Partial nucleotide sequence of a cDNA encoding 18S ribosomal RNA from the coconut rhinoceros beetle, *Oryctes rhinoceros* Linnaeus

Seiichi Furukawa, Masanori Yamamoto, Kiyoko Tanai, Toshio Shono and Minoru Yamakawa

Laboratory of Applied Zoology, Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

1Laboratory of Biological Defense, National Institute of Sericultural and Entomological Science, Tsukuba, Ibaraki 305-8634, Japan

(Received 25 July 1997; Accepted 20 October 1997)

---

**Abstract**

In order to obtain cDNA clones encoding 18S ribosomal RNA or an antibacterial protein, a fat body cDNA library of the coconut rhinoceros beetle, *Oryctes rhinoceros*, was screened using common probes which could hybridize both cDNAs. Partial nucleotide sequencing of two positive clones revealed that both clones encode 18S rRNA but not an antibacterial protein. Comparison of the nucleotide sequence of the two positive clones with those of 18S rRNA genes from two bee species, *Mesopolobus* sp. and *Evania appendigaster* indicated that the partial sequence from *O. rhinoceros* is identical to those of these two bee 18S rRNA genes. This cDNA will be useful as a probe for an internal marker to explore regulatory mechanisms of antibacterial protein gene expression in *O. rhinoceros*.

**Key words:** Molecular cloning, cDNA, nucleotide sequencing, 18S rRNA, Scarbaeidae, *Oryctes rhinoceros*

---

**INTRODUCTION**

In order to elucidate self-defense mechanisms in insects, we have isolated several antibacterial proteins from the silkworm, *Bombyx mori* (Hara et al., 1994; Hara and Yamakawa, 1995a, b). We have also cloned and determined the nucleotide sequences of *B. mori* cDNAs encoding a lectin (Kotani et al., 1995) and antibacterial proteins such as cecropin B (Tanai et al., 1992; Kato et al., 1993), attacin (Sugiyama et al., 1995) and lebocin (Chowdhury et al., 1995). In general, the gene expression of insect self-defense proteins is induced rapidly upon bacterial infection (Boman and Hultmark, 1987). Gene expression is known to be triggered by bacterial cell wall components like lipopolysaccharide (LPS) (Kato et al., 1994a, b; Tanai et al., 1996) and peptidoglycan (Morishima et al., 1992; Iketani and Morishima, 1993). LPS-responsive element (Lenardo and Baltimore, 1989) was identified in the 5'-upstream region of antibacterial protein genes (Tanai et al., 1995).

Moreover, antibacterial protein gene expression is also known to occur tissue-specifically (Kato et al., 1993; Sugiyama et al., 1995; Chowdhury et al., 1995).

We have started to isolate antibacterial proteins from various insects in addition to *B. mori* to understand the regulation of gene expression of antibacterial proteins in insects. The coconut rhinoceros beetle, *O. rhinoceros*, is one source of these proteins, since the larvae are easy to collect in large quantities throughout all seasons in subtropical areas of Japan.

Northern blotting (Alwine et al., 1977) is one of the most useful and popular techniques for the analysis of gene expression. For this analysis it is necessary to have a suitable internal marker to compare the degree of gene expression. rRNA is one standard that can be used as an internal marker.

In the present study, we aimed to clone cDNAs encoding 18S rRNA and/or an antibacterial protein of *O. rhinoceros* using probes which could hybridize both cDNAs. Analysis of
nucleotide sequences of two positive clones obtained indicated that these two clones were identical and encoded 18S rRNA but not an antibacterial protein. Here we present the partial nucleotide sequence of the cDNA and a comparison of this sequence to two bee 18S rRNA genes.

MATERIALS AND METHODS

Insects. The larvae of *O. rhinoceros* were collected from composts of fields at Itoman, Okinawa prefecture, Japan. The 3rd instar larvae were used for this study.

Construction of cDNA library. The larvae were immunized with *Escherichia coli* HB 101 (2 × 10⁷ cells) and fat bodies were excised 10 h after immunization. mRNA was extracted from 570 mg of fat body using a "QuickPrep mRNA Purification Kit" (Pharmacia) and a total of 6.8 µg were obtained. cDNA synthesis was performed with 3.4 µg mRNA using a "cDNA Synthesis Kit" (Pharmacia). Double-stranded cDNA was ligated with EcoRI/NotI adaptors. The cDNA was inserted into EcoRI site of pUC19 vector. *E. coli* JM109 was transformed with the pUC19 plasmids containing the cDNA. The efficiency of construction of the cDNA library was 9.7 × 10⁶ colonies/µg poly(A)+RNA.

