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Identification of a Human cDNA Homologue to the *Drosophila* Translocation Protein 1 (Dtrp1)

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In yeast, several integral membrane proteins such as Sec61p, Sec62p and Sec63p have been reported as the components involved in protein translocation across and into the endoplasmic reticulum (ER) membrane. Among them, the homologues of Sec61p have been found both in bacterias and mammals, whereas those of Sec62p or Sec63p have not. So, Sec61p seem to be the evolutionary conserved component, while Sec62p and Sec63p may not. To date, no homologues of Sec62p have been found in mammals yet. Here, we report a novel human cDNA, HTP1 (for human translocation protein 1), that encodes a protein of 399 amino acids that is 36.3% identical (64.6% similar) to *Drosophila* homologue of Sec62p, *Drosophila* translocation protein 1 (Dtrp1). Northern blot analysis showed two HTP1 transcripts of about 2.8 and 5.5 kb, which were expressed concomitantly in various human tissues such as heart, brain, placenta, liver and pancreas.

In prokaryotes, secretory proteins are transferred directly across the plasma membrane (1,2). In eukaryotes, they are first translocated in an analogous process across the endoplasmic reticulum (ER) membrane, and then transported in vesicles to the plasma membrane (1,2). Similar pathways are also used by membrane proteins (1,2). This process is characterized by two steps, a targeting and a translocation steps. Two pathways, signal recognition particle (SRP) -dependent and -independent pathways, are involved in the targeting step. The SRP-dependent targeting pathway involves the SRP which only recognizes signal sequences of nascent protein bound to the ribosome, and is considered to be the principal mechanism of the ER membrane targeting in mammals (1,2). On the other hand, the SRP-independent pathway, where full-length completed protein are transported to ER membrane by cytosolic chaperones such as SecB in *Escherichia coli* and Hsp 70s in yeast and mammalian cells (3-5), is considered to be a salvage pathway in mammals (1,2).

Details of actual mechanisms of the translocation step are not yet clarified well, but protein transfer is thought to take place through a hydrophilic, proteinaceous 'pore' that is transiently assembled through the interaction of integral membrane proteins (1,2). Genetic analysis in *Saccharomyces cervisia* has identified three integral membrane proteins, Sec61p, Sec62p and Sec63p, that are required for secretory protein translocation (6-8). Sec61p, which is now known to compose Sec61 complex with SSS1p (a suppresser of sec61 mutant) in yeast, is thought to be the aqueous channel that mediates protein translocation (1,2). The homologues of yeast Sec61p and SSS1p are present in both bacterial and mammalian cells (8-11). In yeast, the proteins targeted to the ER membrane by the SRP-independent pathway may interact first with Sec62p, which forms complex with Sec63p within the ER membrane, and then be transferred to Sec61 complex in an ATP-dependent step (1,12,13). But, in mammalian cells, where no homologues of yeast Sec62p and Sec63p have been reported so far, it has been thought that those proteins might interact directly with the Sec61 complex (1). The failure to detect mammalian proteins with homology to either Sec62p or Sec63p had led to a supposition that these might be components of a distinct, perhaps species-specific, translocon (14). But, recently the homologues of Sec62p have been identified in Drosophila (Drosophila translocation protein 1 (Dtrp1)) and *Caenorhabditis elegans* (C18E9.2) (14,15). Here, we report the identification of a human cDNA, HTP1, that encodes a protein with a sequence homology to Sec62p and its homologues (especially to the Dtrp1).

MATERIALS AND METHODS

cDNA analysis. The sequencing strategy used in this study was summarized in Fig. 1. The human cDNA fragments, An-Hp2 and Hp5-

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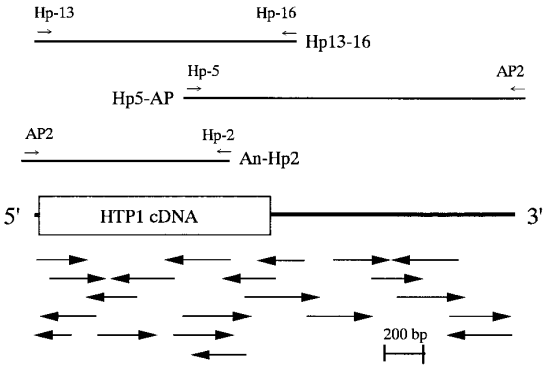

FIG. 1. Sequencing strategy for the HTP1 cDNA. The HTP1 cDNA map was shown in the middle. The open box represents coding region, while the solid bars represent non-coding regions. The lines above the cDNA map represent the three overlapping PCR amplified cDNA fragments, An-Hp2, Hp5-AP and Hp13-16. Arrows below the cDNA map indicate the direction and extent of nucleotide sequence obtained from various sequencing primers. The PCR fragment, Hp13-16, was also used as a probe for northern blot analysis.

