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Isolation, Characterization, and Chromosomal Mapping of a Novel cDNA Clone Encoding Human Selenium Binding Protein

Peter W.G. Chang,[1] Stephen K.W. Tsui,[1] Choong-chin Liew,[2] Cheuk-yu Lee,[1] Mary M.Y. Waye,[1] and Kwok-pui Fung[2]

[1]Department of Biochemistry, The Chinese University of Hong Kong, Shatin, Hong Kong
[2]Department of Clinical Biochemistry, University of Toronto, Toronto, Ontario, Canada

Abstract We have isolated the full-length human 56 kDa selenium binding protein (hSP56) cDNA clone, which is the human homolog of mouse 56 kDa selenium binding protein. The cDNA is 1,668 bp long and has an open reading frame encoding 472 amino acids. The calculated molecular weight is 52.25 kDa and the estimated isoelectric point is 6.13. Using Northern blot hybridization, we found that this 56 kDa selenium binding protein is expressed in mouse heart with an intermediate level between those found in liver/lung/kidney and intestine. We have also successfully expressed hSP56 in *Escherichia coli* using the expression vector-pAED4. The hSP56 gene is located at human chromosome 1q21-22. J. Cell. Biochem. 64:217–224. © 1997 Wiley-Liss, Inc.

Key words: selenium binding protein; human heart cDNA; fluorescent in situ hybridization; chromosome 1

Knowledge of the role of selenium in biology and human physiology is accumulating at a rapid rate [Robberecht and Deelstra, 1994]. Originally recognized for its highly toxic properties, selenium has changed from being perceived as a carcinogenic trace element to an essential nutrient exhibiting potent anti-carcinogenic properties [Medina and Oborn, 1981, 1984; Medina et al., 1985; Vernie, 1984]. The anti-carcinogenic role of selenium is also supported by human epidemiological evidence [Willett and Stampfer, 1986] and cohort studies [Knekt et al., 1990; Salonen et al., 1984; Virtamo et al., 1987]. Deficiency of selenium may cause certain neurological diseases [Emard et al., 1995] including schizophrenia [Berry, 1994]. In 1994, Lehr also demonstrated a beneficial effect of selenium substitution as an adjuvant to anti-arrhythmic therapy. It has been proposed that the effects of selenium in preventing cancer and neurological diseases might be mediated by selenium-binding proteins other than glutathione peroxidase [Medina and Morrison, 1988; Berry, 1994]. The presence of mammalian selenium binding proteins has been documented in numerous organs. These include selenoprotein P [Yang et al., 1987; Read et al., 1990; Hill et al., 1991, 1993], liver fatty acid binding protein [Bansal et al., 1989a], mouse 56 kDa selenium binding protein (mSP56) [Bansal et al., 1990], and 58 kDa selenium binding protein (SP58) [Sinha et al., 1993]. Recently, some experiments have demonstrated that mSP56 is closely related to another 56 kDa protein which was identified by its ability to bind metabolites of acetaminophen (AP56) [Pumford et al., 1992; Bartolone et al., 1992], suggesting that both 56 kDa proteins may be involved in detoxification processes. However, the SP56 and AP56 genes seem to be regulated independently [Lanfear et al., 1993]. Recently, we have cloned and charac-

Abbreviations used: a.a., amino acid; AP56, acetaminophen binding protein; BLAST, basic local alignment search tool; FISH, fluorescent in situ hybridization; hSP56, human 56 kDa selenium binding protein; IPTG, isopropylthio-β-D-galactosidase; mSP56, mouse 56 kDa selenium binding protein; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SP, selenium binding protein; SP56, 56 kDa selenium binding protein; SP58, 58 kDa selenium binding protein.

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*Correspondence to: Dr. Kwok-pui Fung, Department of Biochemistry, Basic Medical Sciences Building, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong.

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terized a human fetal heart cDNA encoding human SP56. In this study, we report the cloning, sequencing, tissue distribution, expression in *Escherichia coli,* and chromosomal mapping of this cDNA.

