Cloning and Characterization of Human Sep1 (hSEPl) Gene and Cytoplasmic Localization of its Product

Yoshiki SATO, 1,2,‡ Akira SHIMAMOTO, 1 Takeo SHOBUIKE, 3 Masanobu SUGIMOTO, 1 Hideo IKEDA, 3 Shigetoshi KURODA, 2 and Yasuhiro FURUICHI1,*

A GENE Research Institute, 200 Kajiwara, Kamakura, Kanagawa, 247-0063, 1 Department of Neuropsychiatry, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700-0914, 2 and Department of Molecular Biology, Institute of Medical Science, University of Tokyo, P. O. Takanawa, Tokyo 108-0074, Japan 3

(Received 21 July 1998; revised 31 July 1998)

Abstract

We isolated and sequenced a human cDNA (designated as hSEPl) encoding both a homologue of mouse Dhm2 and budding yeast SEPl. The gene was shown to be located on the long arm of chromosome 3 (3q25-26.1). The putative hSEPl product (hSEPlp) consisted of 1694 amino acid residues with a molecular mass of about 190 kDa. Northern blot analysis showed a major 10-kb mRNA expressed ubiquitously in various organs as well as a minor 5.5-kb mRNA expressed relatively highly in the testis and placenta. hSEPlp is localized in the cytoplasm as examined by cytochemical and Western blot analyses of fractionated cellular extracts. The biological function of hSEPlp was discussed in correlation with its cytoplasmic localization.

Key words: hSEPl; Dhm2; SEPl; human cell

The SEPl gene was originally isolated from Saccharomyces cerevisiae as a gene coding a strand exchange protein.1,2 However, the SEPl gene/product has been identified by four other unrelated methods, and have been named DST2/Stp3,2 XRN1,3 KEM1,4 and RAR5,5 respectively. In this paper, the gene will be designated SEPl and the protein Sep1p for simplicity. Biochemical and genetic analyses have implicated the roles of Sep1p in various cellular processes other than DNA recombination, such as RNA metabolism, microtubule association, nuclear fusion, meiosis, plasmid DNA replication, telomere function and cellular senescence.1,2,4,10 These multiple roles of Sep1p are difficult to reconcile with a simple model of Sep1p function. Heyer et al.11 reported that Sep1p was mainly localized in the cytoplasm in Sac. cerevisiae. We sequenced and cloned a mouse cDNA homologue to hSEPl designated as Dhm2.12 Bashkirov et al.13 reported that the mouse homologue designated as mXrnlp exists in the cytoplasm and that it has exoribonuclease activity with a preference for G4 tetraplex substrates. In this study, we cloned and sequenced a human cDNA designated as hSEPl corresponding to mouse Dhm2/mXRN1 cDNA and yeast SEPl gene to study its biochemical and biological characteristics.

1. Materials and Methods

1.1. Determination of DNA sequences

A DNA fragment inserted into the pGEM-T vector was amplified using polymerase chain reaction (PCR) using primers set up in the vector. All DNA sequences were determined by PCR-based cycle sequencing using a Prism Sequencing Kit (Perkin Elmer Applied Biosystems Inc., CA) with an automated DNA sequencer (Perkin Elmer Applied Biosystems, Inc.). The sequences were analyzed for homology or identified with known sequences in public databases.

1.2. Western blotting

An 80% confluent culture of 293-EBNA cells was made in a 100-mm plastic dish using Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% fetal calf serum. The cells were transfected with 5 µg of pcDNA3 vector containing hSEPl cDNA with a Flag sequence at the 5’ region together with 80 µl of Lipofectamin (Gibco BRL). hSEPl cDNA was prepared as described in the “Results and Discussion” section. After 5 hr of transfection, the medium was exchanged with fresh medium. The cells were harvested using a scraper at 48 hr after transfection, and were fractionated into cytoplasmic and nuclear fractions as follows. Cells were lysed...
Cloning of Human Sep1 Gene

1.3. Cytochemistry

The hSEPl cDNA prepared as mentioned in the “Results and Discussion” section was inserted into a pEGFP-C3 vector (Clontech) to express the hSEPlp protein tagged with enhanced green fluorescent protein (EGFP). HeLa cells were cultured using DMEM with 10% fetal calf serum. At about 80% confluence, the cells were transfected with 5 μg/ml of mouse monoclonal IgG antibody (M2) against Flag epitope (Eastman Kodak), followed by 1/2000 diluted horseradish peroxidase-conjugated rabbit antibody against mouse IgG (DAKO). We used an ECL system for the detection (Amersham).