AP, were obtained from the human liver Marathon-Ready cDNA library (Clontech) by the method of "rapid amplification of cDNA end (RACE)" (16) with the adapter primers (AP1 and AP2) supplied by the vender, and the gene specific primers, Hp-2 (5′-CTTTTCCCTTCCTTTCCC-CTCCTCAT-3′: the 15 nucleotides at the 3′ end correspond to the nucleotides number 985-971 of the reverse strand of the HTP1 cDNA (figure 2)) and Hp-5 (5′- TTTCTCATCATTTGGCTCATAACTGG-3′: correspond to the nucleotides number 757 - 782), respectively. The primer, Hp-2, was originally made to match the nucleotide sequence of the cDNA clone, HIP23-L1, which was obtained by screening the human liver λgt11 cDNA library (Clontech,) with the monoclonal antibody against the matrix protein (p17) of the human immunodeficiency virus type 1 (HIV-1)(Cellular Product Inc.) to identify the imunoreactive substances observed in normal human tissue from non-HIV-1-infected individuals (17). Unexpectedly, the human cDNA fragment, An-Hp2, obtained by low stringency PCR amplification with the adapter primers and the primer, Hp-2, did not contain any part of the original cDNA clone, HIP23-L1. This cDNA fragment appeared to be the 5′ part of the cDNA encoding the protein very similar to the Dtrp1. So, the 3′ part of this cDNA was then amplified with the adapter primers and another gene specific primer, Hp-5, which was made to match the nucleotide sequence of the cDNA fragment, An-Hp2. The cDNA fragment, Hp5-AP, thus obtained overlapped completely for 230 bp at the 5′ part with the 3′ part of the cDNA fragment, An-Hp2, indicating that these two cDNA fragments came from the 5′ and 3′ part of the same gene, respectively. The nucleotide sequences of the PCR fragments were determined by PCR-direct sequencing method using the cycle sequencing kit (Pharmacia LKB) as recommended by the vender. The primers at the ends of the cDNA fragments could be used to determine about 250 bp of the nucleotide sequence from them by the method. So, twenty sequencing primers matching to the nucleotide sequence of the inside part of the fragments were eventually made before the completion of the sequencing.