MATERIALS AND METHODS

DNA Sequencing of hSP56 cDNA Clone

A unidirectional human fetal heart cDNA library was constructed in the expression vector lambda gt22A using mRNA from 6–12-week-old human fetal hearts [Hwang et al., 1994]. Partial sequencing of cDNA clones isolated from the cDNA library was conducted as described [Liew, 1993; Liew et al., 1994; Hwang et al., 1995; Tsui et al., 1995]. Briefly, phage clones were used to transfect *E. coli* Y1090. Plaques were picked and placed in 50 µl of SM buffer to elute the phage. After 1 h at room temperature, 2 µl aliquots were subjected to polymerase chain reaction (PCR) in the presence of 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 9.0), 200 µM each dNTP, 2U Taq polymerase, and 250 nM each of primers flanking the *Not*I and *Sal*I sites of the λgt22A vector (Forward: 5′-ATTGGTGGCGACGACTCCTGGA-3′; Reverse: 5′-TTTGACACCAGACCAACTGGTA-3′) in a final volume of 50 µl. Samples were then subjected to 5 min denaturation at 94°C, followed by 35 cycles of amplification (95°C, 30 s; 50°C, 30 s; 72°C, 90 s) and a 5 min final extension at 72°C in a thermal cycler (Pharmacia, Piscataway, NJ). PCR products (2.5 µl aliquots) were sequenced directly using a cycle sequencing kit (Life Technologies, Bethesda, MD) in the presence of 160 nM of a fluorescein-conjugated primer nested within the forward PCR primer (Fluorescein-5′-GTTGGCGACGACTCCTGGAGCC-3′). Sequencing reactions were cycled for 25 times at 95°C, 30 s; 50°C, 30 s; 72°C, 90 s, and a 5 min final extension at 72°C. The sequencing products were run and analysed in an automated DNA sequencer (Pharmacia). Sequence comparisons against the GenBank and EMBL nucleotide and protein databases were performed using the BLAST electronic mail server [Altschul et al., 1990]. One of the cDNA clones exhibited DNA sequence similarity to that of the mouse 56 kDa selenium-binding protein (mSP56). The complete sequence of the cDNA was determined by primer walking strategy using dideoxy sequencing with T7 DNA polymerase. The putative protein encoded by this cDNA is named human 56 kDa selenium-binding protein (hSP56).

Cloning and Expression of hSP56

PCR of hSP56 cDNA clone was performed by using a primer specific for the 5′-end of hSP56 (5′-TAGGGC*CATATG*GCTACGAAATGTGGGAATTG-3′) and an oligo dT primer (5′-TAGGGC*GAATTC*TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3′). Both the 5′-primer and the oligo dT primer have an end clamp (TAGGGC) which facilitated cleavage by restriction enzymes [Tsui et al., 1994]. An *Nde*I site and an *Eco*RI site are present in the 5′-primer and the oligo dT primer, respectively. After digestion with *Nde*I and *Eco*RI, the PCR fragment was subcloned into the expression plasmid pAED4. The recombinant plasmid pAED4-hSP56 was transformed into *E. coli* BL21(DE3)pLysS strains. Induction of expression with 0.4 mM IPTG was performed essentially as described by Studier et al. [1990]. The bacterial extract was electrophoresed on 15% SDS-PAGE.

Northern Blot Hybridization

Since the homology between hSP56, mSP56, and AP56 is very high, the hSP56 probe was used to hybridize with total RNA of various mouse tissues. Total RNA was isolated from mouse organs using acid guanidium thiocyanate-phenol-chloroform extraction. The RNA was resolved in 1.5% agarose/2.2M formaldehyde gel and transferred onto nitrocellulose membranes. A radioactively labeled random primed probe was made by using the purified PCR product of hSP56 as the template. Blots were prehybridized for 6 h and hybridized at 42°C for 18–20 h. Membranes were then washed in 1 × SSC twice and washed again in 0.1 × SSC with 0.1% SDS at 42°C to remove nonspecific annealing. Autoradiography was performed at −70°C for 48–72 h.

