Shimane Daigaku Igakubu Kiyo (Bull. Shimane Univ. Fac. Med.) vol.30, pp.71～74, Dec. 2007

Original Paper

Cloning and Sequencing of a Gene Encoding Ribonuclease F1 of *Fusarium moniliforme*

(ribonuclease F1 / genomic DNA sequence / *Fusarium moniliforme*)

Mari IIZUKA* and Hiroshi YOSHIDA*†

Cloning and sequencing of the genomic DNA of *Fusarium moniliforme* encoding ribonuclease F1 was successfully performed. The gene consists of three exons separated with approximately 50-bp long introns. The gene structure was compared with those of several homologous ribonucleases found in the sequence database. It was found that one interesting feature reported for the ribonuclease T1 gene of *Aspergillus oryzae* is conserved among three species of Aspergillus genus and *F. moniliforme*. The feature is that a very short exon encoding the presequence consisting of only five amino acid residues is connected to the next exon in phase 0.

INTRODUCTION

In the course of our study on ribonuclease (RNase) F1 of a phytopathogenic fungus, *Fusarium moniliforme*, we determined its amino acid sequence[1] and carried out the cloning and sequencing of a cDNA encoding the enzyme[2]. In order to gain further insight into the gene structure, we undertook cloning and sequencing of the genomic DNA. Here, we report the structure of the gene encoding RNase F1.

RNase F1 is a homolog of well-known RNase T1 of *Aspergillus oryzae*. Although many other homologs have been studied at the protein level, only the gene structure for RNase T1 has so far been reported[3]. This study is, therefore, the second report on the gene structure for this family of proteins. At present, gene structures for several other homologous RNases are available through genome annotation. Comparison of these gene structures is made in this paper.

The nucleotide sequence reported in this paper has been submitted to the DDBJ Data Bank under accession no. AB355898 with the organism name, *Gibberella fujikuroi*, whose anamorph is called *Fusarium moniliforme.* We have been using the latter name throughout our studies on enzymes of this fungus.

*Division of Chemistry, Department of Life Sciences, Shimane University
*†Present address: 1-15-4-1703 Senda-machi, Naka-ku, Hiroshima 730-0052

MATERIALS AND METHODS

Cloning Methods — The genomic DNA library of *F. moniliforme* was constructed from *Eco*RI digested DNA by ligation to λZAP II (Stratagene) and packaging into λphage. Screening for clones harboring the RNase F1 gene was carried out using a probe obtained by digestion of cDNA clone pUC18F12-1 which had been obtained previously[2]. The fragment was approximately 550 bp-long and contained the entire coding region. The probe was labeled with digoxigenin and was detected immunochemically. Confirmation of isolated clones was carried out by the polymerase chain reaction (PCR) using a sense primer CGCTTCTCTTGCTCTTG and an antisense primer GTTGGTACCACTGCATCCAA, which had been designed on the basis of the cDNA sequence. The primers were synthesized and supplied by Amersham Pharmacia. Positive clones gave a 370-bp long band in the PCR. Inserts of the positive clones were excised and subcloned into pBluescript phagemids with the use of a helper phage. Experimental conditions for the above procedures were described previously[4].

Determination of Nucleotide Sequences — DNA samples were sequenced on both strands with an ABI Prism 310 Genetic Analyzer using the ABI Prism BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) with appropriate oligonucleotide primers that had been synthesized and supplied on order by Amersham Pharmacia.

Sequence Data Search and Analysis — These were carried out at GenomeNet Server (Kyoto Center).

Multiple alignment of amino acid sequences and construction of a phylogenetic tree thereof were accomplished using Clastal W.

RESULTS AND DISCUSSION

Isolation of Genomic DNA Clones Harboring the RNase F1 Gene — Starting from 2 x 10^5 phages of the genomic DNA library, we obtained two positive clones in the form of pBluescript phagemids and named them pBRNFg1 and pBRNFg2. This result does not necessarily mean the low density of the aimed clone in the library, because we encountered difficulties in the screening and excision experiments. In fact, we found three to five positive spots for every 4 x 10^4 phages. The subsequent steps, however, did not proceed as expected but we somehow managed to get the two positive clones. The long size of the insert (9 kbp) may be one of the factors but the reason(s) for the difficulties remain unknown. After digestion with *Eco*RI, their inserts were analyzed for size. Both of them gave a fragment of the same size (9 kbp). Terminal sequence analysis showed that they had the same sequence at both termini. Hence, they were judged to be the identical clone. The nucleotide sequence of the insert of pBRNFg1 was determined on both strands. The sequence was 9204-bp long.