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1    GGAGCGGCCAACATGGCGGAACGCAGGAGACACAAGAAGCGGATCCAGGAAGTTGGTGAACCATCTAAAGAAGAGAAGGCTGTGGCCAAGTATCTTCGATTCAACTGTCCAACAAAGTCC
          M  A  E  R  R  R  H  K  K  R  I  Q  E  V  G  E  P  S  K  E  E  K  A  V  A  K  Y  L  R  F  N  C  P  T  K  S
          1                 10                 20                 30
121  ACCAATATGATGGGTCACCGGGTTGATTATTTTATTGCTTCAAAAGCAGTGGACTGTCTTTTGGATTCAAAGTGGGCAAAGGCCAAGAAAGGAGAGGAAGCTTTATTTACAACCAGGGAG
     T  N  M  M  G  H  R  V  D  Y  F  I  A  S  K  A  V  D  C  L  L  D  S  K  W  A  K  A  K  K  G  E  E  A  L  F  T  T  R  E
          40                 50                 60                 70
241  TCTGTGGTTGACTACTGCAACAGGCTTTTAAAGAAGCAGTTTTTTCACCGAGCCCTAAAAGTAATGAAAATGAAATATGATAAAGACATAAAGAAAGAAAAAGATAAAGGAAAAGCTGAA
     S  V  V  D  Y  C  N  R  L  L  K  K  Q  F  F  H  R  A  L  K  V  M  K  M  K  Y  D  K  D  I  K  K  E  K  D  K  G  K  A  E
          80                 90                 100                110
361  AGTGGAAAAGAAGAAGATAAAAAGAGCAAGAAAGAAAATATAAAGGATGAGAAGACAAAAAAGAAAAAGAGAAAAAAAAAGATGGTGAAAAGGAAGAATCCAAAAAGGAGGAAACTCCA
     S  G  K  E  E  D  K  K  S  K  K  E  N  I  K  D  E  K  T  K  K  E  K  E  K  K  K  D  G  E  K  E  E  S  K  K  E  E  T  P
          120                130                140                150
481  GGAACTCCTAAAAAGAAGGAAACTAAGAAAAAATTCAAACTTGAGCCACATGATGATCAGGTTTTTCTGGATGGAAATGAGGTGTATGTATGGATCTATGACCCAGTTCACTTTAAAACA
     G  T  P  K  K  K  E  T  K  K  K  F  K  L  E  P  H  D  D  Q  V  F  L  D  G  N  E  V  Y  Y  W  I  Y  D  P  V  H  F  K  T
          160                170                180                190
601  TTTGTCATGGGATTAATTCTTGTGATTGCAGTAATAGCGGCCACCCTCTTCCCCCTTGGCCAGCAGAAATGAGAGTAGGTGTTTATTACCTCAGTGTGGGTGCAGGCTGTTTTGTAGCC
     F  V  M  G  L  I  L  V  I  A  V  I  A  A  T  L  F  P  L  W  P  A  E  M  R  V  G  V  Y  Y  L  S  V  G  A  G  C  F  V  A
          200                210                220                230
721  AGTATTCTTCTCCTTGCTGTTGCTCGATGCATTCTATTTCTCATCATTTGGCTCATAACTGGAGGAAGGCACCACTTTTGGTTCTTGCCAAATCTGACTGCTGATGTGGGCTTCATTGAC
     S  I  L  L  L  A  V  A  R  C  I  L  F  L  I  I  W  L  I  T  G  G  R  H  H  F  W  F  L  P  N  L  T  A  D  V  G  F  I  D
          240                250                260                270
841  TCCTTCAGGCCTCTGTACACACATGAATACAAAGGACCAAAAGCAGACTTAAAGAAAGATGAGAAGTCTGAAACCAAAAAGCAACAGAAGTCCGACAGTGAGGAAAAGTCAGACAGTGAG
     S  F  R  P  L  Y  T  H  E  Y  K  G  P  K  A  D  L  K  K  D  E  K  S  E  T  K  K  Q  Q  K  S  D  S  E  E  K  S  D  S  E
          280                290                300                310
961  AAAAAGGAAGATGAGGAGGGGAAAGTAGGACCAGGAAATCATGGAACAGAAGGCTCGGGGGGAGAACGGCATTCAGACACGGACAGTGACAGGAGGGAAGATGATCGATCCCAGCACAGT
     K  K  E  D  E  E  G  K  V  G  P  G  N  H  G  T  E  G  S  G  G  E  R  H  S  D  T  D  S  D  R  R  E  D  D  R  S  Q  H  S
          320                330                340                350
1081 AGTGGAAATGGAAATGATTTTGAAATGATAACAAAAGAGGAACTGGAACAGCAAACAGATGGGGATTGTGAAGAGGATGAGGAAGAGGAAAATGATGGAGAAACACCTAAATCTTCACAT
     S  G  N  G  N  D  F  E  M  I  T  K  E  E  L  E  Q  Q  T  D  G  D  C  E  E  D  E  E  E  E  N  D  G  E  T  P  K  S  S  H
          360                370                380                390
1201 GAAAAATCATAATCTGACTAATTTTGGGACTGAATGAATAAGTACAAGAGGTTGGATTTTCTATGTTGGCTGATTACCATATTGAACACATGGCATTTGTAGCATTCTTTAAATCTATCT
     E  K  S  *
          399
1321 ACTGAGATGTATTTGACATTCAAGCAGTTATATTCGGTCCTTCATTTTATAGAATATTGGCACTATTATTGGTACAGTTTAAAGCCATTAATATGTTTTATCCATTTGATAATTTTACAG
1441 TAAGTAGGTCTCATTCATTTTGACAGTTATCAAAGATGTACTTTCCACAGTTAAATTTACATTAATGGCAATTTTTGATAGTTTTATGGCTTTTTACTGTTAGACTAATCAAAAATAACT
1561 TTAAAAGGAACAAAGAAACTCCAACATTTCACATTATGCATAGTTATGTAGCCATTTCACAGTTTCTTTAAGATGTGTAAACTCATTGTCCTTGATAGTTTTTATTTTTCATTATAAAAT
1681 TATACCAGGAGATTTCTTTTAAGATTCTGAGTTAGCAGAGTTCAAAACTATTTTGTCGAAACAAGCCAACTAGTAACAATGCAGCAACACTTCTGGTTTAGCTAAATTATTTTTCCAATG
1801 TAGGAAATCCACACTGATTTGTACGTCTGACTGAGAGAAAGATGGTCGTCTCCAGCAGAGAAAGTGAACAGCATTTGTTGGAAGGTGATGGCTCTCCCTCCTCCCTCCCCATTTCATTGG
1921 CGTAACGTAAAGTGTATTCTGTACATAATTTACAAATAAAACATTTTATTTTAATTGTTACTTATTATTTAGATATTTCTCAACACTTAAATTCATAAAATTAAGACCATGTAAGGGTAT
2041 GTTTTTAGAGAAATGGAAGTTTGAGTAACCCACAGAACATCTGTGATCTTTCTACAGCAGCTTCAGTTTTCTGTCCAACATTCCATGTATTTTGAATATGAGCAAAAACTGATCTTAAGAG
2161 CAGACTTAAAGTAGCTTTGTACGCCTTAATGTTCATTTTGATTTATTTTAAATCTTTACATTCAGAAATGAGATACTGTATTATCAGACCAGGAGGCATTGCTGTGAAAGATAATTTCCT
2281 ATTCTAAAATATCAAATTTAAAATAAAGATAATGAAAGAAAACATAAGAGAACTATTGGACTCTAATTGCTTTGAACTAGTTTTGCAGTTTGACTTTACGTCTTATACATCTTAGTTACA
2401 AGTCTTTGGAAACTCTTGTTTACTTATGAGCATAATCATTCCTTGGAGTTATACTAACTAATAATGAATATTAAATGATTTTTCTTCAGTC
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FIG. 2. Nucleotide sequence of the 2.5 kb HTP1 cDNA and the predicted amino acid sequence of the corresponding protein. Number in the left-hand column refer to the nucleotide number, with the first nucleotide shown designated as 1. Two potential transmembrane domains, amino acids 196-216 and 225-256, are shown double-underlined. The 399 amino acid begins with the ATG at nucleotide position 13 and terminated with a stop codon at nucleotide position 1210.

