

Tissue specific expression and cDNA structure of a human transcript encoding a nucleic acid binding [oligo(dC)] protein related to the pre-mRNA binding protein K

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ABSTRACT

In human cells at least 20 different proteins or groups of proteins have been identified that are associated with hnRNAs. These proteins (designated A1 – U) are highly abundant in the nucleus. In this study, we present the sequence of a novel cDNA clone, sub2.3, isolated from a human lymphocyte cDNA library. The predicted amino acid sequence shows homology to repeated domains in the human hnRNA binding protein K (hnRNP K), which are believed to be of functional importance. hnRNP K is among the major oligo(rC/dC) binding proteins in vertebrate cells and we show here that the protein product of sub2.3 also binds to oligo(dC). This is shown by a novel approach where we demonstrated specific binding of *in vitro* translated sub2.3 protein to biotinylated oligo(dC) which was immobilized on magnetic streptavidin-coated Dynabeads. Moreover we found that the sub2.3 transcript is expressed in a tissue dependent manner with the highest expression observed in several lymphoid tissues and skeletal muscle. The gene was also abundantly expressed in several lymphoid cell lines and the hepatoma cell line HepG2 while a low expression was observed in the HL60 myeloid cell line and in the HeLa cervical carcinoma cell line. In conclusion, this study presents the cDNA sequence of a novel transcript which shows tissue specific expression and encodes a protein with oligo(dC) binding specificity *in vitro*.

INTRODUCTION

Heterogenous nuclear RNA binding proteins (hnRNP) constitutes a broad class of proteins, designated A1 to U, which are associated to pre-mRNAs during the processing to mRNAs. hnRNPs are believed to influence the structure of hnRNAs, and facilitate or hinder the interaction of hnRNA sequences with other

components that are needed for the processing of pre-mRNAs. In addition, hnRNPs may also play important roles in the interaction between hnRNA and other nuclear structures, in nucleocytoplasmic transport of mRNA, and in other cellular processes (1). hnRNPs are localized in the nucleus and are a major group of proteins there, but there are also examples of hnRNPs that shuttle between nucleus and cytoplasm, leading to the speculation that they also can be involved in the transport of mRNA from the nucleus to the cytoplasm (1,2). The hnRNP J and K proteins have been identified as the major oligo(C) and poly(C) binding proteins in human HeLa cells (3). They are immunologically related to each other and evolutionary conserved among various vertebrates (3). *In vivo* they are believed to bind to cytidine rich polypyrimidin tracts at the 3' ends of introns, but *in vitro* they can bind both to ribopolynucleotides and deoxyribopolynucleotides (3). One can therefore not exclude the possibility that these proteins also bind to DNA. Interestingly, a recent study has shown that hnRNP K can bind to the CT element in the human c-myc promoter, and transfection studies have demonstrated that hnRNP K augments gene expression in a cis-element dependent manner, and may thus play a role in the transcriptional regulation of the human c-myc gene (4).

Several strategies have been employed to isolate genes expressed in specific cells or tissues. One powerful tool is the isolation of genes by different subtraction strategies (5,6,7,8,9,10). In this study we present the sequence of a cDNA clone obtained from cultured lymphocytes by a subtractive strategy. This cDNA clone showed strong homology to a mouse cDNA sequence (GenBank accession no. L19661), designated hnRNP X (11). The hnRNP X cDNA was isolated from a cDNA library generated from the mouse pre-B leukaemia cell line 70Z3. Both the sub2.3 gene product and hnRNP X show homology to repeated domains in hnRNP K (11). In this study we have investigated if sub2.3 has the same binding specificity as hnRNP K, and in addition we also present expression data showing an unique tissue distribution of the sub2.3 transcript.