Chromosomal Mapping of the hSP56

The chromosomal mapping of the hSP56 gene was performed by Dr. H.H.Q. Heng of SeeDNA Biotech Inc. (Ontario, Canada). The pAED4-hSP56 plasmid was biotinylated with dATP using the BRL (Gaithersburg, MD) BioNick labelling kit. The procedure for fluorescent in situ hybridization (FISH) detection was performed essentially as described by Heng et al. [1992] and Heng and Tsui [1993].

RESULTS

Cloning and Sequencing of the hSP56 cDNA

Among the 2,244 human fetal heart cDNA clones that we sequenced, 48.4% (1,085 clones) of them matched to and represented 452 different nuclear genes [Hwang et al., 1995]. Of these 452 distinct known genes, 95 represented human homologues of genes identified in other organisms or new members of gene families previously known in human. One of these human homologues has its DNA sequence resembling mSP56. It was named human 56 kDa selenium-binding protein (hSP56). After subcloning into the expression vector pAED4, the complete cDNA sequence of the hSP56 was determined by automated sequencing. The hSP56 cDNA revealed an open reading frame (ORF) of 1,419 bp encoding 472 amino acids (Fig. 1). The consensus translation initiation sequence RCCAUGG (R represent purine) was present at the start of the ORF. Comparison of the sequence of this cDNA clone with that of mSP56 revealed that the sequence contained the full translated region, a 5′ untranslated region of 15 bp, and a 3′ untranslated region of 234 bp. The calculated molecular weight is 52,250 daltons. The size of deduced protein is close to the molecular weight of 52,362 daltons for mSP56 [Bansal et al., 1990]. The estimated isoelectric point as determined by the software PROSIS is 6.13.

The DNA sequences of hSP56 has a similarity of 87.2 and 86.6% when aligned with those of mSP56 and AP56, respectively (data not shown). When the amino acid sequence of hSP56 was aligned with those of mSP56 and AP56, 87.3% identity, 98.7% similarity and 86.4% identity, 97.9% similarity, respectively, were found (Fig. 2). These results support the fact that the cDNA that we obtained is the human homologue of mouse selenium-binding protein. However, no similarity in DNA sequence can be found between hSP56 and rat selenoprotein P (data not shown).

Expression of hSP56 in *E. coli*

hSP56 cDNA was successfully amplified using a tailor-made cloning primer and an oligo dT primer. The success of the directional cloning was proven by restriction cutting of the putative recombinant plasmid, PCR and automated sequencing. After resolving the crude bacterial extract in 15% SDS-PAGE, it can be shown that the intense band at about 52 kDa is the protein coded by hSP56 cDNA and is absent when it was not induced by IPTG (Fig. 3). The mSP56 protein isolated and purified from mouse liver has a molecular weight of 56 kDa estimated in an SDS-PAGE gel [Bansal et al., 1989b]. The discrepancy between native protein and protein expressed in *E. coli* may be due to the post-translational modifications of the protein in mouse cell, such as phosphorylation or the attachment of carbohydrate linkage.

Northern Blot Hybridization

Since the homology between hSP56, mSP56, and AP56 is very high, the hSP56 probe was used to hybridize with total RNA of various mouse tissues (Fig. 4). However, this probe cannot be used to distinguish between mSP56 and AP56. It was shown that liver, lung, and kidney have the highest signal. A moderate signal could be seen in heart; detectable, but low, signals were found in intestine and spleen. Virtually no signal could be detected in skeletal muscle and brain. Previous results have shown that SP56 and AP56 are expressed at a high level in mouse liver, kidney, and lung, with a lower level in intestine and barely detectable amounts in brain, thymus, muscle, spleen, skin, mammary tissues, testis, and ovary [Lanfear et al., 1993; Morrison et al., 1989]. Such previous results agree well with ours. However, here we further show that the expression of SP56/AP56 in mouse heart is at an intermediate level between those found in liver/lung/kidney and intestine.