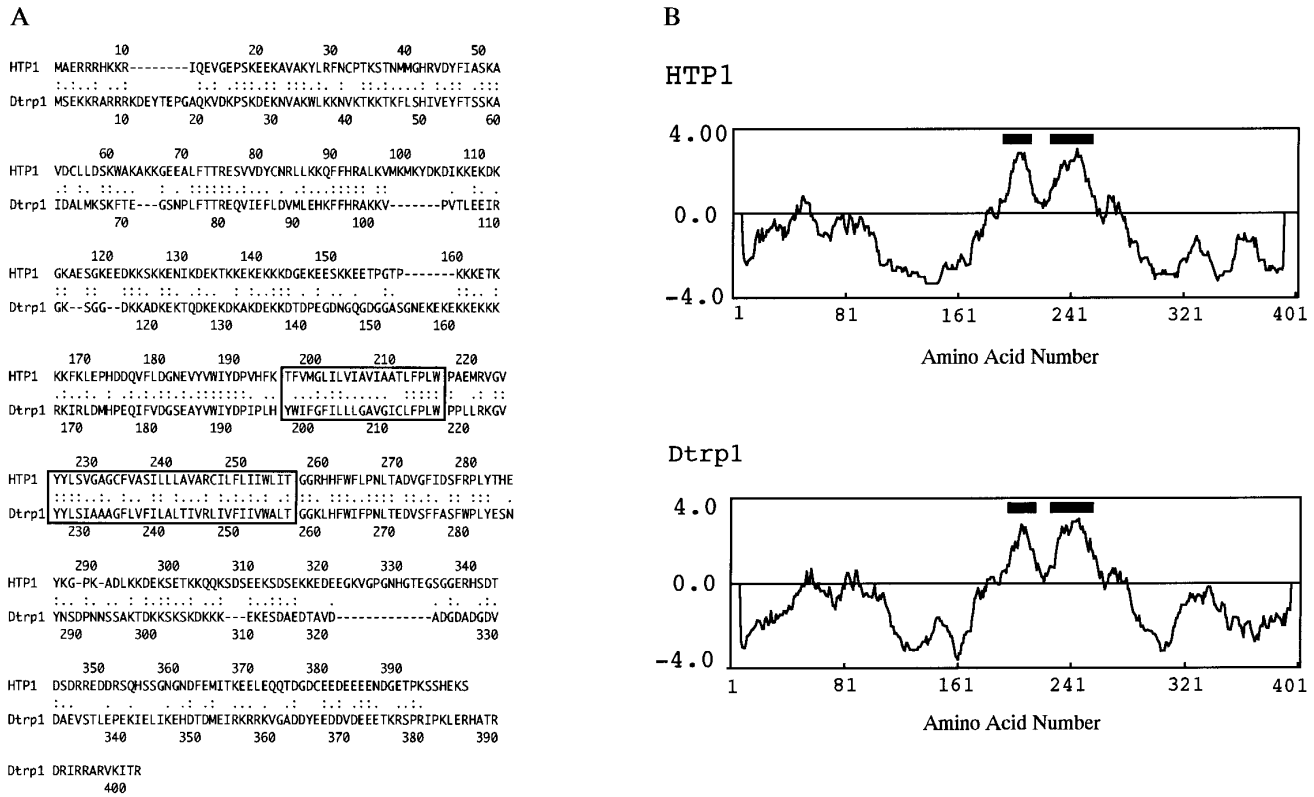
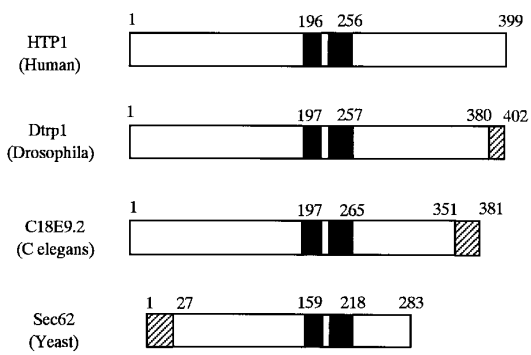


