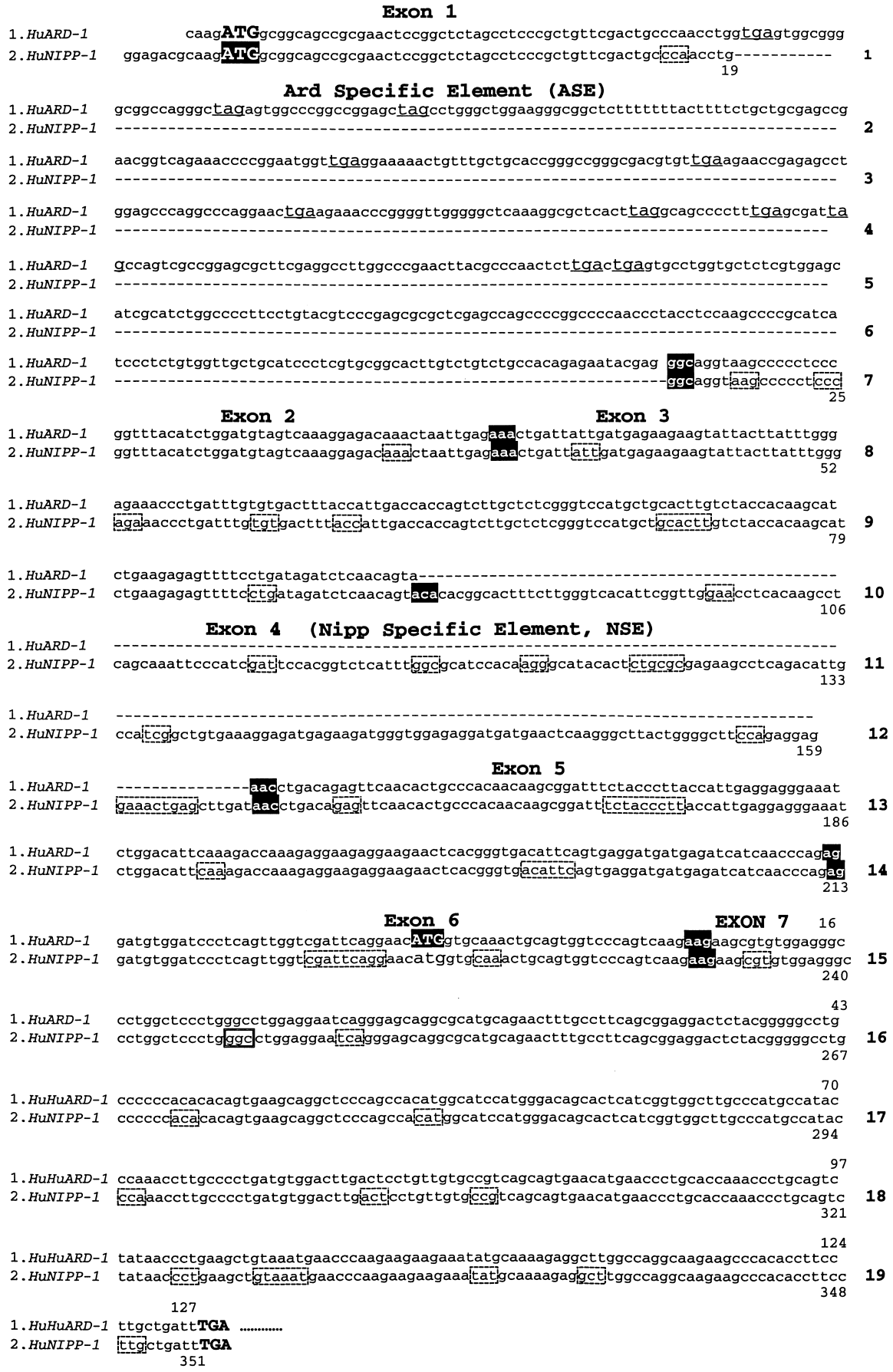
Exon 1
1.HuARD-1 caagATGgcggcagccgcgaactccggctctagcctcccgctgttcgactgcccaacctggtgagtggcggg
2.HuNIPP-1 ggagacgcaagATGgcggcagccgcgaactccggctctagcctcccgctgttcgactgccaacctg---------- 1
19
Ard Specific Element (ASE)
1.HuARD-1 gcggccagggctagagtggcccggccggagctagcctgggctggaagggcggctctttttttactttctgctgcgagccg
2.HuNIPP-1 ---------- 2
1.HuARD-1 aacggtcagaaaccccggaatggtgaggaaaaactgtttgctgcaccgggccgggcgacgtgtgaagaaccgagagcct
2.HuNIPP-1 ---------- 3
1.HuARD-1 ggagcccaggcccaggaactgaagaaacccggggttgggggctcaaaggcgctcactgagcagccccttgagcgatta
2.HuNIPP-1 ---------- 4
1.HuARD-1 gccagtcgccggagcgcttcgaggccttggcccgaacttacgcccaactctgactgagtgcctggtgctctcgtggagc
2.HuNIPP-1 ---------- 5
1.HuARD-1 atcgcatctggccccttcctgtacgtcccgagcgcgctcgagccagccccggccccaaccctacctccaagccccgcatca
2.HuNIPP-1 ---------- 6
1.HuARD-1 tccctctgtggttgctgcatccctcgtgcggcacttgtctgtctgccacagagaatacgag ggcaggtaagccccctccc
2.HuNIPP-1 ----------ggcaggtaagccccctccc 7
25
Exon 2
Exon 3
1.HuARD-1 ggtttacatctggatgtagtcaaaggagacaaactaattgagaaactgattattgatgagaagaagtattacttatttgggg
2.HuNIPP-1 ggtttacatctggatgtagtcaaaggagacaaactaattgagaaactgattattgatgagaagaagtattacttatttgggg 8
52
1.HuARD-1 agaaaccctgatttgtgtgactttaccattgaccaccagtcttgctctcgggtccatgctgcacttgtctaccacaagcat
2.HuNIPP-1 agaaaccctgatttgtgtgactttaccattgaccaccagtcttgctctcgggtccatgctgcacttgtctaccacaagcat 9
79
1.HuARD-1 ctgaagagagttttcctgatagatctcaacagta----------
2.HuNIPP-1 ctgaagagagttttcctgatagatctcaacagtacacacggcactttcttgggtcacattcggttggaacctcacaagcct 10
106
Exon 4 (Nipp Specific Element, NSE)
1.HuARD-1 ----------
