The ribosomal protein S2 from the pacific oyster *Crassostrea gigas*

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Abstract

We report the sequence of a pacific oyster cDNA homologous to the ribosomal protein S2. The entire sequence of the cDNA is 872 nucleotides in length containing an 828 open reading frame. The predicted protein with a deduced molecular weight of 30,101 is very similar to the ribosomal proteins from other animal species. Alignment with the ribosomal proteins of mouse and Drosophila indicates that a glycine-rich sequence in the 5'-end was deleted in the pacific oyster and mouse.

Introduction

The ribosome is a macromolecular assembly which consists of four different molecules of RNA and at least 80 different ribosomal proteins, and is responsible for protein synthesis in all cells (1). Because of the fundamental importance, cloning and sequence analysis of ribosomal proteins have been done in many vertebrates and some invertebrates such as fruit flies, nematodes, and echinoderms (2). Several lines of evidence from these studies indicate that ribosomal proteins has an important role in cell growth, division, and development. For example, a deletion in the gene encoding ribosomal protein S6 in adult mice affects cell cycle (3). In human, a quantitative reduction in synthesis of the ribosomal protein S4 which is linked to X-chromosome is observed in individuals with Turner syndrome having the phenotype, which includes short stature and infertility (4). In *Drosophila melanogaster*, the ribosomal proteins S2 is identified as a gene required for normal development of oocyte (5). The ribosomal proteins S2 gene in *Drosophila* is mapped in *spring of pearls* (*sop*) which is a recessive female sterile mutant isolated in a P element enhancer trap screen. Oogenesis in homozygous sop females arrests at approximately stage 5. In addition, homozygous flies of both sexes have Minute-like characteristics that include reduced bristles, delayed development and larval lethality.

In spite of the importance of the ribosomal protein S2 in cell growth and development, to our knowledge, sequence information is not available in mollusc. Then, here we describe a sequence of the ribosomal protein S2 from the pacific oyster, *Crassostrea gigas*.

Materials and Methods

Sequencing. The nucleotide sequences of cDNAs cloned into pT7Blue and pGEM-T easy TA cloning vectors were determined with a Big-Dye terminator kit and ABI 377 DNA sequencer (PE Biosystems). Each sequence was translated into amino acid sequence in six frames and subjected to search for similarity against protein database using the BLAST program.

5'- and 3'-RACE. RACE methods are performed according to the manufactures instructions (Clontech)

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using 2 μg of mantle poly (A)$^+$RNA. As specific primers in the nucleotide sequence of No.135 cDNA, an antisense primer (5'- ATCTTGTTACCCCAATACCCTCTGC -3') for 5'-RACE and a sense primer (5'- CTGC TACAGATGGCTGGAATTGATG -3') for 3'-RACE were used. The amplified products after PCR were cloned into the pGEM-T easy TA cloning vector.

Results and Discussion

To understand the mechanism of calcification, a suppression subtractive hybridization method was employed to characterize genes expressed in the mantle of the pacific oyster *C. gigas*. This experiment enabled us to isolate a cDNA fragment (No. 135) encoding the ribosomal protein S2. Primers based on its sequence were used to amplify PCR products by the RACE method, which resulted in isolation of a 482 bp fragment for 5'-RACE and a 302 bp fragment for 3'-RACE (Fig. 1). The entire sequence of No. 135 cDNA is 872 bp in length and shows an 828 nucleotide open reading frame coding for a 276 amino acid protein (Fig. 2). The predicted sequence is highly homologous to ribosomal protein S2 genes in various animals. At the amino acid level, it showed 79-82% identity to mouse and Drosophila sequences. Multiple alignment of ribosomal protein S2 in oyster, mouse, and Drosophila indicates that the sequences of oyster and Drosophila have a deletion of a glycine-rich sequence in 5'-end (Fig. 3). This deletion in 5'-end seems to be conserved in protostomes, suggesting that the ancestral Bilateria from which the mollusc and arthropod have derived may have the glycine-rich sequence in 5'-end, and its deletion have occurred in prior to evolution of protostomes. It is also possible to think that the ancestral Bilateria did not contain the glycine-rich sequence in 5'-end, and then, the sequence was inserted in the early stage of diversification of deuterostomes.

It is reported that the ribosomal protein S2 is essential in Drosophila oogenesis, in which the mutation of the ribosomal protein S2 inhibits the progression of cell division. In addition, bristle formation and larval development are perturbed in the homozygous mutant (5). Isolation of the ribosomal protein gene from the mantle by a suppression subtractive hybridization method means that the gene is expressed highly in the mantle. Therefore, the ribosomal protein may have a significant role in cell division and cell differentiation in the mantle of molluscs.