FIG. 3. Amino acid similarity of Human HTP1 to Drosophila Dtrp1. Amino acid sequence and hydrophobicity plot of HTP1 was compared to those of Dtrp1 in panels A and B, respectively. The amino acid residue number is indicated at both ends of the HTP1 and the Dtrp1 sequences. Identical and conservative residues are indicated by a colon and period, respectively. The reported transmembrane domains of Dtrp1 and putative transmembrane domains of HTP1 are boxed in panel A and indicated by solid bar in panel B, respectively. The hydropathy profile was obtained according to the method of Kyte and Doolittle using window of 15 amino acids.

Northern blot analysis. "Human Multiple Tissue Northern (MTN) Blot" containing approximately 2 μg of poly A^+ RNA per lane from eight different human tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas) was obtained from Clontech. The cDNA fragment, Hp13-16, was obtained by PCR amplification of the human liver Marathon-Ready cDNA library using the primers, Hp-13 (5′- ACATGGCGGAACGCAGGAGACACAAG-3′: correspond to the nucleotides number 11 - 36 in figure 2) and Hp-16 (5′- GAAGGACCGAATATAACTGCTTGAAT-3′: correspond to the nucleotides number 1363 - 1348 of the reverse strand), and radiolabeled at the same time by adding [α-^{32}P]dCTP in the PCR reaction mixture. This cDNA fragment, which contains the entire coding region of the protein, was then used as the probe for the northern blot analysis, while β-actin cDNA was radiolabeled by extension of



Identity (Id) and Similarity (Sim) of HTP1

	Transmembrane(%)		Overlap (%)	
	Id	Sim	Id	Sim
Dtrp1	44.3	83.6	36.3	64.6
C18E9.2	41.4	70.0	38.4	67.0
Sec62	31.1	72.1	28.0	59.1

FIG. 4. Similarity of HTP1 amino acid sequence to other homologous proteins. The HTP1 protein and its homologues, Dtrp1, C18E9.2 and Sec 62p, were schematically aligned at left side. The regions with or without homology to the HTP1 protein are shown in open or shaded boxes, respectively. The putative and reported transmembrane domains in the homologous regions are shown in black box. The amino acid number of the start and end points of the transmembrane domains and the homologous region are shown over the boxes. Identities and similarities of the HTP1 amino acid sequence to its homologues were summarized in table at right side.

random hexamers using [α-^{32}P] dCTP and used as the control probe. High stringency hybridization was performed at 68 °C using "Quik-Hyb Hybridization Solution (Stratagene)". The northern blot was then washed twice for 15 min. at room temperature with a 2 × SSC buffer and 0.1 % SDS wash solution and once for 30 min. at 60 °C with a 0.1 × SSC buffer and 0.1 % SDS wash solution.