Structure of the Gene Encoding RNase F1 — The gene, designated as *rnf*, is situated at position 1655-2157 in the determined sequence and consists of three exons separated by approximately 50-bp long introns (Fig. 1). In the promoter region, a TATA box is found 45 bases upstream to the start of the cDNA previously reported[2]. Although the cDNA covered the entire coding region of RNase F1 (hence referred to as full-length), its sequence is thought to lack a short 5'-terminal segment, because the TATA box is generally known to lie approximately 30 bases upstream to the transcription initiation site. The genomic DNA sequence has a possible poly(A) addition signal ATTAAA 28 bases upstream to the poy(A) addition site. Although this signal differs slightly from standard AATAAA, such anomaly has been frequently observed in cDNA sequences of *F. moniliforme* (Reference 4 and direct submission to DDBJ Data Bank, accession numbers AB071859, AB071861, AB071862, and AB092343). There are six conflicts between the previously reported cDNA sequence and the present genomic DNA sequence. They are all in the 3'-untranslated region as follows (expressed as cDNA→genomic DNA): C465

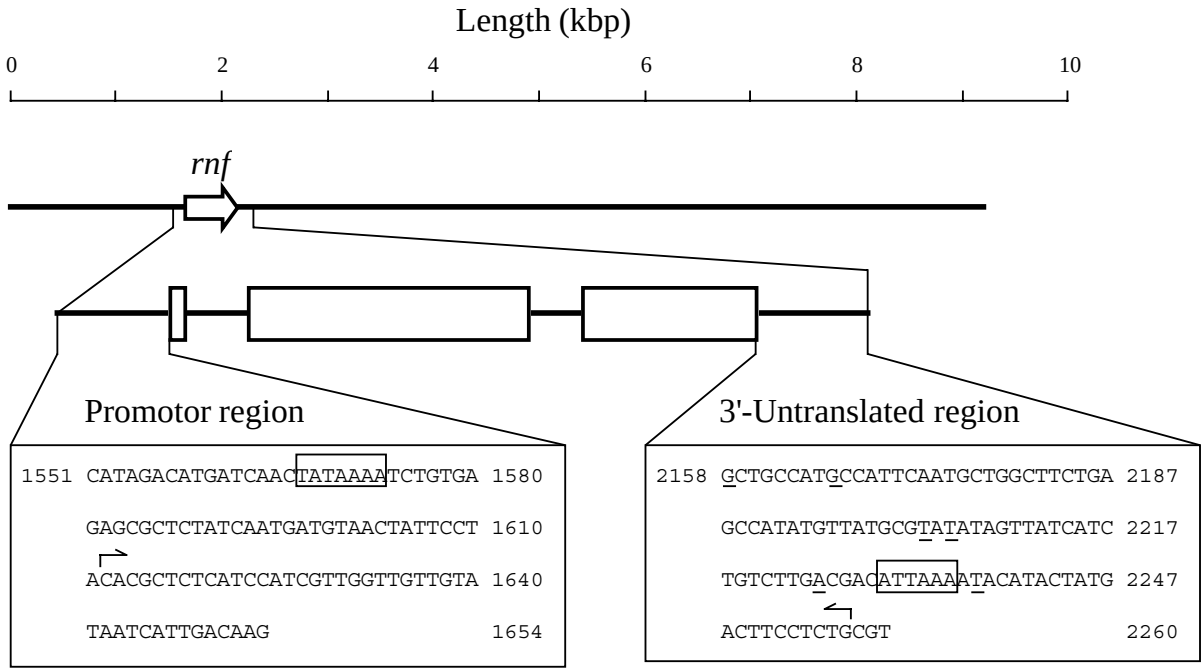

Fig. 1. Structure of gene *rnf* encoding RNase F1 shown in three layers. The top layer shows the position and direction of the gene in the sequenced DNA segment. The scale above is for this layer. The middle layer shows the detailed structure with exons represented by boxes. The bottom layer shows the nucleotide sequences of the promoter and 3'-untranslated regions. In the promotor region, the TATA box is boxed and the start of the cDNA is shown by a hooked arrow. In the 3'-untranslated region, the poly(A) addition signal is boxed and the end of the cDNA (poly(A) addition site) is shown by a hooked arrow. Bases in conflict with the previously reported cDNA sequence are underlined.

→G2158, C473→G2166, A510→T2203, A512→T2205, C532→A2225 and A544→T2237. We have more confidence in the sequencing of the genomic DNA than that of the cDNA, because the former took advantage of advanced technology. Therefore, the cDNA sequence should be corrected as described above.