2.HuNIPP-1 cagcaaattcccatcgattccacggtctcatttggcgcatccacaagggcatacactctgcgcgagaagcctcagacattg 11
133
1.HuARD-1 ----------
2.HuNIPP-1 ccatcggctgtgaaaggagatgagaagatgggtggagaggatgatgaactcaagggcttactggggcttccagaggag 12
159
Exon 5
1.HuARD-1 ----------aacctgacagagttcaacactgcccacaacaagcggatttctacccttaccattgaggagggaaat
2.HuNIPP-1 gaaactgagcttgataacctgacagagttcaacactgcccacaacaagcggatttctacccttaccattgaggagggaaat 13
186
1.HuARD-1 ctggacattcaaagaccaaagaggaagaggaagaactcacgggtgacattcagtgaggatgatgagatcatcaacccagag
2.HuNIPP-1 ctggacattcaaagaccaaagaggaagaggaagaactcacgggtgacattcagtgaggatgatgagatcatcaacccagag 14
213
Exon 6
EXON 7
16
1.HuARD-1 gatgtggatccctcagttggtcgattcaggaacATGgtgcaaactgcagtggtcccagtcaagaagaagcgtgtggagggc
2.HuNIPP-1 gatgtggatccctcagttggtcgattcaggaacatggtgcaaactgcagtggtcccagtcaagaagaagcgtgtggagggc 15
240
43
1.HuARD-1 cctggctccctgggcctggaggaatcagggagcaggcgcatgcagaactttgccttcagcggaggactctacgggggcctg
2.HuNIPP-1 cctggctccctgggcctggaggaatcagggagcaggcgcatgcagaactttgccttcagcggaggactctacgggggcctg 16
267
70
1.HuHuARD-1 ccccccacacacagtgaagcaggctcccagccacatggcatccatgggacagcactcatcggtggcttgcccatgccatac
2.HuNIPP-1 ccccccacacacagtgaagcaggctcccagccacatggcatccatgggacagcactcatcggtggcttgcccatgccatac 17
294
97
1.HuHuARD-1 ccaaaccttgcccctgatgtggacttgactcctgttgtgccgtcagcagtgaacatgaaccctgcaccaaaccctgcagtc
2.HuNIPP-1 ccaaaccttgcccctgatgtggacttgactcctgttgtgccgtcagcagtgaacatgaaccctgcaccaaaccctgcagtc 18
321
124
1.HuHuARD-1 tataaccctgaagctgtaaatgaacccaagaagaagaaatatgcaaaagaggcttggccaggcaagaagcccacaccttcc
2.HuNIPP-1 tataaccctgaagctgtaaatgaacccaagaagaagaaatatgcaaaagaggcttggccaggcaagaagcccacaccttcc 19
348
127
1.HuHuARD-1 ttgctgattTGA .....
2.HuNIPP-1 ttgctgattTGA
351