RESULTS AND DISCUSSION

The nucleotide sequence of the HTP1 cDNA (DDBJ/EMBL/Gen-Bank data base accession number; D87127) is shown in Figure 2. The open reading frame (ORF) begins at position 13 and ends at position 1210, followed by a long (>1300 bp) 3′ untranslated region. The ORF predicts a protein of 399 amino acids. The sequence, AAC, immediately preceding the putative initiator methionine matches to the consensus sequence, A/GNC, for the vertebrate translation initiation site [18]. Hydropaty analysis (Kyte and Doolittle analysis (19)) reveals two centrally located stretches of hydrophobic amino acids long enough to span a lipid bilayer (Figure 3B). These potential transmenbrane domains located at amino acid positions 196-216 and 225-256, respectively. The predicted protein lacks the N-terminal stretch of hydrophobic residues characteristic of signal peptide.

Although the HTP1 cDNA was obtained during a low stringency PCR amplification of the cDNA ends using the primers specific to the cDNA clone, HIP23-L1, which is a candidate encoding a protein immunoreactive with the antibody against p17 of the HIV-1 (see Methods), there was no significant sequence similarity between these cDNAs. However, a search of the protein sequence database with the FASTAP algorithm revealed sequence similarity between the Drosophila translocation protein, Dtrp1 and the deduced product of the HTP1 gene (Figure 3A)(14). There is 36.3% identity and 64.6% overall amino acid similarity across the 410 amino acid overlap region. Beside this sequence similarity, the HTP1 and Dtrp1 proteins share other general structural characteristics. For example, hydropathy analysis revealed the very similar pattern between these proteins (Figure 3B). The membrane-spanning domains of the two proteins, as well as the hinge region, are very similar in size. Neither of them contains a signal sequence. Furthermore, the HTP1 protein showed the sequence and general structural similarities to the other homologous proteins of Dtrp1 such as the yeast Sec62p and the *C. elegans* Dtrp1-like protein C18E9.2 (Figure 4)(15). The identities and similarities between the HTP1 protein and the proteins C18E9.2, and Sec62p are 41.4 and 70.0, and 31.1 and 72.1 %, respectively (Figure 4). Thus, these results strongly indicates that the HTP1 gene is a human homologue to the Dtrp1 gene.

Northern blot analysis using a fragment of the HTP1 cDNA as a probe showed that two transcripts of about 2.8 and 5.5 kb were expressed concomitantly in human

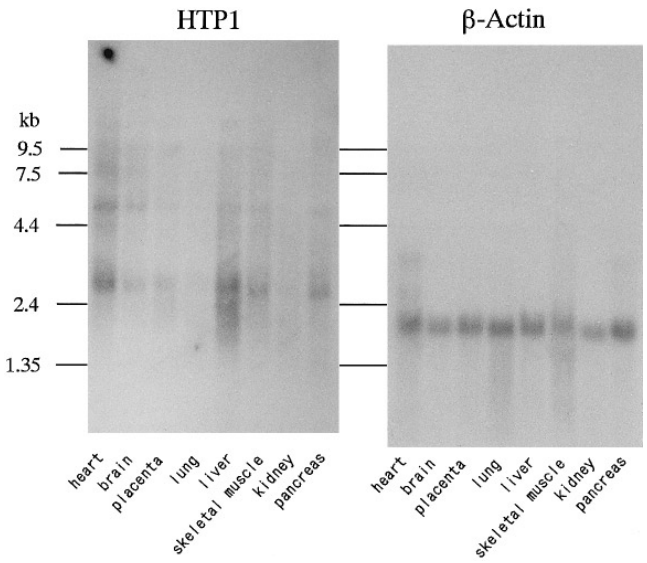


FIG. 5. Expression of HTP1 mRNA in human tissues. Human multiple tissue northern blot were hybridized with a radiolabeled probe made from the HTP1 cDNA fragment (left panel). Right panel is the result of the same membrane hybridized with a human β-actin probe. The numbers on the left of each panel indicate RNA size markers.

heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas (Figure 5). This result indicates that HTP1 is expressed in variety of human tissues. The 2.8 kb transcript appeared to be expressed more than the 5.5 kb transcript in every tissues examined. The expression levels of the transcripts in lung and kidney were very low, while those in heart and liver seemed to be higher than those in the others. Also known is that its homologue, Dtrp1 gene, encodes two transcripts (transcripts of about 1.6 and 2.2.kb)(14). These results indicates that HTP1 is expressed in various human tissues and encode two transcripts at least in these tissues examined, although the expression levels vary among the tissues. The mechanisms involved in the expression of the two HTP1 transcripts may be similar to that used in the case of the Dtrp1 gene, although further experiments are needed to elucidate the mechanisms.