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MATERIAL AND METHODS

Cells

The following human derived cell lines were used in this study; the pre-B cell lines Reh (ATCC CRL 8286) and Nalm-6, The Burkitt's lymphoma cell lines Daudi (ATCC CCL 213) and Raji (ATCC CLL 86), the plasmacytoid cell line U266 (ATCC TIB 196), The T cell line Jurkat (ATCC TIB 152), the myeloid cell line HL60 (ATCC CCL 240), the hepatoma cell line HepG2 (ATCC HB 8065) and the cervical carcinoma cell line HeLa (ATCC CCL 2). All lymphoid cell lines were grown in RPMI 1640 supplemented with 10 % fetal bovine serum at 37 in an humidified atmosphere with 5 % CO₂. HepG2 and HeLa were grown in Dulbecco's Modified Eagles medium supplemented with 10 % Fetal bovine serum.

Isolation of the sub2.3 cDNA clone

The sub2.3 cDNA clone was isolated using a novel subtractive strategy recently described (10). Briefly, biotinylated 1-strand cDNA generated from Reh mRNA was immobilized onto streptavidin-coated magnetic Dynabeads (Dynabeads M-280 Streptavidin; Dynal, Norway). mRNA isolated from TPA stimulated Daudi cells was hybridized to the immobilized 1-strand cDNA. The specific Daudi mRNA left after the subtraction, was converted to a radiolabeled cDNA probe and used for the screening of a cDNA library, generated from TPA stimulated T cells, in bacteria by colony hybridization as described by Grunstein and Hogness (12). The sub2.3 cDNA clone was one of eight cDNA clones isolated from 4×10^4 colonies obtained by this strategy. A near full-length (1600 bp) sub2.3 cDNA clone was isolated from the same cDNA library as described above using the originally isolated sub2.3 cDNA as a probe.

DNA sequencing

Both the original sub2.3 cDNA clone (sub2.3s, 1.1 kb) and the longer near full-length cDNA clone (sub2.3f, 1.6 kb) were sequenced in both directions after restriction fragment subcloning. Overlapping restriction fragments were subcloned into pBluescript KS⁻ (Stratagene, CA). Doublestranded sequencing was performed on plasmid DNA with the Sequenase sequencing kit (USB, Cleveland, OH) according to the manufactures instructions. Database searches was performed using the Blast (National Centre for Biotechnology Information), Fasta and TFasta programs from the GCG program package (13).

In vitro transcription and translation

In vitro transcription and *in vitro* translation were performed with kits obtained from Promega (Madison, USA) according to the manufactures protocol. Briefly, the sub2.3f cDNA clone in pBluescript KS⁻ was linearised with SalI, precipitated and resuspended in RNase free H₂O. *In vitro* transcription was performed for 60 minutes at 37°C, in presence of ribonucleotides, RNasin, DTT, transcription buffer and T7 RNA polymerase. The *in vitro* transcribed RNA was precipitated and subjected to *in vitro* translation by incubation with rabbit reticulocyte lysate (Promega, Madison, USA) in the presence of [³⁵S]-methionine for 60 minutes at 30°C. The *in vitro* translation product was analyzed on a 12 % SDS-polyacrylamide gel. Following electrophoresis, the gel was fixed, dried, and subsequently subjected to autoradiography.

RNA isolation and Northern blot analysis

Total RNA was extracted from cells by the guanidine thiocyanate method (14). 10 µg samples of total RNA were size fractionated on 1 % agarose/formaldehyde denaturing gels, transferred to nylon membranes (Hybond-N nylon, 0.45 micron; Amersham, Arlington heights, IL) and crosslinked to the membranes by UV light. Prehybridization and hybridization was performed in hybridization buffer (5×SSPE, 5×Denhardt, 0.1% SDS and 20 µg/ml salmon sperm DNA) at 65°C and the membranes were hybridized over night with α[³²P]-dCTP-labelled sub2.3 insert (Mega-prime DNA labelling systems, Amersham, Arlington heights, IL). An 19-mer oligonucleotide hybridizing to 18S rRNA (nucleotides 287–305 in the 18S rRNA sequence) was end labelled with [γ-³²P]-ATP (3000 Ci/mM) as described by Sambrook *et al.* (15) and hybridized to the filters over night at 55°C. The membranes were washed with 2×SSC/0.1% SDS at room temperature followed by 1×SSC/0.1 SDS at 65°C. Multiple tissue blots were obtained from Clontech (Multiple tissue blot 1 and 2; Clontech, CA). Each lane contain 2 µg mRNA isolated from different tissues. A third, non-commercial tissue blot, containing 20 µg of total RNA per lane, was a kind gift from Kjetil Taskèn, Institute of Medical Biochemistry, University of Oslo, Norway. These blots were prehybridized and hybridized in hybridization solution (5×SSPE, 5×Denhardt, 50 % formaldehyde and 100 µg/ml salmon sperm DNA) at 42°C. The blots were hybridized with α[³²P]-dCTP labelled sub2.3 cDNA insert over night followed by washing as described by Clontech. All blots were exposed to XAR-5 film (Kodak) at -70°C using DuPont Lightning-Plus intensifying screens (Wilmington, DE).