1.4. Northern blot analysis

Northern blot analysis was made for hSEPl RNAs in different human tissues using a series of human multiple tissue Northern (MTN) blots I, II and human cancer cell line MTN blot (Clontech, Palo Alto, CA). Each lane contained 2 μg of poly(A)-containing RNAs obtained from adult human, and the 5′ region of cDNA of hSEPl (1083 bp) was used as a probe.

1.5. Determination of chromosomal location of hSEPl gene

The location of the hSEPl gene on human chromosome was determined by performing PCRs on the 93 cell lines of the GeneBridge 4 Radiation Hybrid Panel (Research Genetic). The PCR was made by using primers 3′/04/S (5′-ATGATGCTGGATTCTCTTTGC-3′) and 3′/04/AS (5′-GCTCCATATAAAAAATTGGTTTCC-3′) corresponding to the 3′ noncoding region of the gene. The thermal cycle conditions were: (1) initial denaturation at 94 °C for 5 min; (2) 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec, and extension at 72 °C for 30 sec; and (3) a final extension at 72 °C for 5 min. Each reaction mixture contained 25 ng of template genomic DNA in a 12.5-μl reaction buffer. Another set of primers dNA-R (5′-AGCCTGAGTCTAAATTCC-3′) and dN1E (5′-AGAAAGGAAAATCAGTTGCC-3′) were used in the same condition. The hSEPl gene locus in the STS-based map of the human genome was determined from the results of these PCRs using the RHMAPPER program through the World Wide Web server of the Whitehead Institute (http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl).

2. Results and Discussion

2.1. Cloning and sequencing of hSEPl

The cloning of human cDNA homologous to Sac. cerevisiae SEP1 was carried out using the modified method by Shobuike et al. 12,18 Briefly, we first screened the Agt10 cDNA library obtained from human peripheral blood leukocytes (Clontech) by PCR using an oligonucleotide primer corresponding to a conserved amino acid sequence between Sac. cerevisiae Sep1p and Schizosaccharomyces pombe Dhp1 protein (Dhp1p). We obtained a 273-bp fragment highly homologous to mouse Dhmb2 cDNA, which was used as the probe to further screen the cDNA library by plaque hybridization. We obtained a 1083-bp cDNA fragment corresponding to the 5′ region of the mouse Dhmb2 gene. Four ESTs, T66946, H68969, H70945 and T5764, corresponding to the 3′ region of the mouse Dhmb2 gene were also obtained from the public databases. We carried out a PCR using primers set up on these DNA fragments and the cDNA library as a template, and obtained a DNA fragment covering most of the open reading frame (ORF). The parts of the 3′ region including 3′UTR were cloned by the RACE method, and we finally determined the sequence of the whole ORF. The ATG in the sequence CAAAATGGGA is a favorable initiation codon proposed by Kozak. 14 Figure 1 shows the deduced amino acid sequences of hSEPlp and mouse Dhmb2p (mDhmb2p). The predicted hSEPlp consisted of 1694 amino acid residues with a molecular mass of 190 kDa. The top of the sequence showed schemati-
cally the homology between hSEP1p and mDhm2p. The two proteins hSEP1p (1-1313) and mDhm2p (1-1310) were 92% homologous each other in the N-terminal parts. However, the remaining C-terminal portions were less homologous (74%).

2.2. Homology with Sac. cerevisiae Sep1p and Sch. pombe ExoIIP

Figure 2 shows the homology in the amino acid sequence between hSEP1p and yeast homologue proteins, Sac. cerevisiae Sep1p and Sch. pombe ExoIIP. Two significantly conserved regions were found between hSEP1p and Sac. cerevisiae Sep1p: the amino acid sequence between 1 and 392 of hSEP1p showed a 52% homology with the corresponding region of Sac. cerevisiae Sep1p, and the sequence of hSEP1p between 414 and 1174 showed 26% homology with the corresponding region of Sac. cerevisiae Sep1p. Similarly, the two regions of hSEP1p showed homology to the corresponding regions of Sac. pombe ExoIIP. The remaining regions showed no homology.
2.3. Cellular localization of hSEPlp