The identification of this human gene indicates that the homologue to Dtrp1/Sec62p is present also in mammals, and so that the protein transport mechanisms using Sec62p or its homologue are also evolutionary conserved as in the case of Sec61p. These results will provide a useful tool for studying the mechanisms involved in the translocation of the proteins in mammals.

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REFERENCES

1. High, S., and Stirling, C. J. (1993) *Trends Cell Biology* **3,** 335–339.
2. Jungnickel, B., Rapoport, T. A., and Hartmann, E. (1994) *FEBS Lett.* **346,** 73–77.
3. Johnson, K., Murphy, C. K., and Beckwith, J. (1992) *Curr. Opin. Biotech.* **3,** 481–485.
4. Deshaies, R. J., Koch, B. D., and Schekman, R. (1988) *Trends Biochem. Sci.* **13,** 384–388.
5. Wiech, H., Stuart. R., and Zimmermann, R. (1990) *Semin. Cell Biol.* **1,** 55–63.
6. Deshaies, R. J., and Schekman, R. (1987) *J. Cell Biol.* **105,** 633–645.
7. Rothblatt, J. A., Deshaies, R. J., Sanders, S. L., Daum, G., and Schekman, R. (1989) *J. Cell Biol.* **109,** 2641–2652.
8. Stirling, C. J., Rothblatt, J., Hosobuchi, M., Deshaies, R., and Schekman, R. (1992) *Mol. Biol. Cell.* **3,** 129–142.
9. Ito, K., Witterkind, M., Nomura, M., Shiba, K., Yura, T., Miura, A., and Nashimoto, H. (1983) *Cell* **32,** 789–797.
10. Gorlich, D., Prehn, S., Hartmann, E., Kalies, K. U., and Rapoport, T. A. (1992) *Cell* **71,** 489–503.
11. Hartmann, E., Sommer, T., Prehn, S., Gorlich, D., Jentsch, S., and Rapoport, T. A. (1994) *Nature* **367,** 654–657.
12. Musch, A., Wiedmann, M., and Rapoport, T. A. (1992) *Cell* **69,** 343–352.
13. Deshaies, R. J., Sanders, S. L., Feldheim, D. A., and Schekman, R. (1991) *Nature* **349,** 806–808.
14. Noel, P. J., and Cartwright, I. L. (1994) *EMBO J.* **13,** 5253–5261.
15. Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Berks, M., Bonfield, J., Burton, J., Connell, M., Copsey, T., Cooper, J., Coulson, A., Craxton, M., Dear, S., Du, Z., Durbin, R., Favello, A., Fulton, L., Gardner, A., Green, P., Hawkins, T., Hillier, L., Jier, M., Johnston, L., Jones, M., Kershaw, J., Kirsten, J., Laister, N., Latreille, P., Lightning, J., Lloyd, C., McMurray, A., Mortimore, B., O'Callaghan, M., Parsons, J., Percy, C., Rifken, L., Roopra, A., Saunders, D., Shownkeen, R., Smaldon, N., Smith, A., Sonnhammer, E., Staden, R., Sulston, J., Thierry-Mieg, J., Thomas, K., Vaudin, M., Vaughan, K., Waterston, R., Watson, A., Weinstock, L., Wilkinson-Sproat, J., and Wohldman, P. (1994) *Nature* **368,** 32–38.
16. Chenchik, A. Moqadam, F., and Siebert, P. (1995) *CLONTECHniques* **X,** 5–8.
17. Kato, T., Kurita, K., Kawanami, T., and Sasaki, H. (1994) *Neurosci. Lett.* **181,** 134–136.
18. Cavener, D. R., and Ray, S. C. (1991) *Nucleic Acids Res.* **19,** 3185–3192.
19. Kyte, J., and Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132.