Structure Comparison of the Genes Encoding Proteins of RNas T1 Family — RNase F1 is a homolog of well-known RNase T1 of *Aspergillus oryzae*, whose gene structure has been reported by Fujii *et al.*[3]. Gene structures of some other proteins of the RNase T1 family are now available in the sequence database. We selected the followings: RNase U1 homolog of *Ustilago maydis* (TrEMBL Q4PDL2), RNase N1 of *Neurospora crassa* (Swiss-Prot, P09646), hypothetical protein (RNase T1 homolog) of *Aspergillus nidulans* (TrEMBL Q5BCK7),

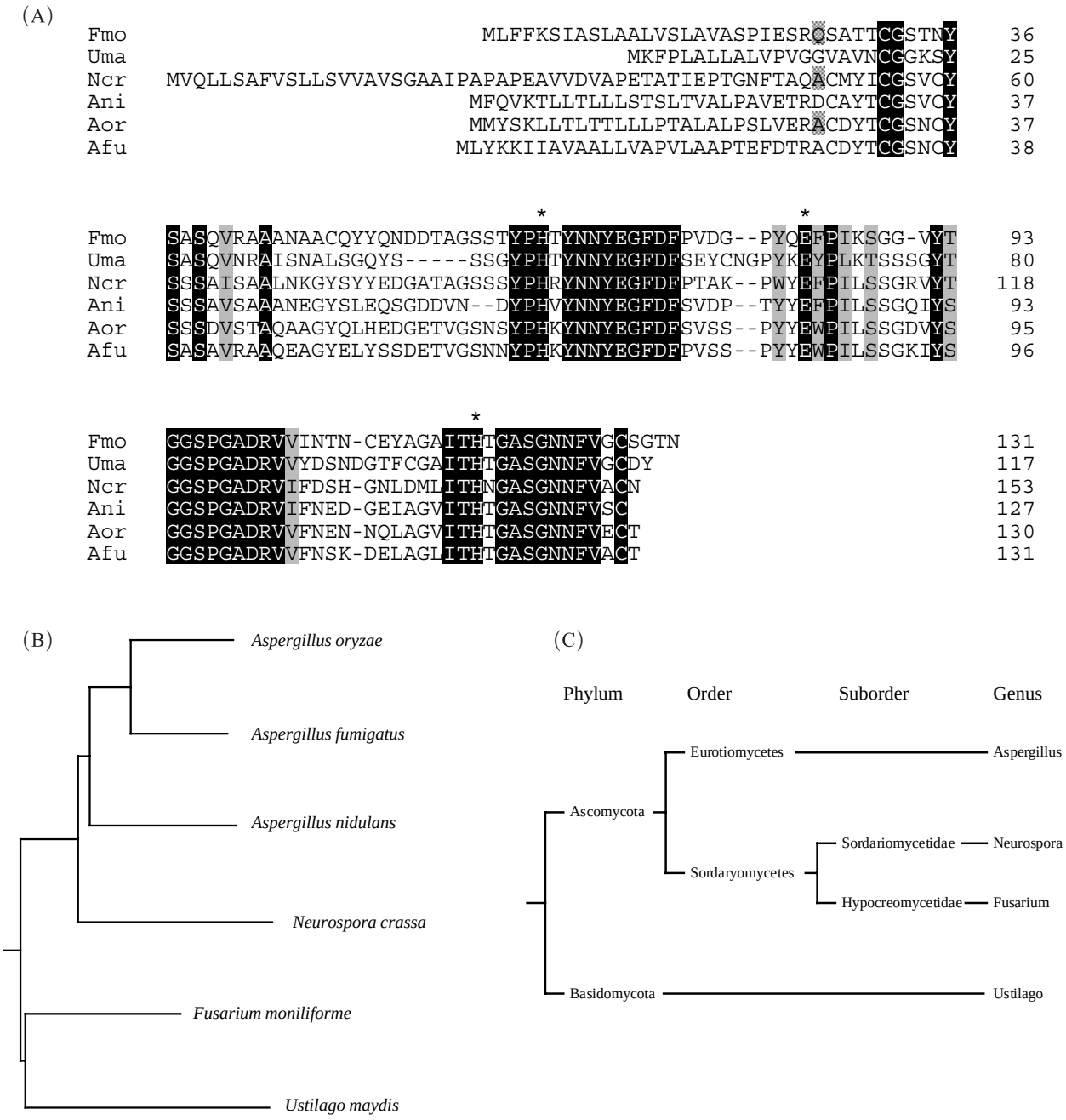

Fig. 2. (A) Multiple sequence alignment of selected RNase T1 homologs. The aligned sequences are homologous RNases from the following fungi: Fmo, *Fusarium moniliforme*; Uma, *Ustilago maydis*; Ncr, *Neurospora crassa*; Ani, *Aspergillus nidulans*; Aor, *Aspergillus oryzae*; and Afu, *Aspergillus fumigatus*. In construction of the alignment, homology among presumed presequences were neglected. Fully conserved residues are highlighted in white letters against dark backgrounds. Conservatively retained residues are shadowed. Asterisks indicate the active site residues. Meshed residues show the N-termini of mature proteins (known for only Fmo, Ncr and Aor), (B) Phylogenetic tree constructed from the multiple alignment. (C) A brief representation of the taxonomy of the fungi. Only relevant divergence points are shown.