Isolation of genomic DNA and Southern blot analysis

Genomic DNA was isolated from the Daudi cell line on a nucleic acid extractor 340A (Applied Biosystem, USA) essentially, according to the manufactures instructions. 7 µg of genomic DNA was subjected to restriction cleavage (HindIII, BamHI, EcoRI, EcoRV or PstI) in appropriate digestion buffer with 4mM spermidin for 3–4 h at 37°C. The reactions were subjected to horizontal agarose gel electrophoresis at 30 volts for 20 h. Denaturation of DNA, and blotting of agarose gel to filter was performed as described by Sambrook *et al.* (15). DNA was crosslinked to the filter (Hybond N+; Amersham) by the combination of baking for 2 h at 80°C and 10 min UV exposure. Sub2.3 insert was labelled with α[³²P]-dCTP (3000 Ci/mM) by random priming as described above. Prehybridization and hybridization of the filter was performed in 5×SSPE, 5×Denhardt, 0.1 % SDS and 20 µg/ml salmon sperm DNA. The probe was hybridized to the filter at 65°C over night. The filters were washed with increasingly stringent washes, dried and subjected to autoradiography.

Nucleic acid-binding assay

The nucleic acid binding assay was performed using a novel approach where oligonucleotides were immobilized to a magnetic solid phase (Dynabeads M-280 streptavidin; Dynal, Norway). The binding and washing conditions in the assay were essentially as described for oligo(dC) binding assays described previously (3,16). The biotinylated oligonucleotides used in this study were a 20-mer oligo(dC) and a control oligonucleotide with the sequence: 5'-GGA-CCT-TCC-TTG-GTG-TGT-TG -3'. 10 µg of each oligonucleotide in PBS was immobilized to 50 µl (10

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sub2.3 ( 1) -----CCTACACCTCCCTCCC-----CCCGCCAGCCG-----CCAAGACTTGACCAGTAACGAG
hnRNP X ( 1) TCCCGCCCTTCCCTTCCCGCCCGCTCCCTTTTCCCTCAGTCGACTCGCGCTCCAGT11TGGCT11CACTCT1CACCAGTGACCAAGACTTGACCCTCAA--AG

sub2.3 ( 53) CCAAAC1CCCCGAACCCCGCCCGCTCGCCATGGATGCCGGTGTGACTGAAAGTGGACTAAATGTGACTCTCACCAT1CGGCT1CTTATGCACGGAAAGGAAGTGG
hnRNP X ( 109) TCCAGCTCCCTGTAA-----CTGCTAGACATGGACACCGGTGTGATTGAAGTGGATAAATGTCACTCTCACCATCAGGCTAC-TATGCATGGAAGGAAGTGG

sub2.3 ( 163) AAGCATCAT1GGGAAGAAAGGGGAGTCCGT1AAGAGGATCCGGGAGGAGTGGCGCGGGATCAACATCTCGGAGGGAA1TGTCCGGAGAGAA1CATCACTCTGACC
hnRNP X ( 209) CAGTATCATCGGAAGAAAGGAGAA1CTGT1AAGAAGATCGCGGAGGAGTGGTGCAGTATCAACATCTCAGAAGGAA1TGTCT1GAGAGAA1TATCACT1TGGCTG