Chromosomal Mapping of hSP56 by FISH

Under the conditions used, the hybridization efficiency was approximately 70% for the probe we used. The hSP56 gene has been mapped to human chromosome by fluorescent in situ hybridization (FISH). The hSP56 gene is located at chromosome 1q21–22 (Fig. 5). No significant FISH signal was observed from other chromosomes.

DISCUSSION

This report describes the isolation and partial characterization of a cDNA encoding the hSP56 protein. To date, this is the first selenium binding protein reported in *Homo Sapiens*. Within the cDNA sequence, there is no in-frame TGA codon which represents the amino

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   1                    M   A   T   K   C   G   N   C   G   P   G      11
   1  TGT ACC AGT CGC AGC ATG GCT ACG AAA TGT GGG AAT TGT GGA CCC GGC      48

  12   Y   S   T   P   L   E   A   M   K   G   P   R   E   E   I   V      27
  49  TAC TCC ACC CCT CTG GAG GCC ATG AAA GGA CCC AGG GAA GAG ATC GTC      96

  28   Y   L   P   C   I   Y   R   N   T   G   T   E   A   P   D   Y      43
  97  TAC CTG CCC TGC ATT TAC CGA AAC ACA GGC ACT GAG GCC CCA GAT TAT     144

  44   L   A   T   V   D   V   D   P   K   S   P   Q   Y   C   Q   V      59
 145  CTG GCC ACT GTG GAT GTT GAC CCC AAG TCT CCC CAG TAT TGC CAG GTC     192

  60   I   H   R   L   P   M   P   N   L   K   D   E   L   H   H   S      75
 193  ATC CAC CGG CTG CCC ATG CCC AAC CTG AAG GAC GAG CTG CAT CAC TCA     240

  76   G   W   N   T   C   S   S   C   F   G   D   S   T   K   S   R      91
 241  GGA TGG AAC ACC TGC AGC AGC TGC TTC GGT GAT AGC ACC AAG TCG CGC     288

  92   N   K   L   V   L   P   S   L   I   S   S   R   I   Y   V   V     107
 289  AAC AAG CTG GTC CTG CCC AGT CTC ATC TCC TCT CGC ATC TAT GTG GTG     336

 108   D   V   G   S   E   P   G   P   Q   K   L   H   K   V   I   E     123
 337  GAC GTG GGC TCT GAG CCC GGG CCC CAA AAG CTG CAC AAG GTC ATT GAG     384

 124   P   K   D   I   H   A   K   C   E   L   A   C   L   H   T   S     139
 385  CCC AAG GAC ATC CAT GCC AAG TGC GAA CTG GCC TGT CTC CAC ACC AGC     432

 140   H   C   L   A   S   G   E   V   M   I   S   S   L   G   D   V     155
 433  CAC TGC CTG GCC AGC GGG GAA GTG ATG ATC AGC TCC CTG GGG GAC GTC     480

 156   K   G   N   G   K   G   G   F   V   L   L   D   G   E   T   F     171
 481  AAG GGC AAT GGC AAA GGG GGT TTT GTG CTG CTG GAT GGG GAG ACG TTC     528

 172   E   V   K   G   T   W   E   R   P   G   G   A   A   P   L   G     187
 529  GAG GTG AAG GGG ACA TGG GAG AGA CCT GGG GGT GCT GCA CCG TTG GGC     576

 188   Y   D   F   W   Y   Q   P   R   H   N   V   M   I   S   T   E     203
 577  TAT GAC TTC TGG TAC CAG CCT CGA CAC AAT GTC ATG ATC AGC ACT GAG     624

 204   W   A   A   P   N   V   L   R   D   G   F   N   P   A   D   V     219
 625  TGG GCA GCT CCC AAT GTC TTA CGA GAT GGC TTT AAC CCC GCT GAT GTG     672

