Isolation of a cDNA Encoding a Homologue of Ribosomal Protein L36 in a Marine Green Alga, Enteromorpha compressa

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The multicellular green alga Enteromorpha (Ul- vales, Chlorophyta) includes some economically valuable species cultivated as "Aonori" (green laver) in Japan. The life cycle of the genus Enteromorpha consists of gametophytic and sporophytic foliaceous thalli, which are indistinguishable in appearance. Both male and female gametes of E. compressa can grow parthenogenetically and can be cloned into gametophytic thalli; thus, separate cultures of male and female gametophytes can be perpetuated from unfused gametes. It is possible to collect a quantity of these clonal gametophytic thalli for the experimental materials in only a few weeks; it completes its life cycle in laboratory culture within a few months and has a comparatively small number of chromosomes (n = 9) in the haploid phase.1 The haploid genome size of E. compressa (0.9 x 10^8 bp) is a little smaller than that of Arabidopsis thaliana (unpublished data). It is easy to induce gametogenesis in E. compressa artificially (Kuwano et al., to be published elsewhere), therefore, we can easily collect experimental materials at synchronized developmental stages. These characters of E. compressa indicate that it is advantageous to study for genetic analysis, including both classical and modern molecular investigations in marine sciences. According to phylogenetic analysis, sequence comparisons suggest that all living green plants belong to one of two major phyla: Streptophyta (land plants and their closest green algal relatives, the charophytes); and Chlorophyta (all other green algae).2 It should be noted that molecular biology studies of the development of Chlorophyta including Enteromorpha are quite limited,3 although its phylogenetic position, which is not linked directly with land plants, is very unique.

We have searched for genes expressed during gametogenesis in E. compressa. Stage specificity of the expression of identical genes should be confirmed using Northern blot analysis with staged RNA samples. Furthermore, to ensure comparable loading of RNA samples, the results of analyses need to be compared with that using an internal standard gene that is constitutively expressed (e.g. β-actin gene in vertebrates). In the present study, a gene that is constitutively expressed during gametophytic generation of E. compressa was identified. This gene encodes a green algal homologue of ribosomal protein L36. This gene was identified during isolation of other gene homologues that are involved in the development of reproductive organs of higher plants.

The determination of EcRL36 cDNA sequence was performed by RT-PCR and RACE (rapid amplification of cDNA ends)-PCR. The cDNA sequence was 530 bp long excluding this string of 26 A's found at the 3' end (Fig. 1). It is not clear whether this string of 26 A's is part of the poly(A) tail, as no typical polyadenylation signal (AATAAA) was found upstream of it. An open reading frame (ORF) of 101 amino acids was found in this cDNA. Using the MOTIF program in the PROSITE pattern of the PROSITE database,5 this predicted protein was found to contain a ribosomal protein L36 signature similar to L36 proteins in other species, and the protein was thus named EcRL36 (Enteromorpha compressa ribosomal protein L36). The EcRL36 protein was the most similar to the L36 protein in A. thaliana (PIR accession number T02526 in a nr-aa of protein sequence database, version 2.0.10). Between EcRL36 and A. thaliana L36, amino acid identity was found at 60 (59.4%) of 101 positions (Fig. 2).

Expression of the EcRL36 mRNA was examined using Northern analysis in vegetative thalli cultured at medium (Vegetative-M) or low temperature (Vegetative-L)6 and in reproductive thalli during gametophytic generation. The major signal band was observed at approximately 0.55 kb (Fig. 3). The size of this band was in fair agreement with that of the cDNA clone (530 bp). The level of hybridization changed (Table 1), as the lowest level (reproductive thalli) was about 50% of the highest level (Vegetative-M). According to one study of Saccharomyces cerevisiae, depression of the relative rates...
Ribosomal Protein Gene from *Enteromorpha*

Figure 1. Nucleotide sequence of the *EcRL36* cDNA from *E. compressa*. The amino acid sequence is shown in one-letter code below the respective codons and an asterisk indicates a stop codon. The ribosomal protein L36e signature is doubly underlined and the *Ec.RL36*-specific probe used for Northern analysis is singly underlined, and *Mbo* II and *Sac* I show restriction enzyme sites. The two gene-specific primer sequences used for the RT-PCR and the RACE-PCR are marked with arrows. For RT-PCR and RACE-PCR, poly(A)+RNA of *E. compressa* strain MGEC-1 was prepared from the reproductive thalli (4-5 cm long) using the Dynabeads mRNA DIRECT Kit (Dynal). Poly(A)+RNA was purified further using Isogen-LS (Wako Pure Chemical Industries). Complementary DNAs from each mRNA sample were synthesized by using the Superscript II reverse transcriptase (Gibco BRL) and original oligo dT primer (adapter primer) [5'-CTAATACGACTCACTATAGGGCTCGAGCG(T) 17-3']. To obtain the cDNA sequence of the 3' region of the *EcRL36* gene, RT-PCR was performed using the cDNA as template, the adapter primer described above, and the 60SF1 gene-specific primer with *GeneTaq* (Wako Pure Chemical Industries). PCR was performed as follows: 5 min at 94°C followed by 30 cycles of 30 sec at 94°C, 30 sec at 57°C, and 1 min at 72°C. The PCR products were separated on 1.5% agarose gels. Fragments of about 300 bp were purified and cloned into the pT7Blue T-vector (Novagen), and sequenced. To obtain cDNA sequence of the 5' region of the *EcRL36* gene, RACE-PCR was carried out using the Marathon cDNA Amplification Kit (Clontech) according to instructions from the manufacturer with 60SR1 gene-specific primer. First strand cDNA was synthesized as described above except that *BcaBEST Polymerase* (Takara) was used instead of Superscript II as the reverse transcriptase. RACE-PCR was performed under the same condition as the RT-PCR.