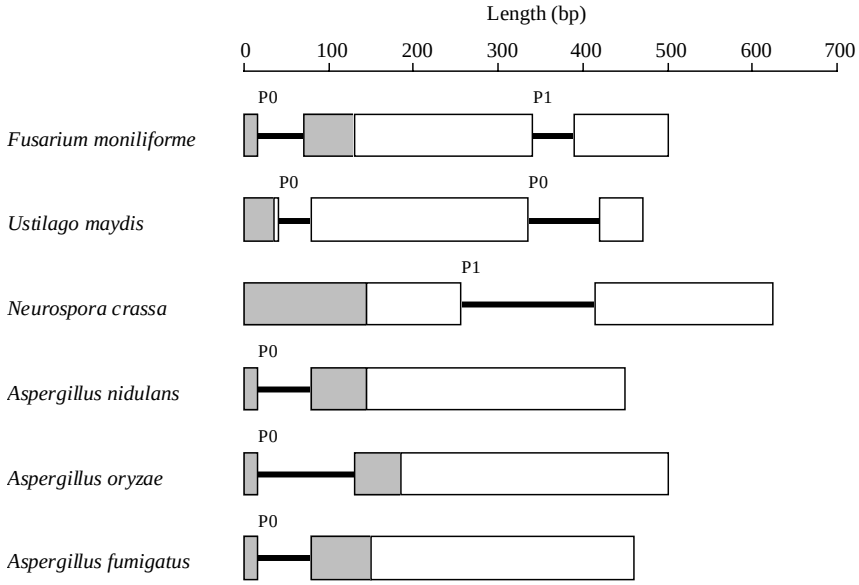

Fig. 3. Structure comparison of the genes encoding RNas T1 homologs. Exons and introns are shown by boxes and thick lines, respectively. P0 and P1 mean that the exon junction at the indicated site is in phase 0 and phase 1, respectively. Phase 2 has not been observed in these genes. The parts of exons that encode the presequences are shadowed.

and RNase T1 homolog of *Aspergillus fumigatus* (TrEMBL Q4W9Z2). Although the indicated accession numbers are for amino acid sequences, the corresponding gene structures are accessible through those entries. We then created the multiple alignment of the amino acid sequences of these RNases (Fig. 2A) and obtained a phylogenetic tree thereof (Fig. 2B). The tree is in good agreement with the taxonomy for these fungi which is briefly described in Fig. 2C. The gene structures for these RNases shown in Fig. 3, however, are not so clearly interpreted as in most cases for genes of homologous proteins. They differ with respect to the number, position, and length of introns as well as the phase of exon junction. Phase 0 signifies that the junction is just between the two adjacent codons, whereas, phase 1 and phase 2 mean that the junction lies after the first and the second letter of a codon, respectively. The only one feature commonly observed among several fungi is that a very short exon encoding the presequence consisting of only five amino acid residues is connected to the next exon in phase 0. This feature is observed in the fungi of Aspergillus genus and *F. moniliforme* but not in *N. crassa*, which is considered to be closer to Aspergillus fungi than *F. moniliforme* from the viewpoint of Fig. 2B. This finding may imply close relationship between Aspergillus and Fusarium genera, although any definitive conclusion cannot be drawn at present on the evolution of the gene structure for this family of proteins.

ACKNOWLEDGMENTS

We thank Ms. Y. Tanabe of Department of Biosignaling and Radioisotope Experiment, Center for Integrated Research in Science, Shimane University for her skillful technical assistance in DNA sequencing.

REFERENCES

1. Hirabayashi, J. and Yoshida, H. (1983) The primary structure of ribonuclease F1 from *Fusarium moniliforme.* Biochem. Int. **7**, 255-262
2. Yoshida, H., Iizuka, M., Norioka, N., Norioka, S. and Sakiyama, F. (1998) Cloning and sequencing of cDNA encoding ribonuclease F1 from *Fusarium moniliforme*. Biochem. Mol. Biol. Int. **45**, 555-560
3. Fujii, T., Yamaoka, H., Gomi, K., Kitamoto, K. and Kumagai, C. (1995) Cloning and nucleotide sequence of the ribonuclease T1 gene (*rntA*) from *Aspergillus oryzae* and its expression in *Saccharomyces cerevisiae* and *Aspergillus oryzae*. Biosci. Biotech. Biochem. **59**, 1869-1874
4. Yoshida, H., Iizuka, M., Narita, T., Norioka, N. and Norioka, S. (2006) Cloning and sequencing of cDNA and genomic DNA encoding PDM phosphatase of *Fusarium moniliforme*. J. Biochem. **140**, 813-823

(Received 2007/9/11)