 220   E   A   G   L   Y   G   S   H   L   Y   V   W   D   W   Q   R     235
 673  GAG GCT GGA CTG TAC GGG AGC CAC TTA TAT GTA TGG GAC TGG CAG CGC     720

 236   H   E   I   V   Q   T   L   S   L   K   D   G   L   I   P   L     251
 721  CAT GAG ATT GTG CAG ACC CTG TCT CTA AAA GAT GGG CTG ATA CCC TTG     768

 252   E   I   R   F   L   H   N   P   S   A   T   Q   G   F   V   G     267
 769  GAG ATC CGC TTC CTG CAC AAC CCA AGT GCC ACC CAG GGT TTT GTA GGC     816

 268   C   A   S   A   P   N   I   Q   R   F   Y   K   T   R   E   G     283
 817  TGT GCC TCA GCT CCA AAC ATC CAG CGC TTC TAC AAA ACG AGG GAA GGT     864

 284   T   W   S   V   E   K   V   I   Q   V   P   P   K   K   V   K     299
 865  ACA TGG TCA GTG GAG AAG GTG ATC CAG GTG CCC CCC AAG AAA GTG AAG     912

 300   G   W   L   L   P   G   V   P   G   L   I   T   D   I   L   L     315
 913  GGC TGG CTG CTG CCA GGG GTG CCA GGC CTG ATC ACC GAC ATC CTG CTC     960

 316   S   L   D   D   R   F   L   Y   F   S   N   W   L   H   G   D     331
 961  TCC CTG GAC GAC CGC TTC CTC TAC TTC AGC AAC TGG CTG CAT GGG GAC    1008

 332   L   R   Q   Y   D   I   S   D   P   Q   R   P   R   L   T   G     347
1009  CTG AGG CAG TAT GAC ATC TCT GAC CCA CAG AGA CCC CGC CTC ACA GGA    1056

 348   Q   L   F   L   G   G   S   I   V   K   G   G   P   V   Q   V     363
1057  CAG CTC TTC CTC GGA GGC AGC ATT GTT AAG GGA GGC CCT GTG CAA GTG    1104

 364   L   E   D   E   E   L   K   S   Q   P   E   P   L   V   V   K     379
1105  CTG GAG GAC GAG GAA CTA AAG TCC CAG CCA GAG CCC CTA GTG GTC AAG    1152

 380   G   K   R   V   A   G   G   P   Q   M   I   Q   L   S   L   D     395
1153  GGA AAA CGG GTG GCT GGA GGC CCT CAG ATG ATC CAG CTC AGC CTG GAT    1200

 396   G   K   R   L   Y   I   T   T   S   L   Y   S   A   W   D   K     411
1201  GGC AAG CGC CTC TAC ATC ACC ACG TCG CTG TAC AGT GCC TGG GAC AAG    1248

 412   Q   F   Y   P   D   L   I   R   E   G   S   V   M   L   Q   V     427
1249  CAG TTT TAC CCT GAT CTC ATC AGG GAA GGC TCT GTG ATG CTG CAG GTT    1296

 428   D   V   D   T   V   K   G   G   L   K   L   N   P   N   C   L     443
1297  GAT GTA GAC ACA GTA AAA GGA GGG CTG AAG TTG AAC CCC AAC TGC CTG    1344

 444   V   D   F   G   K   E   P   L   G   P   A   L   A   H   E   L     459
1345  GTG GAC TTC GGG AAG GAG CCC CTT GGC CCA GCC CTG GCT CAC GAG CTT    1392

 460   R   Y   P   G   G   D   C   S   S   D   I   W   I   *             473
1393  CGC TAC CCT GGG GGC GAT TGT AGC TCT GAC ATC TGG ATT TGA ACT CCA    1440