Table 2
Sizes of introns and the nucleotide sequences at the splice site junctions in the *NIPP-1/ARD-1* gene

Intron name[a]	5′ Splice site sequence		Intron (kb)	3′ Splice site sequence	
	Exon	Intron		Intron	Exon
1_{Nipp}	AACCTG	GTGAGTGGCGG	2.1	ATTTTTATTTTTCAG	GGCAGGTAA
1_{Ard}	ACGAGG	GTAGGTTAGCG	1.7	ATTTTTATTTTTCAG	GGCAGGTAA
1^{*}_{Ard}[b]	CTTCCT	GTACGTCCCGA	1.6	ATTTTTATTTTTCAG	GGCAGGTAA
2	ATTGAG	GTATGGAAAT	5.2	TTTAATATTTATTTAG	AAACTGATT
3	ACAGTA	GTAAGTAACT	2.0	ATTTGGTTATTCCCAG	CACACGGCA
4	CTTGAT	GTAATTCCCT	1.6	ATGAACCCTGGCTTAG	AACCTGACA
5	ACCCAG	GTGAGGTATC	2.8	TCTTCTCTTTTTGCAG	AGGATGTGG
6	CTCAAG	GTAGGAAGCA	3.1	TCATCGTGAACTGTAG	AAGAAGCGT
Consensus[c]	NAG	GTAAGT		CCTTTTTTTTNCAG	G

[a] The numbering of introns is indicated in Fig. 4, line B.
[b] 1_{Ard*} indicates the sequence of variant splice junction of the cDNA clone isolated from Soares parathyroid tumor (GenBank accession No. W44872).
[c] The consensus sequences at the exon/intron junction.

a result of extensive secondary structure in this region and consequent aberrant cDNA synthesis during the reverse transcriptase reaction or synthesis of the second strand cDNA (e.g., see Loewen and Switala, 1995). The 5′-non-coding sequences and the regions of overlap in the putative protein-coding regions of *ARD-1* and *NIPP-1* cDNAs derived from human cells are identical.

3.3. Structural organization of the human ARD-1 and NIPP-1 genes

Primers complementary to segments of the ASE were used to screen a human genomic DNA PAC library by PCR to identify specific clones containing ASE sequences. Analysis of restriction-enzyme-generated DNA fragments by Southern blotting using the ASE, NSE, and Z probes indicated that both of two ASE-containing clones also included *NIPP-1*-specific sequences and sequences common to both *ARD-1* and *NIPP-1*, suggesting that ARD-1 and NIPP-1 may be encoded by a single locus. This interpretation was confirmed by PCR analysis. To further characterize this locus, DNA segments of one of the clones were amplified using primers corresponding to specific regions of *ARD-1* and *NIPP-1*, and the resulting PCR products were separated by gel electrophoresis, purified from gels, and sequenced. The combined results of Southern blotting, PCR analysis, and sequencing (Fig. 4) showed that the *NIPP-1/ARD-1* gene is contained within a 20 kb DNA fragment that includes seven exons separated by six introns. The last exon, which is 1438 bp in length, encodes the entire ARD-1 peptide except for the first 10 amino acids (i.e., the C-terminal region of NIPP-1) as well as a 3′-untranslated region containing a sequence common to both *ARD-1* and *NIPP-1* transcripts. The other protein-coding exons, which range in length from 57 bp to 221 bp, are separated by introns longer than 1 kb (Fig. 4B).

