Sequence Analysis and Expresion of a RecQ Gene Homologue from *Lentinula edodes*

Shiho Katsukawa; Kazuo Shishido

Department of Life Science, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 226-8501, Japan

We cloned and sequenced a *recQ* gene homologue from *Lentinula edodes*. This gene, named *Le.recQ*, was found to have a coding capacity of 945 amino acids (aa). The deduced Le.RECQ protein was clearly smaller than other fungal RecQ proteins such as *Neurospora crassa* QDE3 (1955 aa), *Schizosaccharomyces pombe* Rqh1 (1328 aa), and *Saccharomyces cerevisiae* SGS1 (1447 aa). It exhibited the highest homology to the *Arabidopsis thaliana* RecQl4A protein (1182 aa) in its size and aa sequence. Northern-blot analysis showed that the *Le.recQ* gene is transcribed at similar levels during mycelial development in *L. edodes* fruiting-body formation. The *L. edodes* dikaryotic mycelial cells were found to contain a clearly larger amount of *Le.recQ* transcript than the *L. edode*s two compatible monokaryotic mycelial cells. Results in situ RNA-RNA hybridization showed that subhymenium and outer region of trama contain larger amounts of *Le.recQ* transcript. Expression of *Le.recQ* cDNA in *S. cerevisiae* might partially complement defects associated with the loss of its homologue *S. cerevisiae SGS1* gene.

1. Introduction

RecQ helicases, a group of DNA helicases with a remarkable sequence conservation within all seven helicase motifs first reported in *Escherichia coli* RECQ (Nakayama et al. 1884; Irino et al. 1986), are widely found in organisms from bacteria to human. Whereas in *E. coli* and yeast (Gangloff et al. 1994; Stewart et al. 1997) only one RecQ protein is present, five different RecQ homologues have been found so far in human (Ellis et al. 1995; Yu et al. 1996, Puranam et al. 1994; Kitao et al. 1998) and six different RecQ homologues in plant *Arabidopsis thaliana* (Hartung et al. 2000).

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RecQ helicases have been reported to be involved not only in recombination, as in *sgs1* mutants in *S. cerevisiae* and *rqh1* mutants in *S. pombe* and humans affected by Bloom (BLM) and Werner (WRN) syndromes, but also in re-initiation of replication following DNA damages, as found in *E. coli*, *S. cerevisiae*, human etc. (reviewed by Cobb et al. 2002 and Wu and Hickson 2002). *N. crassa* QDE3 has been shown to be involved in post-transcriptional gene silencing, as the first evidence of a new function for a DNA helicase (Cogoni et al. 1999). All these evidences are related to the fundamental genetic processes: replication, recombination, repair and transcription. Expression in different tissues of *A. thaliana* six *RecQ* genes has been analyzed by RT-PCR method, showing that the expression of *RecQl1*, *RecQl2*, *RecQl4A* and *RecQl4B* genes is higher in shoots and flowers than in leaves and seedlings, but the expression of *RecQl3* gene does not differ much between all examined tissues (Hartung et al. 2000).

Although the genomic DNA fragment containing recQ sequence (not entire recQ gene) has been isolated from Ustiligo maydis, belonging to protobasidiomycetes (Sanchez-Alonso et al. 1998), there is no report on isolation of recQ homologue from the eubasidiomycetes. This led us to attempt to isolate recQ gene homologue(s) from Lentinula edodes, one of the typical eubasidiomycetes from which we have previously isolated various genes and analyzed their functions (Hori et al. 1991; Kajiwara et al. 1992; Endo et al. 1994; Kondoh et al. 1995; Kaneko et al. 1998; Zhou et al. 1998; Kaneko and Shishido 2001; Akiyama et al. 2001; Nishizawa et al. 2002), and to attempt to study the expression in L. edodes of recQ gene homologue in the course of fruiting-body formation, in both vegetatively growing binucleate-celled dikaryon and uninucleate-celled monokaryon and also in hymenophore (gill tissue). We also attempted to express the recQ homologue in S. cerevisiae and study whether the recQ homologue complements defects associated with the loss of S. cerevisiae SGS1 (recQ homologue) gene.