1441  CCC TCA TCA CCC ACA CTC CCT ATT TTG GGC CCT CAC TTC CTT GGG GAC    1488

1489  CTG GCT TCA TTC TGC TCT CTC TTG GCA CCC GAC CCT TGG CAG CAT GTA    1536

1537  CCA CAC AGC CAA GCT GAG ACT GTG GCA ATG TGT TGA GTC ATA TAC ATT    1584

1585  TAC TGA CCA CTG TTG CTT GTT GCT CAC TGT GCT GCT TTT CCA TGA GCT    1632

1633  CTT GGA GGC ACC AAG AAA TAA ACT CGT AAC CCT GTC                   1668
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Fig. 1. The cDNA and amino acid sequences of hSP56. The nucleotide sequence data has been submitted to the GenBank/EMBL Data Libraries under the accession number U29091. In the DNA sequence, the start codon (ATG), stop codon (TGA), and polyadenylation signal (AATAAA) are underlined.

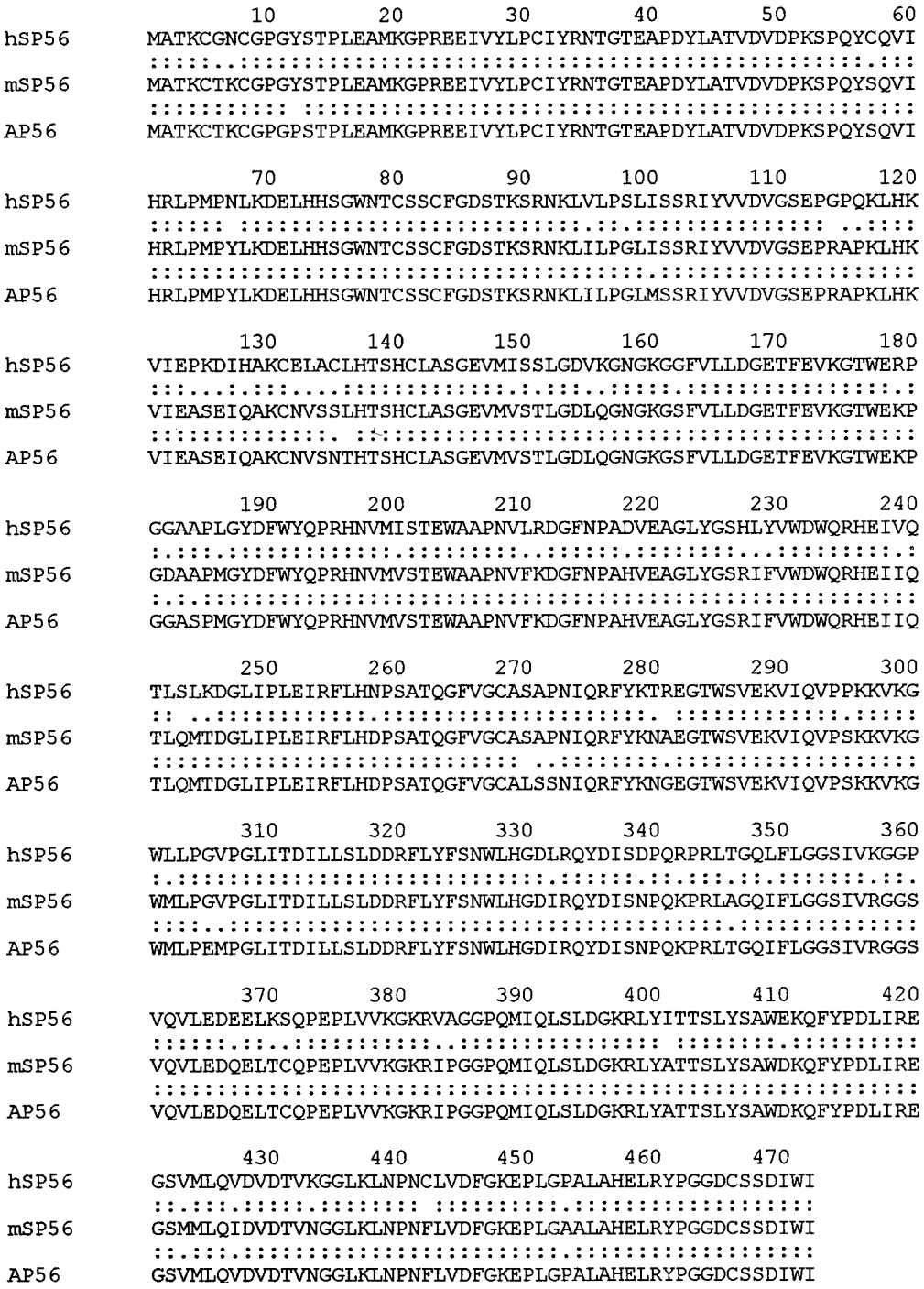

Fig. 2. Comparison of amino acid sequences of hSP56, mSP56, and AP56. Amino acids that are identical between the two sequences are marked by (:) while those that are similar are marked by (.).

acid selenocysteine [Chambers et al., 1986]. Therefore, hSP56 may interact with selenium in a manner different from the selenoproteins glutathione perioxidase and formate dehydrogenase. This result is in accordance to that of mouse SP56 [Bansal et al., 1990]. Concerning the amino acid sequence, the putative hSP56 protein is similar to both mouse SP56 and AP56, with a slightly higher similarity to the former. It seems that hSP56 protein is the human homolog of mouse SP56.

The results for the tissue distribution of the homolog of hSP56 in mouse tissues are very similar to that reported previously for mSP56

and AP56 whose expressions in liver, kidney, and lung are the highest [Lanfear et al., 1993]. However, we further reported that significant levels of SP56 and AP56 are present in heart. This finding may help to further elucidate the function of this protein.