Comparison of *ARD-1* and *NIPP-1* cDNA sequences with the genomic DNA sequence indicates that the *ARD-1* and *NIPP-1* transcripts are generated by alternative splicing of a single precursor RNA. The sequences at the exon/intron junctions of this mRNA are shown in Table 2. The splice junction sequence corresponds to the consensus sequence of splice junctions (Shapiro and Senapathy, 1987). Exon 1 of the *ARD-1* transcript includes a retained intron (the ASE segment) that is spliced out in *NIPP-1* transcripts. At the junction of exon 1 and the ASE segment, there is an in-frame translational stop codon. The retained intron contains 11 separate stop codons which are present in all three reading frames, ensuring that any protein synthesis

Fig. 3. Comparison of the cDNA sequences of human *ARD-1* (*HuARD-1*), human *NIPP-1* (*HuNIPP-1*) and bovine *NIPP-1* (*BoNIPP-1*). The translational start codon (ATG) for human *NIPP-1* is shown in a black box in line 1, the start codon for *ARD-1* is similarly shown in line 15, and the termination codons for the two peptides are shown in line 20 in bold letters. Line numbers are indicated in bold type at right. The termination codons present in the ASE segment, as described in the text (lines 1–5), are underlined. The dotted boxes indicate the positions of silent nucleotide differences between human NIPP-1 and bovine NIPP-1 (not shown), and the box marked with double solid lines in line 16 indicates the position of the only amino acid difference detected between the human and bovine NIPP-1 peptides. Complete bovine sequence information can be accessed through the Genbank, Acc. No. Z50748. The black boxes containing lower-case letters represent codons located at the splice junctions of transcripts. The numbers below each line represent the position of that specific amino acid codon in the human NIPP-1 peptide. The numbers above the line represent the positions of amino acid codons in the ARD-1 peptide. The dashed lines indicate the absence of a sequence. The 3′- untranslated region of human *ARD-1* is represented by dots. The full sequence information for human ARD-1 and NIPP-1 can be obtained from the Genbank, Acc. Nos. U14575 and AF064751–AF064757. The sequence of the 3′- untranslated region of human *NIPP-1* has not been determined. The exons are indicated in bold type.