2. Results

2.1. *Cloning and ucleotide sequence(nt) analysis of* Le.recQ *gene*

L. edodes genomic DNA was digested with *Bam*HI, *Eco*RI or *Hin*dIII and the resulting digests were put through Southern-blot analysis at higher

(65°C) and lower (58°C) temperatures using the probe of the PCR-amplified 0.7-kb *recQ* conserved sequence (Probe 1 of Fig. 1). A single signal was detected in all three digests and at both higher and lower temperatures: 9.0 kb for *Bam*HI, 3.2 kb for *Eco*RI, and 8.0 kb for *Hin*dIII. We cloned the 3.2-kb *Eco*RI-*Eco*RI fragment (Clone 1 of Fig. 1). The nt sequence analysis suggested that the cloned 3.2-kb fragment contains the sequences encoding all seven RecQ helicase motifs (I, Ia, II, III, IV, V, and VI)(see Figs. 1 and 2), but it lacks 5'-terminal coding and promoter regions of *Le.recQ* gene (see Fig. 1). To clone these missing sequences of *Le.recQ,* the following inverse PCR was carried out. The *L. edodes* genomic DNA was digested with *Sal*I, *Sph*I, *Xba*I, or *Xho*I, all of which cut the aforementioned 3.2-kb *Eco*RI-*Eco*RI fragment at a single site. The resulting digests were subjected to Southern-blot analysis using the ^{32}P-labelled 1.2-kb *Eco*RI-*Sal*I fragment (Probe 2 of Fig. 1) within the 3.2-kb *Eco*RI-*Eco*RI fragment. A single signal was detected in all four digests: 1.4 kb for *Sal*I, 6.4 kb for *Sph*I, 15 kb for *Xba*I, and 10 kb for *Xho*I. Based on these data, a restriction map was constructed as shown in Fig. 1.

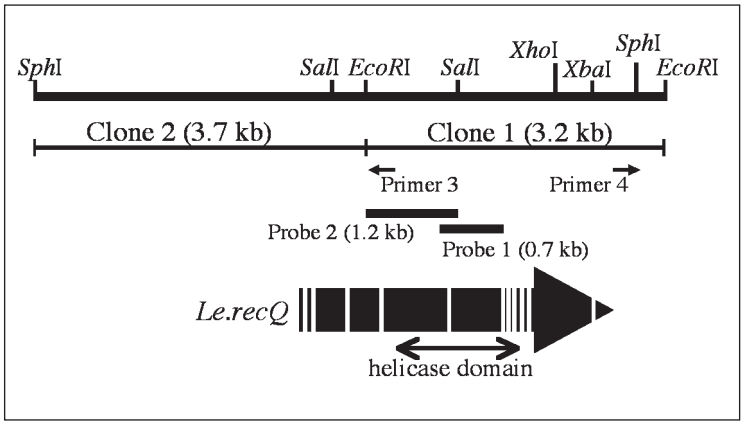

Figure 1. Restriction and gene maps of the region containing *Le.recQ* gene on the chromosome of *L.edodes. Le.recQ* gene is represented by arrow

The *Sph*I-digested *L. edodes* genomic DNA fragments were circularized by self-ligation and the resulting circular DNAs were subjected to inverse PCR using the primers 3 and 4, isolating the 3.7-kb *Sph*I-*Eco*RI fragment

(Clone 2 of Fig. 1). This 3.7-kb fragment was restriction mapped and sequenced. Based on the nt sequences of *Le.recQ* gene, we attempted to synthesize its cDNA by RT-PCR method using the total RNA prepared from *L. edodes* mature fruiting bodies. We succeeded in isolation of 3.2-kb cDNA sequences. The 6.9-kb genomic sequences (of 3.2-kb *Eco*RI-*Eco*RI fragment and 3.7-kb *Sph*I-*Eco*RI fragment) were compared with the cDNA sequences. Perhaps of the *Le.recQ* gene contains 3,396-bp coding region interrupted by 11 small (nt 49-59) introns and encodes 945 amino acids (aa). Putative transcription termination signal of AATAAA and the signal-like sequence of AATACAA were found between translation stop codon (TAG) and poly(A)-addition site.

2.2. *The transcription start point (tsp) of* Le.recQ *gene.*

To confirm the translation start codon of *Le.recQ* gene and analyze the structural feature of *Le.recQ* promoter, we determined the tsp of *Le.recQ* gene by primer extension method. The primer extension product of the *Le.recQ* transcript isolated from the *L. edodes* mature fruiting bodies gave a clear band at the position of 117-nt upstream of the suggested translation start codon (data not shown). The promoter region of *Le.recQ* gene contained a TATA-like sequence (TATACTAT) 40-nt upstream from the tsp, but not other eukaryotic (fungal) promoter consensus sequences such as GC-box, CAAT-box and CT-stretch.

