Expression Profiles of a Human Gene Identified as a Structural Homologue of Meiosis-specific recA-like Genes

Shusei SATO,¹ Naohiko SEKI,¹ Yasuo HOTTA,² and Satoshi TABATA¹,*
Kazusa DNA Research Institute, 1532-3 Yanauchino, Kisarazu-shi, Chiba 292, Japan ¹ and
Nara Institute of Science and Technology, 8916-5 Takayama-cho, Ikoma-shi, Nara, 630-01 Japan ²

(Received 18 August 1995)

Abstract

Using the cDNA clone from mouse testis which carries the conserved sequences among meiosis-specific recA-like genes, a highly homologous cDNA clone was isolated from a cDNA library of human testis. The clone had the coding capacity of a protein consisting of 340 amino acid residues, which coincides with the average size of putative eukaryotic recA-like proteins. When expression of the corresponding gene, named HsLIM15, in various tissues was examined by reverse transcription-PCR, products of two different sizes were detected in testis: While the longer was predominantly seen in the testis, the shorter was commonly to all the tissues including the testis. Analysis of the sequences indicated that the longer product corresponded to the above cDNA clone, and the shorter one was its deletion product missing an internal 165 bp portion. The result strongly suggests that the mRNA species coding for the putative meiosis-specific RecA-like protein in human is predominantly expressed in testis possibly as an alternative splicing product of a ubiquitously expressed gene.

Key words: recA-like gene; DMC1; LIM15; meiotic recombination; human

To better understand the molecular mechanisms of homologous recombination during meiosis, it is necessary to first characterize the components which participate in this process. Since RecA-like proteins are considered to have an essential role in the recombination reactions, attempts have been made to identify the corresponding genes from both genetical and biochemical approaches. Two recA-like genes, DMC1 and RAD51 were first identified in the budding yeast, Saccharomyces cerevisiae. DMC1 is expressed exclusively in meiotic cells,² participating in the formation of synaptonemal complexes and repair of double-strand breaks at recombination hotspots.¹ Therefore, this gene seems to be essential for progression of meiosis.¹,² Although RAD51 has significant structural homology to DMC1,³ it seems to be phylogenetically distinct from DMC1 and in fact, its expression is seen in cells where DNA repair and/or recombination are expected to occur.³ We previously found that a gene named LIM15, which was identified by isolation of a meiosis-specific cDNA clone from microsporocytes of Lilium longiflorum, was a homologue of DMC1.⁷ Using the conserved sequences in DMC1 and LIM15, we isolated their structural homologues from other eukaryotes including ArLIM15⁸ from Arabidopsis thaliana and MmLim15⁹ from mouse. DNA sequence data indicate that the sizes of the putative gene products are nearly identical and that their amino acid sequences are well conserved among the homologues. Thus, we tentatively defined these homologues as meiosis-specific recA-like genes. In this paper, we identified a human homologue of this class of genes, named HsLIM15, by isolation of a cDNA clone from a human testis cDNA library, and its sequence as well as transcriptional specificity were examined.

1. Isolation of Human cDNA Clones Carrying the recA-like Sequence

The cDNA clones carrying the recA-like sequence were isolated by screening a human testis cDNA library (Clontech, USA) with the previously isolated cDNA clone of MmLim15 as probe. Four independent clones showing positive signals were obtained from approximately 3 x 10⁶ plaques. The human gene corresponding to the cDNA clones was termed HsLIM15.

The entire nucleotide sequence of one of the cDNA clones, hsc01, is shown in Fig. 1; it was 1,627 bases long, and contained a large open reading frame (ORF), which can code for a protein of 340 amino acids. The 3'-untranslated sequence (530 bases) which followed the ORF was fairly long compared with those of MmLim15 (126 bases).

The predicted amino acid sequence was highly homol-
Expression of a Human recA-like Gene

had identities of 60.1% and 53.2% to those of Liml5 genes was lesser but significant: The HsLiml5 sequence of similarity to the putative products of other recA-like homologues to that of MmLiml5 (96.8% identity). The extent of conservation in various tissues using the same primer pairs indicated by underlining in Fig. 1). We found that another splicing product of a ubiquitously expressed gene was missing (nucleotide numbers 498 to 662 indicated by underlining in Fig. 1). As shown in Fig. 4, a distinct band of the expected length was observed in the lane of the testis, although faint bands with the same length were also seen in the lanes of the spleen and the ovary. An unanticipated finding was that an additional band, 165 bases shorter than expected, was detected in all the tissues including the testis. Nucleotide sequence analysis of this band revealed that the internal 165 nucleotides of the longer product was missing (nucleotide numbers 498 to 662 indicated by underlining in Fig. 1). We found that another cDNA clone, hsc02, contained a shorter cDNA species, of which the sequence was identical to that of the longer cDNA species, except for the deleted portion. This result strongly suggests that the mRNA species coding for the human homologue of meiosis-specific RecA-like proteins is predominantly expressed in the testis as an alternative splicing product of a ubiquitously expressed gene. Although no information is available regarding the functional role of the ubiquitously expressed mRNA species, the size of the protein encoded is smaller than those for the putative eukaryotic RecA-like proteins reported so far.