sub2.3 ( 273) GCCCCACCAATGCCATCT1TAAGGCT11CGCTATGATCATCGACAAGCTGGAGGAAGATATCAACAGCTCCATGACCAACAGTACCGCGGCCAGCAGGCCCCCGGTACC
hnRNP X ( 319) GACCGACTAA1GCCATCT1CAAAGCC11TGC1ATGATCAT1GACAAACTGGAAAGGACAT1AAGCAGCTCT1ATGACCAATAGTACAGCTGCCAGTAGACCCCGGTACC

sub2.3 ( 383) CTGAGGCTGGTGGTCCCGCCACCCAGTCCGGCTCCCTGAT1GGGAAAGCGGGTGTAAAGTCAAAGAGATCCCGGAGAGTACGGGGCCAGGTCCAGGTGGCGGGGA
hnRNP X ( 429) TTACGGCTGGTGGT1CCTGCTAGTCACTGTGGCTCTCT1AT1TGGAAAAGTGGT1GCAAGATCAAGGAA1AAGAGAGAGTACAGGGCTCAGGTCCAGGTGGCAGGGGA

sub2.3 ( 493) TATGCTGCCAAC1CCACCGAGCGGGCCAT1CACCATCGCTGGCGTCCCGAGTCTGTCAACAGAT1TGCCTGGTCA1GCTGGAGACGCT1CCCACT
hnRNP X ( 539) TATGCTGCCAAC1CAACTGAGCGGGCACTCACT1TGC1GGCAT1CCCGAGTCCATCAT1GAGTGTGTCAAACAGATCTGCTGGTCA1G1TGGAGTCCCCCC-----

sub2.3 ( 603) CTCGCAAGGGAGAGT1CATGACCA11CCGTACCAGCCCATGCCCGCCAGT1CCCGAGTCACT1CGCGGGGGCCCAAGATCGGT1-----GCAGCGAGCT1CGGGC
hnRNP X ( 644) -----GAAGGGCT1GACCA1CCCGTACCGGCCCAAGCC-1CCAGT1CTCCAGTCACT1TGCAGGTGGT1CAGGACAGGTACAGCACAGGCAGCGACAGT1CGGAC

sub2.3 ( 704) TACCCCATGCCACCA-----TGACCTGGAGGACCACCTCTAGATGCCTACTCGAT1CAAGGACAACACACC-AT11CTCCGCTCGAT1TGCCA
hnRNP X ( 743) 111CCCCACACCCCGTCCAT1TGCC1CAACCTCAGCTGGAGGGACCACCTCTAGAGGCC1T1ACCAT1CAAGGACAGT1TGCCAT1CCACAGCCAGAT1TGACCA

sub2.3 ( 795) AGCTGAACAGGTGGCAAGACAACAGTCTCACT11GCCATGATGCACGGGGACCGGAT1CGCGGAA1TGACTCCAGCTCTCCAGAGT1GAAAGGCTA11GGGCAAGT
hnRNP X ( 853) AGCTGACACAGT1GGCAATGCAACAGTCTCAT111CCCATGACCA1GGCAACCCCGAT1CAGTGGCA1TGAATCCAGCTCTCCAGAGT1GAAAGGCTA11GGGCAAGT

sub2.3 ( 905) TTGGATGCATCTACTCAAACCACC---CATGAACTCACCAT1CCAAATAACT1AAATGGCTGCATAATCGGGG-----CCAACATTAATGAGATCCGCCAGATGTC
hnRNP X ( 963) TTGGATGCATCTGCTCAAACACTTCTCATGAACTCACCAT1CCAAATGAT1TGAATGGCTGCATAATCGGGGCTCAAGGCCCAAAATCAATGAGATCCGTCAGATGTC