2.3. *Comparison of the amino acid (aa) sequences of Le.RECQ and other RecQ proteins*

To determine the relationship between *Le.recQ* and other *recQ* genes, their derived aa sequences were compared (Figs. 2 and 3). *N. crassa* QDE3, *S. pombe* Rqh1, *S. cerevisiae* SGS1, *A. thaliana* RecQl4A, *E. coli* RECQ, Homo sapiens BLM, and Homo sapiens WRN consist of 1955, 1328, 1447, 1182, 610, 1417, and 1432 aa, respectively. Among these RecQ-type proteins, the *A. thaliana* RecQl4A was most homologous to the *Le.recQ* gene product, Le.RECQ (945 aa), in size. The RecQ-type helicases are known to have a

remarkably conserved helicase domain. So the aa sequences of the helicase domain of Le.RECQ were compared with those of other RecQ proteins. The *N. crassa* QDE3, *S. pombe* Rqh1, *S. cerevisiae* SGS1, *A. thaliana* RecQl4A, *E. coli* RECQ, H. sapiens BLM, and H. sapiens WRN showed 50%, 49%, 48%, 50%, 44%, 49%, and 36% identity to the Le.RecQ protein, respectively (Fig. 3). These data indicate that the *A. thaliana* RecQl4A protein is the most homologous to the Le.RECQ protein in its size and aa sequence (of the helicase domain). The RecQ-type proteins have been reported frequently to contain acidic aa-rich sequence(s) usually in their N-terminal region and C-terminal (to the helicase domain) conserved region. It was found that the Le.RECQ possesses both acidic aa-rich sequence and C-terminal conserved region.

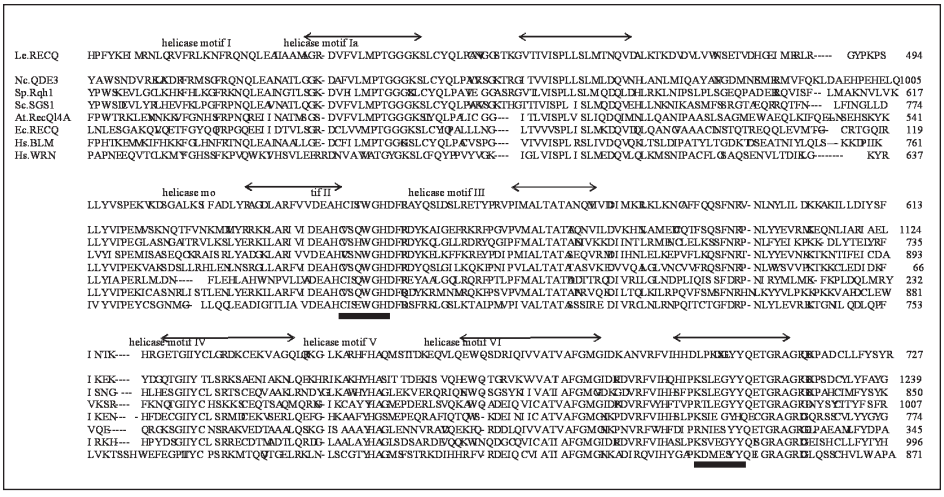

Figure 2. Comparison of the amino acid sequences of *L. edodes* RECQ (Le.RECQ) and other RecQ helicase proteins in their helicase domains

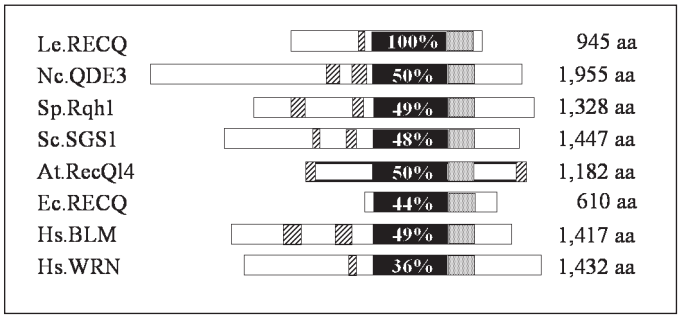

Figure 3. Schematic representation of members of the RecQ Helicase family of Le.RECQ, Nc.QDE3, Sp.Rqh1, Sc.SGS1, At.RecQl4A, Ec.RECQ, Hs.BLM and Hs.WRN. Acidic aa-rich sequences and C-terminal conserved regions are shown by striped bars and light-shaded bars respectively