A human cell line, 293-EBNA cell line (Invitrogen), was transfected with plasmid DNA containing the hSEPlp cDNA with the sequence coding for an N-terminal Flag peptide with an amino acid sequence DYKDDDDK. After the transient expression of the hSEPlp/Flag protein, the cells were separated into cytoplasmic and nuclear fractions, and the protein was extracted from them. The proteins were separated by SDS-PAGE, and the hSEPlp/Flag protein was detected by Western blot analysis using anti-Flag antibodies. The whole lysate showed a single band at around 208 kDa (Fig. 3). A clear band was found only in the cytoplasmic fraction of the protein extracts, but not in the nuclear fraction. These results showed that hSEPlp is expressed mainly in the cytoplasm. We also transiently expressed in HeLa cells the hSEPlp tagged with EGFP at its N-terminus. The EGFP/SEPlp protein was mainly expressed in the cytoplasm, especially at the nuclear periphery (Fig. 4), which is consistent with the above biochemical finding that hSEPlp was mainly expressed in the cytoplasm. We also did a similar experiment using the 293-EBNA cell line, but the expression of EGFP/SEPlp protein was poor in this cell line. Our results as a whole are consistent with those obtained for the cytoplasmic localization of Sep1p in Sac. cerevisiae and in mouse cells.

Although we extensively searched for the presence of a nuclear localization signal (NLS) consisting of an array of basic amino acids, such as NKRRCF, in the amino acid sequence of human Werner's syndrome helicase, we could not find any type of NLS signal. This result is also consistent with the view that hSEPlp is expressed and has a biological role in the cytoplasm. In this case, hSEPlp may be involved in processing and/or degrading RNA as proposed for the mouse cell system.

2.4. Expression of hSEPlp gene in various human organs

To measure the levels of expression of the hSEPlp gene in human organs, as well as in various tumor cell lines, a multiple tissue Northern blot analyses were performed (Fig. 5). The bands at ca. 10 kb are the major bands in all the cells and organs, except for the lung, liver and kidney and lung carcinoma A549, and are considered to correspond to the main hSEPlp mRNA. The 10-kb hSEPlp mRNA was expressed ubiquitously in the various tissues and organs examined, including the heart, brain, pancreas, spleen and testis. One or two minor bands at around 5.5 kb were also observed in the testis and placenta, which may be products of alternative splicing as discussed in the mouse. The 10-kb band was a major band in hSEPlp mRNAs, different from the mouse homologue Dhm2, in which the 5.8-kb band was a major band in the testis.
Figure 4. Intracellular localization of hSEPlp.

Figure 5. Northern blot analysis of hSEPl RNAs in different human tissues using a series of human multiple tissue Northern (MTN) blots I, II, and human cancer cell line MTN blot (Clontech, Palo Alto, CA). PBL, peripheral blood leukocyte; HL-60, promyelocytic leukemia; K-562, chronic myelogenous leukemia; MOLT-4, lymphoblastic leukemia; Raji, Burkitt’s lymphoma; SW480, colorectal adenocarcinoma; G361, melanoma.

2.5. Chromosomal location of hSEPl

Chromosomal mapping of the hSEPl gene was done by performing PCRs using a primer set of 3'/04/S and 3'/04/AS on the radiation hybrid cell lines of the GeneBridge 4 panel. The results (data not shown) were formatted as a vector “0” and “1” for clear results, and as a vector “2” for unclear results, as follows: 10000 00010 01000 00111 11001 02010 01101 00201 00101 00001 11101 01000 01110 10010 00000 10111 00001 01010 111. These data were e-mailed to the WICGR Mapping Service for the analysis by the RHMAPPER program. The obtained results showed that the hSEPl gene was located in human chromosome 3, between STS markers D3S1309 and D3S1569 (Fig. 6), that is, 3q25-26.1.

2.6. Discussion

Although Sac. cerevisiae Sep1p has been suggested to have multiple roles, a body of data has recently accumulated indicating that Sep1p of Sac. cerevisiae and its mouse homologue is the major 5’ → 3’ exoribonuclease in cytoplasmic mRNA turnover. The localization...
of hSEPlp in the cytoplasm, as shown in this study, is consistent with this view.

2.7. Conclusion

The hSEPl gene was highly homologous to mouse Dhm2, which is the homologue of Sac. cerevisiae SEPl. The hSEPl gene product, hSEPlp, was mainly localized in the cytoplasm, consistent with the view that hSEPlp is mainly involved in RNA metabolism. hSEPl is located at chromosome 3q25-26.1, where no noticeable disease genes have been reported yet.

References

1. Tishkoff, D. X., and Johnson, A. W., Kolodner R. D. 1991, Molecular and genetic analysis of the gene encoding the Saccharomyces cerevisiae strand exchange protein Sep1, Mol. Cell. Biol., 11, 2593-2608.