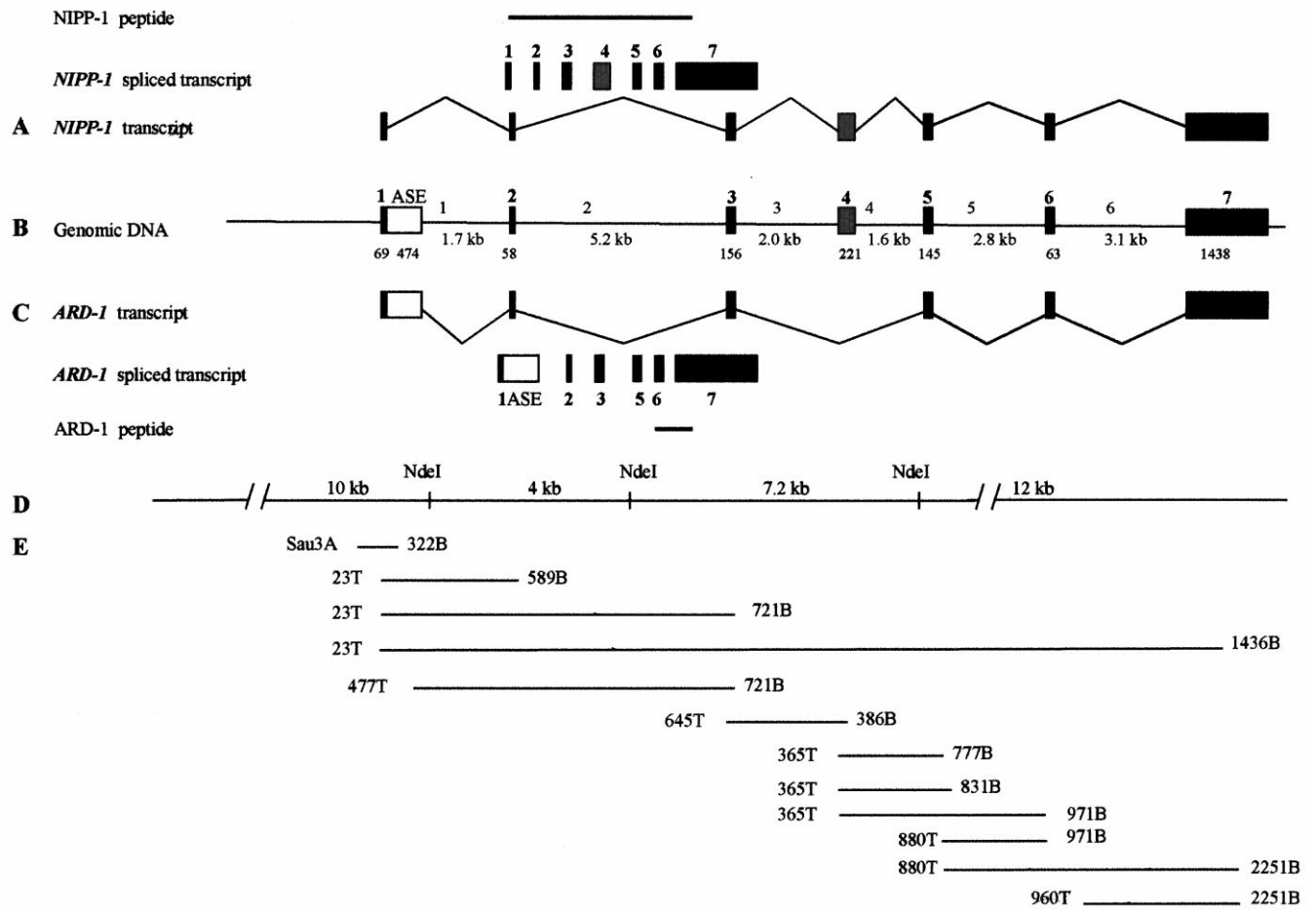

Fig. 4. Structural organization of the human *NIPP-1*/*ARD-1* gene. Line B shows the genomic map of *NIPP-1*/*ARD-1*. The introns are represented by a line; the black boxes represent common sequences present in *ARD-1* and *NIPP-1* transcripts. ASE (*ARD-1*-specific element) is shown by an unfilled box and NSE (*NIPP-1*-specific element) is shown by a grey box. The numbers (bold type) above the boxes indicate specific exons and the numbers above the line indicate the introns. The numbers below the line indicate the distance between the exons in kilobase pairs and the numbers below the boxes indicate the sizes of the exons in base pairs or nucleotides. Lines A and C represent *NIPP-1* and *ARD-1* transcripts. Boxes above line A and below line C represent the spliced transcripts of *NIPP-1* and *ARD-1*, respectively. The thick lines represent the coding regions of NIPP-1 and ARD-1 peptides on the transcripts. Line D: *Nde*I restriction sites for the DNA segment encoding *NIPP-1*/*ARD-1*. Line E: PCR primer pairs used for amplification of DNA fragments which were then purified and sequenced for the determination of sequences at the splice junctions. The primers are represented by numbers; 'T' and 'B' indicate top and bottom strand primers, respectively. The sequences of the PCR primers are shown in Table 1.

initiated spuriously in 5′-region of *ARD-1* transcript will not continue.

In *NIPP-1* transcripts, the junction between exons 1 and 2 occurs at the 3′-end of a sequence in common with *ARD-1* transcript, whereas in *ARD-1* mRNA, the 5′-end of exon 2 is spliced to the ASE. A BLAST search using the ASE sequence as a bait identified a cDNA clone isolated from Soares parathyroid tumor (GenBank accession No. W44872) containing a truncated ASE segment (shown in Table 2, line 3) that was spliced to exon 2. In all cases, the splice junction sequence 3′-to the exon is GT. Another distinction between *ARD-1* and *NIPP-1* transcripts occurs at exon 3. In *NIPP-1* transcripts this exon is joined to a sequence (exon 4; NSE) that is absent in *ARD-1* mRNA, where exon 3 is instead directly spliced to exon 5. All of the exon/intron junction sequences of *ARD-1* and *NIPP-1* transcripts correspond to canonical splice sites (Shapiro and Senapathy, 1987), as shown in Table 2.

4. Discussion

The results reported here establish that ARD-1, a mammalian cell endoribonuclease having RNase E-like activity (Wang and Cohen, 1994; Claverie-Martin et al., 1997), and NIPP-1, an inhibitor of protein phosphatase 1 (Van Eynde et al., 1995; Jagiello et al., 1995, 1997; Trinkle-Mulcahy et al., 1999), are encoded by a single gene, and that ARD-1 and NIPP-1 peptides are produced in human cells as discrete proteins from the same alternatively spliced pre-mRNA.

In order to investigate the presence of ARD-1-like proteins in human cells, antigen-purified polyclonal anti-

body to ARD-1, which is identical to the C-terminal sequence of NIPP-1, was raised and used for Western blotting. This antibody detected proteins having the same size as NIPP-1 and approximately the same size as purified histidine-tagged protein encoded by *ARD-1* cDNA in bacteria. ARD-1 was present in T-lymphocytes and 293 embryonic kidney cells (data not shown), as well as in both pre-B-cells (Nalm-6) and mature B-cells (Raji and DHL-9). The slightly slower migration of endogenous mammalian ARD-1 in comparison with bacterially expressed ARD-1 may indicate that ARD-1 synthesized in human cells is post-translationally modified.