Table 1. Quantification of relative levels of the *EcRL36* mRNA.

<table>
<thead>
<tr>
<th>Stage</th>
<th>mRNA level*</th>
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<tbody>
<tr>
<td>Vegetative-M</td>
<td>100</td>
</tr>
<tr>
<td>Reproductive thalli</td>
<td>52.3</td>
</tr>
<tr>
<td>Vegetative-L</td>
<td>97.0</td>
</tr>
</tbody>
</table>

*Levels of the *EcRL36* mRNA in the Northern blot shown in Fig. 3 were quantified using a FLA 2000 computerized densitometer scanner (Fuji Film). The level of *EcRL36* mRNA at the "Vegetative-M" stage was arbitrarily set at 100.*
EcRL36  
Arabidopsis L36  

Figure 2. Alignment of the amino acid sequences between EcRL36 and Arabidopsis thaliana L36. EcRL36 was aligned using the CLUSTAL W program with ribosomal protein L36 from A. thaliana (PIR accession number T02526). An asterisk indicates an exact match between residues; a colon indicates a match between highly similar amino acids; a dot indicates a match between similar amino acids; a dash indicates a gap added to the sequence.

Figure 3. Northern analysis of the EcRL36 mRNA from E. compressa. A: Total RNA (15 μg per lane) from gametophytes: the "Vegetative-M" (4–5 cm long: lane 1), reproductive thalli (4–5 cm long: lane 2), and "Vegetative-L" (4–5 cm long: lane 3) were run on a 1.5% formaldehyde-agarose gel, transferred to a NYTRAN membrane (Schleicher & Schuell) and hybridized with 153 bp Mbo II-Sac I fragments (see Fig. 1) that were radio-labeled with [α-32P]dATP. Conditions for the hybridization and subsequent washing have been described previously. Total RNA was prepared by using the CTAB method that was modified as follows: the extraction buffer was used at a ratio of 1:14 (w/v), and total RNA was purified further by using Isogen-LS (Wako Pure Chemical Industries). Positions of four molecular weight markers (4.40, 2.73, 1.35, and 0.24 kb) are indicated. B: Ethidium bromide staining of 28S and 18S rRNAs demonstrates comparable loading of the RNA samples.

was performed (Fig. 4). The phylogenetic trees, which were constructed by the neighbor-joining method, the most parsimony and the maximum likelihood methods, showed similar tree topology. The organisms used in the present study formed the following three groups: 1) fungi, Saccharomyces cerevisiae A, S. cerevisiae B, Candida albicans, Schizosaccharomyces pombe; 2) animals, Mus musculus, Rattus norvegicus, Drosophila melanogaster; 3) plants, Daucus carota, A. thaliana, E. compressa. The unique representative of Chlorophyta, E. compressa formed a sister group of plant lineage, although we did not obtain a high bootstrap value (77%). This analysis of the ribosomal protein L36 sequences shows that E. compressa may represent a coherent and unique lineage relative to plants.

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Figure 4. Phylogenetic tree inferred from an alignment of the amino acid sequences of ribosomal proteins L36. The tree was constructed by the neighbor-joining method. The number at each node shows the bootstrap value of the branch, which was calculated from 1000 bootstrap replications. The scale bar represents 0.1 mutations per site. Branch lengths are drawn to scale. Species [with SWISS-PROT or EMBL (for Arabidopsis thaliana and Enteromorpha compressa) accession numbers in parentheses] are as follows: Saccharomyces cerevisiae A (baker's yeast; P05745); Saccharomyces cerevisiae B (baker's yeast; O14455); Candida albicans (yeast; P47834); Schizosaccharomyces pombe (fission yeast; Q92365); Mus musculus (mouse; P47964); Rattus norvegicus (rat; P39032); Drosophila melanogaster (fruit fly; P49630); Caenorhabditis elegans (nematode; P49181); Daucus carota (land plant; P52866); A. thaliana (land plant; T02526); E. compressa (green alga; AB045113; this report).

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References