Screening and nucleotide sequencing. In the homology analysis of the possible nucleotide sequence deduced from amino acid sequences of an *O. rhinoceros* antibacterial protein, we found that a part of the sequence was partially identical with that of 18S rRNA gene of a bee, *Mesopolobus* sp. (Carmean et al., 1992). We tried to screen cDNA clones encoding 18S rRNA and an antibacterial protein using probes, which could simultaneously hybridize both cDNA clones. For preparation of the probes we synthesized two DNA oligomers (38 mers) based on the amino acid sequence of an isolated antibacterial protein from the hemolymph of *O. rhinoceros* (unpublished results). The sequences of the synthetic oligomers were as follows: 5'-C-A(A/G)CCGCGGCCTC-3' and 5'-CA(A/G)CGCCGCGGCCTC-3'. The probes were labeled with fluorescein-11-dUTP using an "ECL 3'-Oligolabeling System" (Amersham). Screening procedures were as described previously (Kato et al., 1993). The nucleotide sequence of the cDNAs was determined by dye-terminator cycle sequencing method using a DNA sequencer (ABI 373A).

RESULTS

Molecular cloning of cDNAs encoding 18S rRNA and an antibacterial protein

We screened a total of 22,000 colonies with the mixed probes and obtained two positive clones. As the two clones (designated SF-1 and SF-2) were confirmed to be positive in the 2nd screening, we isolated the plasmids and digested them with EcoRI to compare the sizes of the cDNA inserts. The sizes of the inserts from SF-1 and SF-2 were found to be identical by 4% agarose gel electrophoresis (Fig. 1). Partial nucleotide sequences of SF-1 and SF-2 clones were determined and the results indicated that these clones contain identical sequences.

Comparison of the nucleotide sequence of positive clones with those of other insect 18S rRNA genes

The two cDNA clones appeared not to encode the objective antibacterial protein but to be homologous to 18S rRNA genes from two bee spe-

Fig. 1. Electrophoresis of cDNA inserts from SF-1 and SF-2 clones. One microgram of purified plasmid DNA was digested with EcoRI and separated by 4% agarose gel electrophoresis. After electrophoresis, DNA fragments were visualized by staining with ethidium bromide. Lane 1: 100 base pair (bp) ladder as molecular mass markers (Pharmacia), lane 2: SF-1 non-digested, lane 3: SF-1 digested with EcoRI, lane 4: SF-2 non-digested, lane 5: SF-2 digested with EcoRI. Arrowhead indicates the position of the cDNA inserts (ca. 250 bp).
cies, *Mesopolobus* sp. and *Evania appendigaster* (Carmean et al., 1992) (Fig. 2). The identity at the nucleotide sequence level was 100% between the positive cDNA clones and the 18S rRNA genes. This result suggests that the nucleotide sequence of the 18S rRNA gene is well conserved through evolution in three different insect species. The cDNA encoding 18S rRNA of *O. rhinoceros* is long enough to serve as a probe for Northern blotting and will be useful as an internal marker to study the regulation of gene expression of antibacterial proteins in this insect. On the contrary, cDNA encoding an antibacterial protein was not obtained in the present study.

**DISCUSSION**

The mRNA purification method employed in this study depended on a poly(A) sequence at the 3' end. Namely, mRNAs in the total RNA fraction were purified through oligo(dT)-cellulose column chromatography. Theoretically, all rRNAs are excluded by this method and cDNA library does not contain rRNA clones. However, it is very difficult to isolate mRNAs without rRNA contamination, because of their enormous quantity as compared to that of mRNAs. We took advantage of this fact and assumed that cDNA synthesis based on the rRNAs as templates should occur under the standard conditions with oligo(dT) primers, since long rRNA sequences should contain oligo(A) stretches, which can hybridize oligo(dT) primers. One purpose of this study was to obtain a cDNA clone encoding rRNA of *O. rhinoceros* as a probe for an internal marker, therefore we did not think it necessary to screen a full size cDNA. Thus, it did not matter where these oligo(A) stretches were localized and that they hybridize oligo(dT). This assumption was confirmed to really occur by the fact that our cDNA library contained rRNA clones. Thus, our strategy to isolate cDNA clones encoding 18S rRNA was effective. To the best of our knowledge, nucleotide sequences of the cDNA encoding 18S rRNA from *O. rhinoceros* is the first report.

On the other hand, no cDNA clones encoding an antibacterial protein were obtained this time. We speculate that a number of the colonies screened might be too small because of the presence of a low level of the antibacterial protein cDNAs as compared to the presence of an abundant amount of 18S rRNA clones in the fat body cDNA library used in this study. Accordingly, increasing the number of colonies screened should help in obtaining the objective clones.
The results obtained in this study are the first step toward our goal to explore the regulatory mechanisms of gene expression of antibacterial proteins in *O. rhinoceros*. Systematic isolation of various antibacterial protein cDNAs and genes from this insect is presently in progress in our laboratory.

**ACKNOWLEDGEMENTS**

We thank Mr. K. Kuramori and Mr. S. Hanashiio, Okinawa Prefectural Plant Disease and Insect Control Station for collecting the coconut rhinoceros beetles. We are also indebted to Dr. D. Taylor for critical reading of this manuscript. This work was partly supported by Enhancement of Center of Excellence, Special Coordination Funds for Promoting Science and Technology, Science and Technology Agency, Japan and by Grants-in-Aid (Bio Media Program, BMP 97-V-1-5-3) from the Ministry of Agriculture, Forestry and Fisheries, Japan.

**REFERENCES**