2.4. *Transcriptional expression in* L. edodes *of* Le.recQ *gene*

Fruiting body was formed on saw-dust-corn bran medium. Total cellular RNA was isolated from preprimordial aggregated mycelia, primordia, immature fruiting bodies and mature fruiting bodies and subjected to Northern-blot analysis using ^{32}P-labelled probes of the PCR-amplified 0.7-kb *Le.recQ* conserved sequence (Probe 1 of Fig. 1) and the cDNA (1.2 kb) of *Le.ras*, which has been shown to be transcribed at similar levels during mycelial development in fruiting-body formation of *L. edodes* (Hori et al. 1991). The specific radioactivities of the two probes were almost the same. A single signal of 3 kb, corresponding to the size of *Le.recQ* cDNA, was detected in all RNA blots and the signal intensities were similar, though they were significantly weaker than those of the 1.2-kb *Le.ras* signals (Fig. 4A). The intensities of the *Le.ras* signals were similar in all RNA blots, ensuring an equal loading and transfer of RNA preparations. These results indicate that *Le.recQ* is constitutively transcribed during the fruiting-body formation of *L. edodes*, but the transcript levels are relatively low.

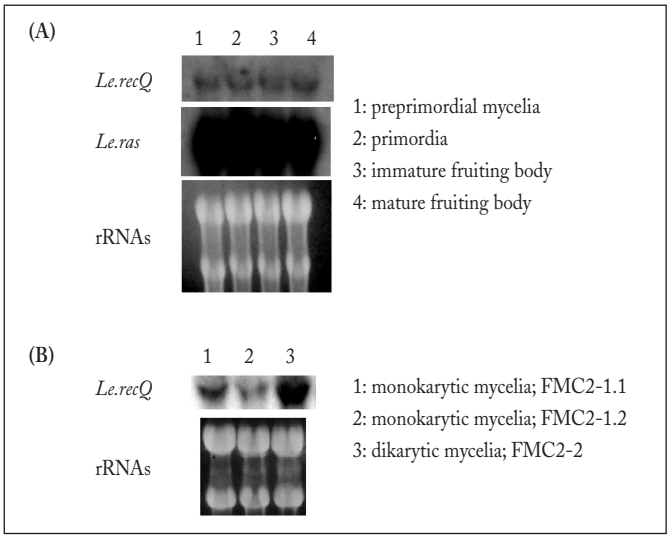

Figure 4. Transcriptional expression of *Le.recQ* gene in the course of fruiting-body formation (A) and in vegetatively growing dikaryotic and monokaryotic strains of *L. edodes* (B)

We investigated the transcript levels of *Le.recQ* gene in vegetatively growing mycelial cells of two compatible monokaryotic strains of FMC2-1.1 and FMC2-1.2 (Yasuda and Shishido, 1999) and dikaryotic strain FMC2-2 obtained by crossing FMC2-1.1 and FMC2-1.2. These strains were cultured in liquid SMY medium. As shown in Fig. 4B, FMC2-2 (lane 3) contained several times larger amount of *Le.recQ* transcript as compared with FMC2-1.1 (lane 1) and FMC2-1.2 (lane 2). It was also shown that FMC2-2 grown in the liquid medium with shaking (lane 3 of Fig. 4B) contains clearly larger amount of *Le.recQ* transcript than FMC2 (parental strain of FMC2-2) grown on the solid medium (lane 1 of Fig. 4A). These results suggest that *Le.recQ* gene might function much more actively in the binucleate-celled dikaryon in which growth is faster than the uninuleate-celled monokaryons (FMC2-2 grows approximately 1.3 times faster than FMC2-1.1 and 2 times faster than FMC2-1.2).

Quantitative RT-PCR analysis demonstrated that *Le.recQ* transcript is present under high density in hymenophore (gill tissue), which contains a large amoun of total RNA. Results in situ RNA-RNA hybridization showed

that subhymenium (on the top of which hymenium is formed) and outer region of trama (the region branching out into the suybhymenium) contain larger amounts of *Le.recQ* transcript (Fig. 5).