We previously examined expression of the mouse homologue, MmLim15, in various tissues using the same RT-PCR method, and detected only a single unique band of the expected length in the testis but not in other tissues (see Fig. 4 in ref. 9). Although the result apparently contradicts the present observation, we assume that in the previous analysis, one of the primers used was included in the deleted portion, so that the shorter cDNA species was not detected. This can be confirmed by comparison of the sequences of cDNA and genomic DNA. The functional role of the putative products of the shorter cDNA species also remains to be clarified.

Acknowledgments: We thank Dr. T. Kobayashi for valuable discussion and S. Sasamoto for technical assistance.

Figure 1. Nucleotide sequence of hsc02 cDNA. The predicted amino acid sequence is shown under the nucleotide sequence. The 5' terminus of the cDNA, and the first methionine downstream of the in-frame termination codon indicated by double underlining are numbered as +1. The positions of primers for RTPCR for quantitation of the transcript and for chromosome mapping are indicated by arrows. The region not found in the cDNA species also remains to be clarified.

2. Analysis of Transcripts

The tissue specificity of the expression of HsLim15 was examined by reverse-transcription (RT)-PCR using poly(A)+ RNAs from human brain, heart, lung, liver, kidney, spleen, thymus, testis, and ovary as templates. PCR was carried out first with the pair of distal primers, fp11 plus rp13, followed by the second-round amplification with the pair of the proximal primers, fp12 plus rp12 (Fig. 1), and the products were separated by agarose gel electrophoresis.

As shown in Fig. 4, a distinct band of the expected length was observed in the lane of the testis, although faint bands with the same length were also seen in the lanes of the spleen and the ovary. An unanticipated finding was that an additional band, 165 bases shorter than expected, was detected in all the tissues including the testis. Nucleotide sequence analysis of this band revealed that the internal 165 nucleotides of the longer product was missing (nucleotide numbers 498 to 662 indicated by underlining in Fig. 1). We found that another cDNA clone, hsc02, contained a shorter cDNA species, of which the sequence was identical to that of the longer cDNA species, except for the deleted portion. This result strongly suggests that the mRNA species coding for the human homologue of meiosis-specific RecA-like proteins is predominantly expressed in the testis as an alternative splicing product of a ubiquitously expressed gene. Although no information is available regarding the functional role of the ubiquitously expressed mRNA species, the size of the protein encoded is smaller than those for the putative eukaryotic RecA-like proteins reported so far.

We previously examined expression of the mouse homologue, MmLim15, in various tissues using the same RT-PCR method, and detected only a single unique band of the expected length in the testis but not in other tissues (see Fig. 4 in ref. 9). Although the result apparently contradicts the present observation, we assume that in the previous analysis, one of the primers used was included in the deleted portion, so that the shorter cDNA species was not detected. This can be confirmed by comparison of the sequences of cDNA and genomic DNA. The functional role of the putative products of the shorter cDNA species also remains to be clarified.

Acknowledgments: We thank Dr. T. Kobayashi for valuable discussion and S. Sasamoto for technical assistance.

The chromosomal location of HsLim15 was analyzed by PCR using a panel of human-rodent somatic cell hybrids and a pair of primers complementary to the 3'-untranslated region of the cDNA (mp01 and mp02 indicated in Fig. 1). As shown in Fig. 3, a specific product was detected only in the hybrid containing human chromosome 22.

The predicted amino acid sequence is shown under the nucleotide sequence. The 5' terminus of the cDNA, and the first methionine downstream of the in-frame termination codon indicated by double underlining are numbered as +1. The positions of primers for RT-PCR for quantitation of the transcript and for chromosome mapping are indicated by arrows. The region not found in the cDNA species also remains to be clarified.

2. Analysis of Transcripts

The tissue specificity of the expression of HsLim15 was examined by reverse-transcription (RT)-PCR using poly(A)+ RNAs from human brain, heart, lung, liver, kidney, spleen, thymus, testis, and ovary as templates. PCR was carried out first with the pair of distal primers, fp11 plus rp13, followed by the second-round amplification with the pair of the proximal primers, fp12 plus rp12 (Fig. 1), and the products were separated by agarose gel electrophoresis.

As shown in Fig. 4, a distinct band of the expected length was observed in the lane of the testis, although faint bands with the same length were also seen in the lanes of the spleen and the ovary. An unanticipated finding was that an additional band, 165 bases shorter than expected, was detected in all the tissues including the testis. Nucleotide sequence analysis of this band revealed that the internal 165 nucleotides of the longer product was missing (nucleotide numbers 498 to 662 indicated by underlining in Fig. 1). We found that another cDNA clone, hsc02, contained a shorter cDNA species, of which the sequence was identical to that of the longer cDNA species, except for the deleted portion. This result strongly suggests that the mRNA species coding for the human homologue of meiosis-specific RecA-like proteins is predominantly expressed in the testis as an alternative splicing product of a ubiquitously expressed gene. Although no information is available regarding the functional role of the ubiquitously expressed mRNA species, the size of the protein encoded is smaller than those for the putative eukaryotic RecA-like proteins reported so far.