sub2.3 (1004) CGGGGCCAGATCAAAAT1GCCAACCCAGTGGAAAGGCTCCTCTGGTAGGCAAGTFACTATCACTGGCTCTGCTGCCAGTATTAGTCTGGCCAGTATCTAATCAATGCCA
hnRNP X (1073) TGGGGCCAGATCAAAAT1GCCAACCCAGTGGAAAGGATCTACTGATCGGCAAGT1ACCATCACTGGATCTGCTGCCAGCAT1AGCCTGGCTCAGTATCTAATCAATGTC

sub2.3 (1114) GGC11TCTCTGAGAAGGG---CATGGGTGACAGT1GAAAGTGTAGG1TCCCTCAAT1AACCCT11TCTGCTG11TCTCC-----ATGATCCAAGTGTGTAAT11CTG--
hnRNP X (1183) GGC11TCTCTGAGACAGGTGGCAT1GGGAGGAGT1GAAAGTGTAGG1TCCCTCAAT1AACCCT11TCTGCTG11TCTCC-----ATGATCCAAGTGTGTAAT11CTG--

sub2.3 (1215) --GTCAGTGT1CCAGG11TAAA-TAAT11GTAAGTGT1CAAGT1TCTACACAAC11TATCATCCGCTAAGAA11TAAAAATCACA-----T1CTCTGTTT
hnRNP X (1288) CAGTCAGCGACTCCAGG11TAAAA1AG11TGTAAAT1TTC-AG11TCTACAC-ACT11TATCATCCACTCATGAT11T1TAA1TAAAGTGT11TAAAT1TCTCTGTTT

sub2.3 (1309) AGCTGT1TAATGCTGGGAT--CCATAT1-AGT11TATAAGCT11TCCCTG11T1TAGT11TGT11TGGG1111TGGCTCATGAAT11TAT11TCTG11TGTGTAAGAAA
hnRNP X (1396) AGCAGT1-ACGCTGAGATATCCATAT1TAGT11TATAAGCT11TCCCTG11T1TAGT11TGT11TGGG1111TGGCTCATGAAT11TAT11TCTG11TGTGTAAGAAA

sub2.3 (1416) TGTAAGAGTGGAA1TGT1TAAAT11TCACT11TAGT11TCTGTAATGTCAGAA11TAAAGAA1TAAAAACGGAT1GGT1TAAAAATGCT1CATAT11TAAAAAGCT1GGGA1
sub2.3 (1526) TGCTGCT1TAAAAA

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Figure 1. Comparison of the cDNA sequences of sub2.3 and hnRNP X. The difference in the 3' UTR between sub2.3s and sub2.3f, as referred to in the text under RESULTS, is indicated with a solid underline. The poly A tail of sub2.3f is shown in the figure, while the poly A tail of sub2.3s starts from nucleotide number 1492.

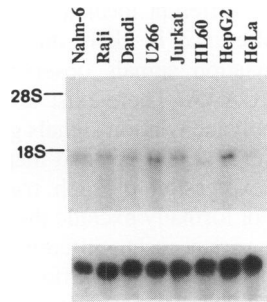


Figure 4. Expression of the sub2.3 transcript in different cell lines. Upper panel: hybridization with the sub2.3 cDNA, lower panel: hybridization with an 18S rRNA probe.

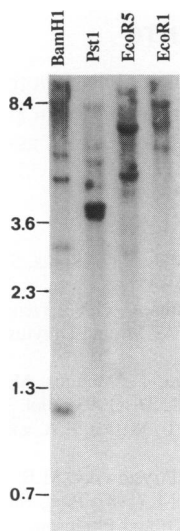


Figure 5. Southern blot analysis of genomic DNA isolated from Daudi cells. The DNA was cleaved with the indicated restriction enzymes as described in Material and Methods. Molecular weights in kb are indicated to the left in figure.