At present, the physiological role of hSP56 is unknown. Evidence has been presented previously for a putative role of a 58 kDa selenoprotein in growth control in mammalian cells [Morrison et al., 1988]. When the mouse cDNA of SP56 was cloned and sequenced, it was also considered to be a growth regulatory protein [Bansal et al., 1990]. However, the fact that SP56 and AP56 cannot be detected in mammary cells or mammary cell lines [Lanfear et al., 1993] implies that neither SP56 nor AP56 is the 58 kDa selenoprotein detected in mammary cells. Based on the similarity of protein sequences, we believe that SP56 and AP56 are functionally similar. Various hypotheses have been proposed on the role of AP56 and SP56 in acetaminophen-induced hepatoxicity: AP56 and SP56 may have some important functions which are inhibited by acetaminophen-binding and this results in cell death; alternatively, AP56 may have a protective role as a scavenger of toxic electrophiles or oxidant species such as acetaminophen metabolites [Pumford et al., 1992; Lanfear et al., 1993]. Previous reports have suggested that acetaminophen detoxification may depend on the selenium status of the animal, since acetaminophen-induced hepatotoxicity and lipid peroxidation seem to be decreased by selenium administration [Schnell et al., 1988; Wendel and Feuerstein, 1981]. Most recently, Burk et al. [1995] reported that selenoprotein P, another selenium binding protein, mediates the protective effect of selenium supplement treatment on liver necrosis induced by free radicals in selenium deficient rat. We report here that hSP56 is present in mouse heart in significant amounts. It is of interest to test whether hSP56 can mediate the protective effect of selenium supplement treatment against free-radicals-induced necrosis of heart tissue in selenium deficient animals. Clinically, deficiency of selenium can cause heart failure [Yang et al., 1984] and the diseased condition can be alleviated by selenium supplementation. It will be of potential clinical significance to investigate the protective role of hSP56 throughout the selenium supplement treatment of the selenium deficient patients.