We previously examined expression of the mouse homologue, MmLim15, in various tissues using the same RT-PCR method, and detected only a single unique band of the expected length in the testis but not in other tissues (see Fig. 4 in ref. 9). Although the result apparently contradicts the present observation, we assume that in the previous analysis, one of the primers used was included in the deleted portion, so that the shorter cDNA species was not detected. This can be confirmed by comparison of the sequences of cDNA and genomic DNA. The functional role of the putative products of the shorter cDNA species also remains to be clarified.

Acknowledgments: We thank Dr. T. Kobayashi for valuable discussion and S. Sasamoto for technical assistance.

The chromosomal location of HsLim15 was analyzed by PCR using a panel of human-rodent somatic cell hybrids and a pair of primers complementary to the 3'-untranslated region of the cDNA (mp01 and mp02 indicated in Fig. 1). As shown in Fig. 3, a specific product was detected only in the hybrid containing human chromosome 22.

The predicted amino acid sequence is shown under the nucleotide sequence. The 5' terminus of the cDNA, and the first methionine downstream of the in-frame termination codon indicated by double underlining are numbered as +1. The positions of primers for RT-PCR for quantitation of the transcript and for chromosome mapping are indicated by arrows. The region not found in the cDNA species also remains to be clarified.
Figure 2. Comparison of the amino acid sequences of the putative products of HsLIM15 and other recA-like genes in eukaryotes. The entire amino acid sequences of the putative gene products of HsLIM15, MmLiml5 in mouse, LIM15 in Lilium longiflorum, DMC1 in S. cerevisiae, and a human homologue of RAD51 (HsRAD51) are aligned. The positions of amino acids identical with those of HsLIM15 are indicated by colons.

Figure 3. Chromosome assignment using a monochromosomal hybrid cell panel. PCR was carried out with primers mpOl and mpO2 (shown in Fig. 1) using genomic DNA samples from human (Hum), mouse (Mo), Chinese hamster (Ha), and monochromosomal (1-22, X, and Y) hybrid cells purchased from Coriell Inst. as templates. The products were separated on 2.5% agarose gels with a 1 kb ladder DNA marker.

Figure 4. Quantitative measurement of the HsLIM15 transcript in various tissues. Transcripts of A. HsLIM15 and B. glyceraldehyde 3-phosphate dehydrogenase (G3PDH) were detected by RT-PCR in brain, heart, lung, liver, kidney, spleen, thymus, testis, and ovary. RT-PCR was carried out as detailed earlier. Poly(A) RNA was purchased from Clontech, USA. As a positive control, and hscOl DNA was used as a template. A portion of the transcript of G3PDH was also amplified as a control by using a G3PDH primer set (Clontech, USA). The DNA size marker is a 100-bp DNA ladder.
References

1. Bishop, D. H., Park, D., Xu, L., and Kleckner, N. 1992, DMC1: A meiosis-specific yeast homolog of E. coli recA required for recombination, synaptonemal complex formation, and cell cycle progression, Cell, 69, 439-456.

2. Kobayashi, T., Hotta, Y., and Tabata, S. 1993, Isolation and characterization of a yeast gene which is homologous with a meiosis-specific cDNA from a plant, Mol. Gen. Genet., 237, 225-232.

3. Shinohara, A., Ogawa, H., and Ogawa, T. 1992, Rad51 protein involved in repair and recombination in S. cerevisiae is a RecA-like protein, Cell, 69, 457-460.

4. Ogawa, T., Yu, X., Shinohara, A., and Egelman, E. H. 1993, Similarity of the yeast Rad51 filament to the bacterial RecA filament, Science, 259, 1896-1899.

5. Sung, P. 1994, Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast Rad51 protein, Science, 265, 1241-1243.

6. Shinohara, A., Ogawa, H., Matsuda, Y., Ushio, N., Ikeda, K., and Ogawa, T. 1993, Cloning of human, mouse and fission yeast recombination genes homologous to RAD51 and recA, Nature Genet., 4, 239-243.

7. Kobayashi, T., Kobayashi, E., Sato, S. et al. 1994, Characterization of cDNAs induced in meiotic prophase in lily microsporocytes, DNA Res., 1, 15-26.

8. Sato, S., Hotta, Y., and Tabata, S. 1995, Structural analysis of a recA-like gene in the genome of Arabidopsis thaliana, DNA Res., 2, 89-93.

9. Sato, S., Kobayashi, T., Hotta, Y., and Tabata, S. 1995, Characterization of a mouse recA-like gene specifically expressed in testis, DNA Res., 2, 147-150.

10. Yoshimura, Y., Morita, T., Yamamoto, A., and Matsushiro, A. 1993, Cloning and sequence of the human RecA-like gene cDNA, Nucleic Acids Res., 21, 1665.

11. Dubois, B. L. and Naylor, S. L. 1993, Characterization of NIGMS human/rodent somatic cell hybrid mapping panel 2 by PCR, Genomics, 16, 315-319.