mRNA expression

The expression pattern of the sub2.3 gene in different human tissues was investigated using two commercial Northern blots obtained from Clontech (multiple tissue blots 1 and 2) and one non-commercial multiple tissue blot (multiple tissue blot nr 3). Tissue blot number 1 shows a high expression of sub2.3 in skeletal muscle, low expression in heart and almost no expression in brain, placenta, lung, liver, kidney and pancreas (Fig.3A). Results from the hybridization of tissue blot number 2 with sub2.3 demonstrates a high expression in two lymphoid tissues (thymus and peripheral blood leucocytes) and lower expression in spleen, prostate, testis, ovary, small intestine and colon (Fig.3B). In the third, non-commercial tissue blot, sub2.3 shows high expression in skeletal muscle, a lower expression in liver, spleen, adrenal gland, and thyroid gland, and almost no detectable expression in brain and transverse colon (Fig.3C). We have also hybridized the sub2.3 cDNA clone to a multiple cell line blot, containing RNA isolated from different cell lines (Fig.4). The results show that the gene is expressed in the B lymphoid cell lines Nalm6, Daudi, Raji, U266, in the T cell line Jurkat and in the hepatoma

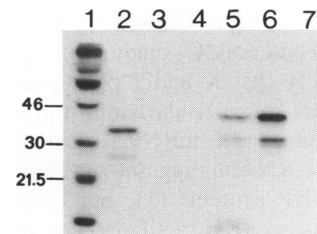


Figure 6. Nucleic acid binding assay. Lane 1: molecular weight standard. Lanes 2-4: β -lactamase after *in vitro* translation, after incubation with control oligonucleotide and after incubation with oligo(dC), respectively. Lane 5-7: sub2.3 protein after *in vitro* translation, after incubation and binding to oligo(dC) and after incubation with control oligonucleotide, respectively.

cell line HepG2, while a very low expression is observed in the myeloid cell line HL60 and the cervical carcinoma cell line HeLa. This blot has been reprobed with a oligonucleotide against 18S rRNA to compare the amounts of total RNA applied.

Southern blot analysis of genomic DNA

Southern blot analysis of genomic DNA isolated from the cell line Daudi shows the occurrence of several bands when cleaved with different restriction enzymes (Fig.5). The occurrence of only 2 bands after cleavage with EcoRV indicates that the gene exists in not more than two copies in the genome. We have also performed the same analysis on genomic DNA obtained from peripheral blood leucocytes from healthy individuals without any observed difference in the restriction pattern.

Nucleic acid-binding properties of the sub2.3 protein

Based on the partial homology to hnRNP K, which binds both to oligo(rC) and oligo(dC) (3), we wanted to investigate whether sub2.3 had the same nucleotide binding specificity. The sub2.3 gene product and a control protein, β -lactamase from *E.coli*, were synthesized *in vitro* as described in Material and Methods, and their binding to oligo(dC) was assayed using an approach adapted from the isolation of DNA binding transcription factors (18), based on the immobilization of oligonucleotides to a magnetic solid phase (magnetic streptavidin Dynabeads). *In vitro* translated sub2.3 and β -lactamase were incubated with a oligo(dC) 20-mer or a control oligonucleotide, both immobilized onto streptavidin Dynabeads, under conditions described for the binding of proteins (hnRNPs) to oligo(dC) affinity columns (16). The results obtained show that *in vitro* translated sub2.3 binds to oligo(dC), but not to the control oligonucleotide (Fig.6). The specificity of the experiment is confirmed by the observation that β -lactamase binds neither to oligo(dC) nor to the control oligonucleotide (Fig.6).

DISCUSSION

We present here the sequence of a cDNA (sub2.3) isolated from a human T cell cDNA library. sub2.3 shows strong homology to a mouse cDNA sequence proposed to encode a nucleic acid binding protein (hnRNP X; 11), and is partially homologous to human hnRNP K at the amino acid level. sub2.3 is most likely the human homolog to hnRNP X. The sub2.3 sequence has an open reading frame of 897 nucleotides initiated by a strong consensus initiation sequence predicting the production of a 299 amino acid long polypeptide. hnRNP K has been shown to bind