While the possibility that the endogenous ARD-1 protein we observed is a stable proteolytic degradation product from NIPP-1 cannot be excluded, especially since NIPP-1 is known to be susceptible to proteolytic degradation (Van Eynde et al., 1995), the presence of the discrete ARD-1 protein in human cells is consistent with our detection of transcripts expected to encode such a protein. We noticed during our experiments that it was difficult to detect ARD-1 by Western blotting of gels using a standard protocol (Tris/glycine/methanol, pH 8.6, 2 h at 100 V) and also that it was necessary to load excess protein onto gels in order to see the ARD-1 band, which Western blots indicated was present at no more than one-hundredth the concentration of NIPP-1. Together, these findings may explain the failure of studies by others to detect ARD-1 protein in Western blots using a polyclonal NIPP-1/ARD-1 antibody (Van Eynde et al., 1995, 1999). Preliminary results indicate that ARD-1 is found mostly in the cytoplasm (data not shown), whereas NIPP-1 is located primarily, but not exclusively, in the nucleus (see also Jagiello et al., 1997).

cDNA analyses indicate that *ARD-1* and *NIPP-1* mRNAs diverge at the 3′-end of exon 1, where a 474 nt sequence (the ASE) is retained as part of the 5′-untranslated region of *ARD-1* mRNA, but is excised from the transcript encoding NIPP-1. Alternative splicing is a well-recognized means of production of protein isoforms (Smith et al., 1989) such as NIPP-1 and ARD-1, which share a common domain but perform different functions. Transcript features implicated in alternative splicing include sequestration of splice acceptor or donor sites in regions of secondary struture, and *cis* elements containing pentameric repeats (Aoufouchi et al., 1996; Hedjran et al., 1997). Additionally, the reading frame of the precursor mRNA (pre-mRNA) or the presence of a translation stop codon (Dietz et al., 1993) can influence splice site selection. The ASE contains 11 separate translational stop codons, which are present in all three reading frames; the absence of this segment in NIPP-1 transcripts is a prerequisite for continuation of synthesis of the nascent NIPP-1 protein and may also affect *NIPP-1*/*ARD-1* splicing.

Multiple studies have shown that eukaryotic transcripts containing premature termination codons (PTC) commonly are subject to a RNA degradative process known as nonsense-mediated decay (NMD) (Maquat, 1995; Hentze and Kulozik, 1999). *ARD-1* mRNA shares common features with transcripts known to trigger NMD; i.e., an in-frame nonsense codon followed by a retained intron, the ASE segment. As observed for transcripts previously shown to undergo NMD, the steady-state level of the *ARD-1* transcript is at least 20-fold less than that of *NIPP-1* as seen by Northern blotting (Fig. 2).

In transcripts having retained introns, the peptide encoded by the longer message can be expressed during certain stages of development or be cell-type-specific, as shown for the two PDZ domains of periaxin genes that result from alternative splicing and intron retention (Dytrych et al., 1998). Essential for expression of *ARD-1* is a translational re-initiation site at the 3′-side of the nonsense codon; several groups have shown such translational reinitiation in mammalian messages (e.g., Zhang and Maquat, 1997). Zhang and Maquat showed also that translation reinitiation increases the stability of messages that are destined for NMD. In *ARD-1* transcripts, five translational initiation codons are present at the 3′-side of the ASE segment, which may help stabilize the mRNA until the ribosomes encounter the AUG that will translate the ORF of *ARD-1*. The 3′-exon/intron junction of *ARD-1* transcripts is not fixed, as seen from cDNA derived from Soares parathyroid tumor, where a truncated ASE was found to be spliced to exon 2.

ARD-1 shares common characteristics with proteins that are involved in mRNA splicing (e.g., ASF/SF2, 9G8 and 70K snRNP), including an RNA binding site at the N terminus of the peptide and a highly charged carboxyl terminus (Ge et al., 1991; Golovkin and Reddy, 1996). Interestingly, transcript species containing retained introns have been detected also for these splicing-related genes, and proteins expressed from their alternatively spliced transcripts are involved in regulating the cellular concentration of the protein isoforms they encode. For all of the above mRNAs, the shorter of two alternatively spliced transcripts encodes a protein involved in a splicing complex, and this may also be the case for NIPP-1 (Trinkle-Mulcahy et al., 1999). The longer transcript includes a segment which, like the ASE, contains a translation termination codon that yields a trunctated protein that is an isoform of the full-length protein. The 70K snRNP has an RNA recognition domain that is followed by a glycine-rich region and a highly charged carboxyl-terminus (Krainer et al., 1991). ARD-1 is a proline-rich protein containing a RNA recognition motif at amino acid residues 15–38, followed by a glycine-rich region and a very basic lysine-rich carboxyl terminus.