2. Dykstra, C. C., Kitada, K., Clark, A. B., Hamatake, R. K., and Sugino A. 1991, Cloning and characterization of DST2, the gene for DNA strand transfer protein β from Saccharomyces cerevisiae, Mol. Cell. Biol., 11, 2583-2592.

3. Larimer, F. W., Hsu, C. L., Maupin, M. K., and Stevens, A. 1992, Characterization of the XRNI gene encoding a 5 → 3' exoribonuclease: sequence data and analysis of disparate protein and mRNA levels of gene-disrupted yeast cells, Genes, 120, 51-57.

4. Kim, J., Ljungdahl, P. O., and Fink, G. R. 1990, kem mutations affect nuclear fusion in Saccharomyces cerevisiae, Genetics, 126, 790-812.

5. Kipling, D., Tambini, C., and Kearsey, S. E. 1991, rae mutations which increase artificial chromosome stability in Saccharomyces cerevisiae identify transcription and recombination proteins, Nucleic Acids Res., 19, 1385–1391.

6. Hsu, C. L. and Stevens, A. 1993, Yeast cells lacking 5' → 3' exoribonuclease 1 contain mRNA species that are poly(A) deficient and partially lack the 5' cap structure, Mol. Cell. Biol., 13, 4826-4835.

7. Muhirad, D., Decker, C. J., and Parker, R. 1995, Turnover mechanisms of the stable yeast PGK1 mRNA, Mol. Cell. Biol., 15, 2145–2156.

8. Interthal, H., Bellocq, C., Bahler, J., Bashkirov, V. I., Edelstein, S., and Heyer, W. D. 1995, A role of Sep1 (=Kem1, Xrnl) as a microtubule-associated protein in Saccharomyces cerevisiae, EMBO J., 14, 1057–1066.

9. Tishkoff, D. X., Rockmill, B., Roeder, G. S., and Kolodner, R. D. 1995, The sep1 mutant of Saccharomyces cerevisiae arrests in pachytene and is deficient in meiotic recombination, Genetics, 139, 495–509.

10. Liu, Z., Lee, A., and Gilbert, W. 1995, Gene disruption of a G4-DNA-dependent nuclease in yeast leads to cellular senescence and telomere shortening, Proc. Natl. Acad. Sci. USA, 92, 6002–6006.

11. Heyer, W. D., Johnson, A. W., Reinhart, U., and Kolodner, R. D. 1995, Regulation and intracellular localization of Saccharomyces cerevisiae strand exchange protein 1 (Sep1/Xrnl/Kem1), a multifunctional exonuclease, Mol. Cell. Biol., 15, 2728–2736.

12. Shobuik, T., Sugano, S., Yamashita, T., Ikeda, H. 1997, Cloning and characterization of mouse Dhm2 cDNA, a functional homolog of budding yeast SEP1, Gene, 191(2), 161–166.

13. Bashkirov, V. I., Scherthan, H., Solinger, J. A., Buerstedde, J. M., and Heyer, W. D. 1997, A mouse cytoplasmic exoribonuclease (mXRN1p) with preference for G4 tetraplex substrates, J. Cell. Biol., 136, 761–773.

14. Kozak, M. 1986, Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes, Cell, 44, 283–292.

15. Matsumoto, T., Shimamoto, A., Goto, M., and Furuchi, Y. 1997, Impaired nuclear localization of defective DNA helicases in Werner's syndrome, Nat. Genet., 16, 335–336.

16. Stevens, A. 1978, An exoribonuclease from Saccharomyces cerevisiae: effect of modifications of 5' end groups in the hydrolysis of substrates to 5'-mononucleotides, Biochem. Biophys. Res. Commun., 81, 656–661.

17. Stevens, A. 1980, Purification and characterization of a Saccharomyces cerevisiae exoribonuclease which yields 5'-mononucleotide by a 5'-3' mode of hydrolysis, J. Biol. Chem., 255, 3080–3085.

18. Walter, M. A., Spillett, D. J., Thomas, P., Weissenbach, J., and Goodfellow, P. N. 1994, A method for constructing radiation hybrid maps of whole genomes, Nat. Genet., 7, 22–28.

19. Shobuik, T., Sugano, S., Yamashita, T., and Ikeda, H. 1995, Characterization of cDNA encoding mouse homolog of fission yeast dhp1+ gene: structural and functional conservation, Nucleic Acids Res., 136, 357–361.

20. Szankasi, P and Smith, G. R. 1996, Requirement of Sch. pombe exonuclease II, a homologue of Sac. cerevisiae Sep1p, for normal mitotic growth and viability, Curr. Genet., 30, 284–293.