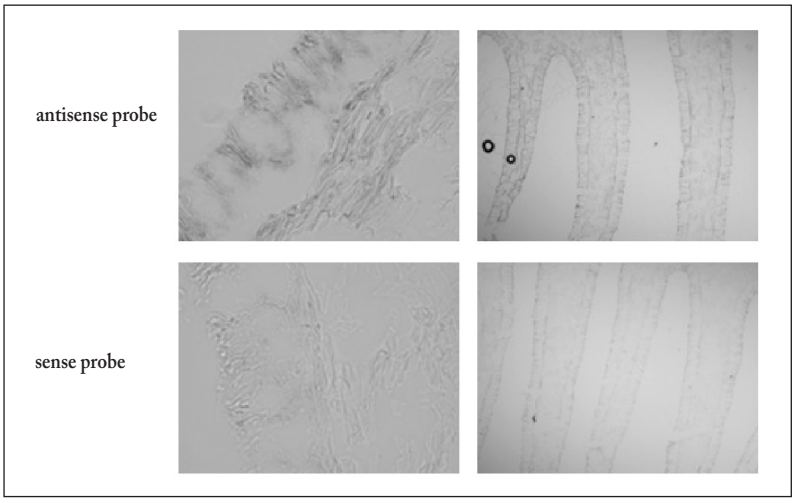

Figure 5. Expression of the *Le.recQ* gene in hymenophores of *L. edodes*

2.5. *Functional complementation of* Le.recQ *cDNA in* S. cerevisiae sgs1 *mutant*

S. cerevisiae possesses one *RecQ*-type gene, *SGS1*. The *sgs1* defective mutation causes *S. cerevisiae* a slight delay in growth and a sensitivity to DNA-damaging compound, methylmethanesulfonate (MMS) (Miyajima et al. 2000). We examined whether *Le.recQ* gene is able to complement these phenotypes of the *sgs1* mutant. *S. cerevisiae* 966NS-1 carrying the *Le.recQ* cDNA expression plasmid pYES2-Le.recQ, i.e., the 966NS-1[pYES2-Le.recQ] and the 966NS-1 carrying the *S. cerevisiae SGS1* expression plasmid pYES2-SGS1, i.e., the 966NS-1[pYES2-SGS1] were used for the experiments. The 966NS-1 carrying the vector pYES2, i.e., the 966NS-1[pYES2] was used as a control. The growth rates of these three strains were analyzed in the CM (without uracil) medium containing 2% raffinose and 0.2% galactose under the absence and presence of 0.001% MMS. The growth rate in the absence of MMS of the 966NS-1[pYES2-Le.recQ] was similar to that of the

966NS-1[pYES2-SGS1], and was higher than that of the 966NS-1[pYES2] (data not shown). In the presence of MMS, on the other hand, the 966NS-1[pYES2-Le.recQ] grew faster than the 966NS-1[pYES2], but slower than the 966NS-1[pYES2-SGS1] (data not shown). These results suggest that the *Le.recQ* cDNA can complement *sgs1* mutation of *S. cerevisiae*, but the complementation is not as efficient as that given by *S. cerevisiae SGS1* gene.

3. Discusion

As for the problem whether eubasidiomycete *L. edodes*, a multicellular filamentous fungus, possesses plural number of *recQ* gene, the following data appear likely to imply the presence of a single *recQ* gene homologue on *L. edodes* genome. Southern hybridization at higher (65˚C) and lower (58˚C) temperatures of *Bam*HI-, *Eco*RI-, or *Hin*dIII-digested *L. edodes* genomic DNA using the probe of 0.7-kb *recQ* conserved sequence gave a single signal. The 0.7-kb band in agarose gel of the PCR-amplified product was cut out from the gel and inserted into the pBluescript II vetor, followed by transformation of *E. coli*. Total 12 clones were selected and sequenced. The 10 fragments have an identical nt sequence of *Le.recQ* and other 2 fragments the nt sequences unrelated to *recQ* gene (data not shown). To verify the presence of a single *recQ* gene in *L. edodes*, other approaches, including a whole genome sequence analysis, are necessary. There exists a correlation between *Le.recQ* transcription level and growth rate of the mycelial cells. Efficient expression of *Le.recQ* gene is considered to be required for good growth of mycelial cells, implying a role in DNA replication.

A functional complementation test indicated that *Le.recQ* cDNA does complement slow growth phenotype of *S. cerevisiae sgs1* mutant, implying that *Le.recQ* play a role in DNA synthesize and cell divisions of the yeast. On the other hand, *Le.recQ* only partially complements the MMS-sensitivity of the *sgs1* mutant. The biological significance of *Le.recQ* gene in *L. edodes* totally remains to be determined.

4. Acknowledgements

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