PP-1 is known to participate in the regulation of important events in RNA metabolism; because NIPP-1 is a regulatory subunit of PP-1 as well as an RNA-

binding protein, it has been proposed that NIPP-1 targets PP-1 to RNA-associated substrates (Jagiello et al., 1997). Since PP-1 can modulate alternative splice site selection (Cardinali et al., 1994), it may be implicated in the alternative splicing of ARD-1 and NIPP-1 transcripts. ARD-1, which binds to RNA as a discrete protein (Claverie-Martin et al., 1997), also imparts RNA-binding capabilities to NIPP-1; antibodies directed against the carboxy-terminal part of NIPP-1 (i.e., the part that comprises ARD-1) (Jagiello et al., 1997) or deleting the ARD-1 segment (Trinkle-Mulcahy et al., 1999) abolish RNA binding by NIPP-1. It is tempting to speculate that ARD-1 and NIPP-1 may compete in splicesosomes for the same RNA substrates and that NIPP-1 may target PP-1 to the substrate, perhaps in a phosphorylation-dependent manner, without cleaving it.

After completion of this work, a paper published by Dr M. Bollen and co-workers appeared (Van Eynde et al., 1999), reporting a gene structure for *NIPP-1/ARD-1* similar to the one found by us, and also describing *NIPP-1*-derived transcripts having the characteristics of those reported here. However, in contrast to our findings, Van Eynde et al. did not detect a discrete ARD-1 protein, and also concluded that the presence of *ARD-1* (in their paper termed *NIPP-1γ*) mRNA is restricted to human transformed B-lymphocytes. The basis for these different findings has not been determined.

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References

Aoufouchi, S., Yelamos, J., Milstein, C., 1996. Nonsense mutations inhibit RNA splicing in a cell-free system: recognition of mutant codon is independent of protein synthesis. Cell 85, 415–422.

Cardinali, B., Cohen, P.T., Lamond, A.I., 1994. Protein phosphatase 1 can modulate alternative 5′ splice site selection in a HeLa splicing extract. FEBS Lett. 352, 276–280.

Church, G.M., Gilbert, W., 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA 81, 1991–1995.

Claverie-Martin, F., Wang, M., Cohen, S.N., 1997. *ARD-1* cDNA from human cells encodes a site-specific single-strand endoribonuclease that functionally resembles *Escherichia coli* RNase E. J. Biol. Chem. 272, 13 823–13 828.

Coburn, G.A., Mackie, G.A., 1999. Degradation of mRNA in *Escherichia coli*: an old problem with some new twists. Prog. Nucleic Acid Res. Mol. Biol. 62, 55–108.

Dietz, H.C., Calle, D., Francomano, C.A., Kendzior, R.J., Pyeritz, R.E., Cutting, G.K., 1993. The skipping of constitutive exons in vivo induced by nonsense mutations. Science 259, 680–683.

Dytrych, L., Sherman, D.L., Gillespie, C.S., Brophy, P.J., 1998. Two PDZ domain proteins encoded by the murine *Periaxin* gene are the result of alternative intron retention and are differentially targeted in Schwann cells. J. Biol. Chem. 273, 5794–5800.

Ge, H., Zuo, P., Manley, J.L., 1991. Primary structure of the human splicing factor ASF reveals similarities with *Drosophila* regulators. Cell 66, 373–382.

Gilbert, D.M., Cohen, S.N., 1987. Bovine papilloma virus plasmids replicate randomly in mouse fibroblasts throughout S phase of the cell cycle. Cell 50, 59–68.

Golovkin, M., Reddy, A.S., 1996. Structure and expression of a plant U1 snRNP 70K gene: alternative splicing of U1 snRNP 70K pre-mRNAs produces two different transcripts. Plant Cell 8, 1421–1435.