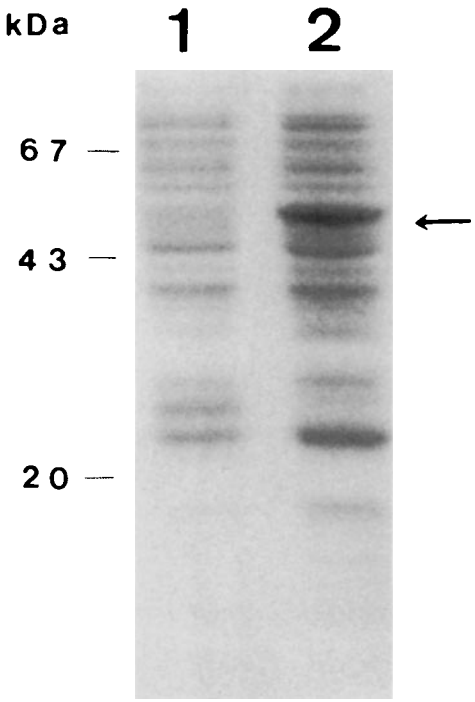

Fig. 3. Expression of hSP56 in *E. coli*. **Lane 1:** Uninduced recombinant bacterial extract. **Lane 2:** Induced recombinant bacterial extract.

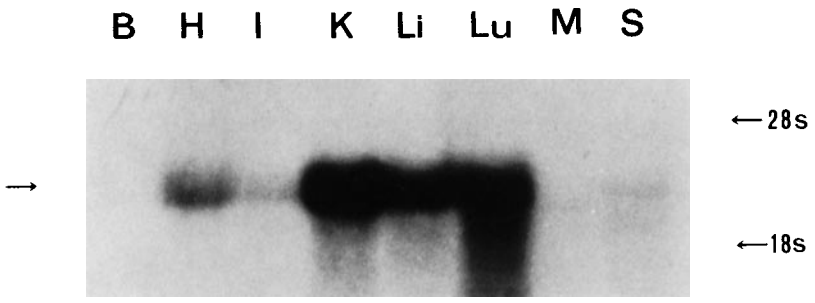

Fig. 4. Northern hybridization. Key for mouse RNA samples: B, brain; H, heart; I, intestine; K, kidney; Li, liver; Lu, lung; M, muscle; S, spleen.

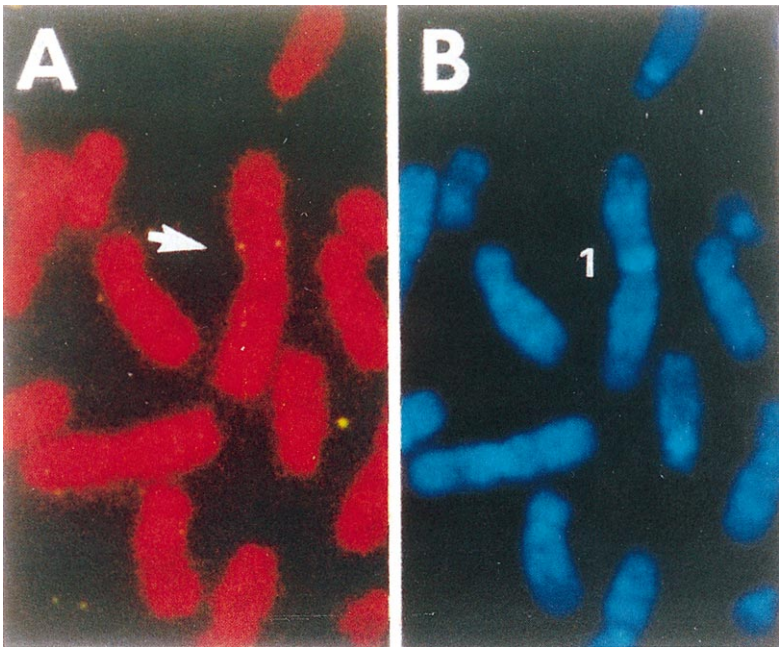

Fig. 5. FISH mapping. **A:** FISH signals on chromosomes one. **B:** The same mitotic figures stained with DAPI to identify chromosome 1.

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