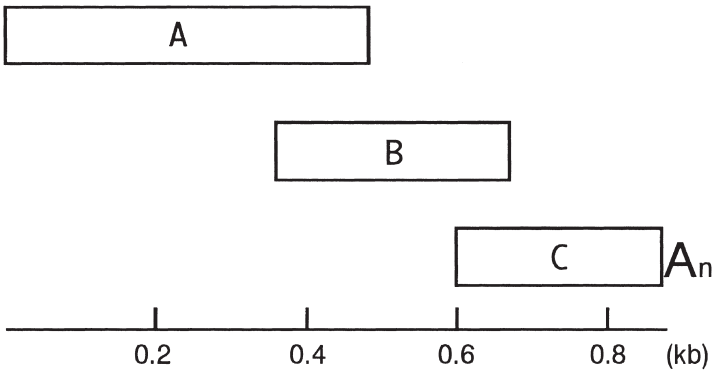

Fig. 1. Cloning of the cDNAs encoding the ribosomal protein S2 in the pacific oyster. A: 5'-RACE product. B: No. 135 cDNA isolated by the SSH method. C:3'-RACE product followed by poly-A tail.

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  1  CCAATGGCGGACGCACCAGCCGGTGGTAGAGGAGGTTTCCGGGGTGGATTCGGCTCTGGTGGTAGAGGCCGAGGCAGGGGACGCGGTCGT
        M  A  D  A  P  A  G  G  R  G  G  F  R  G  G  F  G  S  G  G  R  G  R  G  R  G  R  G  R     29

 91  GGACGAGGCCGTGGAAGGGGGCGTGGTGCCAAGGAGGGAGAGAAGGAGTGGATGCCAGTGACTAAACTTGGCAGGCTTGTGAAAGACCAG
     G  R  G  R  G  R  G  R  G  A  K  E  G  E  K  E  W  M  P  V  T  K  L  G  R  L  V  K  D  Q     59

181  GAAATCAAATCATTACAAGAAATCTACCTTTTCTCCTTGCCAATTAAGGAGTTTGAGATCATTGATTATTTCCTTGGACCATCTCTGAAG
     E  I  K  S  L  Q  E  I  Y  L  F  S  L  P  I  K  E  F  E  I  I  D  Y  F  L  G  P  S  L  K     89

271  GATGAAGTTTTGAAAATCATGCCTGTGCAGAAACAGACACGGGCTGGTCAAAGAACCAGATTCAAGGCTTTTGTCGCCATCGGAGACCAC
     D  E  V  L  K  I  M  P  V  Q  K  Q  T  R  A  G  Q  R  T  R  F  K  A  F  V  A  I  G  D  H    119

361  GATGGACACGTAGGTTTGGGAGTTAAATGCTCCAAGGAAGTAGCCACAGCTATCCGGGGTGCCATCATTCTGGCTAAGCTAACAGTCATC
     D  G  H  V  G  L  G  V  K  C  S  K  E  V  A  T  A  I  R  G  A  I  I  L  A  K  L  T  V  I    149

451  CCTGTGCGCAGAGGGTATTGGGGTAACAAGATCGGAAAACCCCACACTGTACCATGCAAGGTGACAGGAAAATGTGGCAGTGTTGTGGTG
     P  V  R  R  G  Y  W  G  N  K  I  G  K  P  H  T  V  P  C  K  V  T  G  K  C  G  S  V  V  V    179

541  AGGTTGATCCCAGCACCCAGAGGTACTGGTATTGTCAGTGCTCCTGTACCCAAGAAACTGCTACAGATGGCTGGAATTGATGATTGCTAC
     R  L  I  P  A  P  R  G  T  G  I  V  S  A  P  V  P  K  K  L  L  Q  M  A  G  I  D  D  C  Y    209

631  ACCTGTGCCAGGGGATGTACAGCTACGCTTGGCAACTTTGCAAAAGCAACATACGCTGCCATTGCCAACACTTATTCTTACTTGACCCCT
     T  C  A  R  G  C  T  A  T  L  G  N  F  A  K  A  T  Y  A  A  I  A  N  T  Y  S  Y  L  T  P    239

721  GACCTGTGGAAGGAGACTGCCTTCACCAAGTCTCCATACCAGGAGTTCACTGATTACCTGGCCAAGAACCACACCCGCCAGCCCGGACAG
     D  L  W  K  E  T  A  F  T  K  S  P  Y  Q  E  F  T  D  Y  L  A  K  N  H  T  R  Q  P  G  Q    269

811  AGACAGGAGCAGAAGGTGTACTAAGCTTATTAAACCATTGAAATAAAGGCTTATAAACCCTG
     R  Q  E  Q  K  V  Y  *                                                                      276
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Fig. 2. Nucleotide and deduced amino acid sequence of the ribosomal protein S2 of C. gigas. The asterisk indicates a stop codon. A conceptual polyadenylation signal is underlined.

Fig. 3. Alignment of the ribosomal protein S2 sequences from C. gigas, Drosophila, and mouse. The residues are boxed when conserved in the sequences. A dash indicates a gap added to the sequence.

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和 文 抄 録

マガキ *Crassostrea gigas* のリボソームタンパク質 S2

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マガキ（*Crassostrea gigas*）の外套膜から単離したリボソームタンパク質 S2 をコードする cDNA（872 塩基）の塩基配列を決定した。予想されるアミノ酸配列は分子量 30,101 で他の多くの動物種由来のリボソームタンパク質 S2 と高い相同性を示した。マウスおよびショウジョウバエのリボソームタンパク質 S2 との比較を行ったところ、マガキとショウジョウバエでは、5' 端領域においてグリシンリッチな配列が欠失していることが明らかとなった。