Hedjran, F., Yeakley, J.M., Huh, G.S., Hynes, R.O., Rosenfeld, M.G., 1997. Control of alternative pre-mRNA splicing by distributed pentameric repeats. Proc. Natl. Acad. Sci. USA 94, 12 343–12 347.

Hentze, M.W., Kulozik, A.E., 1999. A perfect message: RNA surveillance and nonsense-mediated decay. Cell 96, 307–310.

Huang, H., Liao, J., Cohen, S.N., 1998. Poly(A)- and poly(U)-specific RNA 3′ tail shortening by *E.coli* ribonuclease E. Nature 391, 99–102.

Jagiello, I., Beullens, M., Stalmans, W., Bollen, M., 1995. Subunit structure and regulation of protein phosphatase-1 in rat liver nuclei. J. Biol. Chem. 270, 17 257–17 263.

Jagiello, I., Beullens, M., Vulsteke, V., Wera, S., Sohlberg, B., Stalmans, W., von Gabain, A., Bollen, M., 1997. NIPP-1, a nuclear inhibitory subunit of protein phosphatase-1, has RNA-binding properties. J. Biol. Chem. 272, 22 067–22 071.

Krainer, A.R., Mayeda, A., Kozak, D., Binns, G., 1991. Functional expression of cloned human splicing factor SF2: homology to RNA-binding proteins, U1 70K and *Drosophila* splicing regulators. Cell 66, 383–394.

Loewen, P.C., Switala, J., 1995. Template secondary structure can increase the error frequency of the DNA polymerase from *Thermus aquaticus*. Gene 164, 59–63.

Maquat, L.E., 1995. When cells stop making sense: effects of nonsense codons on RNA metabolism in vertebrate cells. RNA 1, 453–465.

Nierlich, D.P., Murakawa, G.J., 1996. The decay of bacterial messenger RNA. Prog. Nucleic Acid Res. Mol. Biol. 52, 153–216.

Ross, J., 1995. mRNA stability in mammalian cells. Microbiol. Rev. 59, 423–450.

Schägger, H., Von Jagow, G., 1987. Tricine–sodium dodecyl sulfate–polyacrylamide gel electrophoresis for the separation of protins in the range from 1 to 100 kDa. Anal. Biochem. 166, 368–379.

Sedmak, J.J., Grossberg, S.E., 1977. A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue G250. Anal. Biochem. 79, 544–552.

Shapiro, M.B., Senapathy, P., 1987. RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. Nucleic Acids Res. 15, 7155–7174.

Smith, C.W.J., Patton, J.G., Nadal-Ginard, B., 1989. Alternative splicing in the control of gene expression. Annu. Rev. Genet. 23, 527–577.

Trinkle-Mulcahy, L., Ajuh, P., Prescott, A., Claverie-Martin, F., Cohen, S., Lamond, A.I., Cohen, P., 1999. Nuclear organisation of NIPP1, a regulatory subunit of protein phosphatase 1 that associates with pre-mRNA splicing factors. J. Cell Sci. 112, 157–168.

Van Eynde, A., Wera, S., Beullens, M., Torrekens, S., Van Leuven, F., Stalmans, W., Bollen, M., 1995. Molecular cloning of NIPP-1, a nuclear inhibitor of protein phosphatase-1, reveals homology with

polypeptides involved in RNA processing. J. Biol. Chem. 270, 28 068–28 074.

Van Eynde, A., Perez-Callejon, E., Schoenmakers, E., Jacquemin, M., Stalmans, W., Bollen, M., 1999. Organization and alternate splice products of the gene encoding nuclear inhibitor of protein phosphatase-1 (NIPP-1). Eur. J. Biochem. 261, 291–300.

Vulsteke, V., Beullens, M., Waelkens, E., Stalmans, W., Bollen, M., 1997. Properties and phosphorylation sites of baculovirus-expressed nuclear inhibitor of protein phosphatase-1 (NIPP-1). J. Biol. Chem. 272, 32 972–32 978.

Wang, M., Cohen, S.N., 1994. ard-1: A human gene that reverses the effects of temperature sensitive and deletion mutations in the *Escherichia coli rne* gene and encodes an activity producing RNase E-like cleavages. Proc. Natl. Acad. Sci. USA 91, 10591–10595.

Wera, S., Hemmings, B.A., 1995. Serine/threonine protein phosphatases. Biochem. J. 311, 17–29.

Zhang, J., Maquat, L.E., 1997. Evidence that translation reinitiation abrogates nonsense-mediated mRNA decay in mammalian cells. EMBO J. 16, 826–833.