to both oligo(dC) and oligo(rC), and is together with hnRNP J the major oligo(C) and poly(C) binding protein in human HeLa cells (3). It is likely that K and J play a role in the nuclear metabolism of hnRNAs, particularly of pre-mRNAs that contain cytidine rich sequences (3). hnRNP K does not contain a RNP consensus sequence RNA binding domain, found in many of the characterized hnRNP proteins (1), but contains a conserved 45-amino-acid sequence (KH motif; 19) and gly-arg-gly-gly or gly-lys-gly-gly sequences (3). The KH motif overlaps a set of direct repeats described by Matunis *et al.* (3), and this motif has been suggested to be important for RNA binding (19). The sub2.3 sequence also contains a set of direct repeats of 20 amino acids with 85 % homology to one of the internal repeats in hnRNP K (3). The strong homology between these repeats can indicate that these sequences are important for the binding specificity. Like hnRNP K sub2.3 does not contain a consensus RNA binding domain found in most hnRNPs (1).

Because of the homology of sub2.3 with repeated domains in hnRNP K, suggested to interact with RNA (19), we wanted to test for oligo(dC) binding of the sub2.3 protein product. Using an approach where oligo(dC) was immobilized to a solid magnetic phase, we showed that the protein encoded by sub2.3 bound to oligo(dC) but not to a control oligonucleotide. A control protein, β -lactamase, did neither bind to the oligo(dC) nor to the control oligonucleotide. These experiments indicate that, like hnRNP K and J, the sub2.3 protein product has oligo(dC) binding specificity. It has recently been shown that hnRNP K can bind to the CT-element in the c-myc promoter and may have a role in the transcriptional regulation of this gene, leading to a potentially dual role for hnRNP K binding to both hnRNA and DNA (4). The nucleic acid binding specificity of the sub2.3 protein has to be evaluated further to investigate if sub2.3 belongs to a new class of hnRNA binding proteins and if it is involved in binding to DNA.

The expression pattern of the sub2.3 transcript varies considerably between different tissues. It is abundantly expressed in skeletal muscle, thymus and peripheral blood leucocytes while a lower expression is observed in prostate, spleen, testis, ovary, small intestine, heart, liver, adrenal and thyroid glands. In contrast, it is only faintly expressed or not detectable in brain, kidney lung, placenta and transverse colon. Sub2.3 is expressed in different lymphoid cell lines, in normal B and T lymphocytes (data not shown), in addition to the hepatoma cell line HepG2. Sub2.3 is expressed at a low level in the HL60 cell line and in HeLa cells. To our knowledge, no previous studies have demonstrated a differential tissue expression pattern of hnRNPs in humans. The hnRNP X transcript was found to be expressed in numerous tissues and cell types without further specification (11). In a study from tobacco plant tissue-specific alternative splicing is shown for two pre-mRNA binding proteins (20) and a study from *Drosophila melanogaster* demonstrates that the expression of the Hrb87f transcript, encoding a pre-mRNA binding protein related to hnRNP A and B, is regulated during embryogenesis, larval and pupae stage (21). The functional consequence of the differential expression pattern of sub2.3 has to be investigated further.

We have isolated two sub2.3 cDNAs, one near full-length cDNA clone (sub2.3f) corresponding to the size of the transcript observed on Northern analysis, and one shorter cDNA clone (sub2.3s), truncated in the 5'-untranslated region. Apart from being shorter at the 5' end, the sub2.3s cDNA clone also differs from sub2.3f in a stretch of 36 nucleotides at the 3' end. The

poly A tail shows a different localization in these two cDNA clones, and the difference is likely due to the usage of two different polyadenylation signals observed in the sequence, AAUAAA and AUUAAA. There exist several examples of the usage of different polyadenylation signals generating transcripts that differ in length (22,23,24,25) and there are also examples of tissue specific expression of such transcripts (21,22,23). However, we can not formally exclude the possibility that these two forms are splice variants using different exons. Whether there is any functional importance of this difference, or whether these two transcripts are differently regulated, is not addressed here.

In conclusion our data presents the identification of a novel human protein with oligo(dC) binding specificity. One very interesting feature of the sub2.3 gene is the tissue specific expression pattern which indicates a tissue-dependent function of this protein.

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