

Conserved Ribosomal Protein Sequences S5, S18, S27, S30 in the Pacific Oyster *Crassostrea gigas* and *Crassostrea virginica*

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Abstract

cDNA fragments encoding ribosomal proteins were isolated from the mantle of pacific oyster *Crassostrea gigas* by the subtractive hybridization method. The sequence information was used to isolate entire cDNAs, and the predicted amino acid sequences were shown to be very similar to the 40S ribosomal protein genes (S5, S18, S27, and S30) of *Crassostrea virginica*. Northern blot hybridization revealed that S5, S18, and S30 were predominantly expressed in the digestive gland, the gill, and the mantle. On the other hand, S27 was highly expressed in the adductor muscle.

1. Introduction

The mantle surrounds the whole body of a mollusc and is responsible for shell formation. Its important role in shell formation is to produce organic matrix proteins that are secreted into the extrapallial space and then regulate the crystallization of calcium carbonate. The process of shell formation, calcification, begins with the determination of which type of crystal, aragonite or calcite, should be made. Some organic matrix proteins have been isolated⁽¹⁻¹⁰⁾ and shown to regulate the formation of the two types of calcium carbonate crystal^(11,12). These studies indicate that various organic matrix proteins have been found and have evolved in molluscs. We have been undertaking, by use of suppression subtractive hybridization (SSH) method, a study to identify the genes expressed in the mantle of the pacific oyster *Crassostrea gigas*⁽¹³⁾. In the analyzed sequences, 4 ribosomal proteins (S5, S18, S27, S30) were identified. Ribosomes consist of many different proteins, of which sequences are reported mainly in vertebrates and several model species. In contrast, comparatively little is known about ribosomal protein sequences in molluscs. We report here the cDNA sequences of the four ribosomal proteins. Ribosomal protein S27, which has a zinc finger domain, is expressed abundantly in the adductor muscle.

2. Results and Discussion

Because the 4 cDNAs encoding ribosomal protein were partial, to determine the whole nucleotide sequences, we isolated the 5'-end and 3'-end cDNAs by the RACE method using oligonucleotides specific to the 4 cDNAs as primers. The nucleotide sequence of the ribosomal protein S5 showed an open reading frame consisting of 612 bp followed by a canonical polyadenylation signal and poly (A) tail (Fig. 1). The predicted amino acid sequence showed 99% identity to the *C. virginica* S5 sequence (Fig. 5). *C. gigas* S5 was nearly equal in identity to human and mouse S5 (91% and 90% respectively), while it showed 84% identity to *Drosophila* S5, which is slightly less than the identity between the human and *Drosophila* sequences. The sequenced cDNA of S18 is 523bp in length and showed a 456 nucleotide open reading frame coding for a 152 amino acid sequence (Fig. 2). The predicted protein showed 99% identity to *C. virginica* S18 (Fig. 5) and 83% identity to human and mouse S18. To *Drosophila* S18, it showed 78% identity, which is slightly more than the identity between the human and *Drosophila* sequences. The sequenced cDNA of S27 contained a single ATG-initiated open reading frame of 252bp (Fig. 3). The predicted protein showed 100% identity to *C. virginica* S18 (Fig. 5) and 93% identity to human and mouse S27. To *Drosophila* S27, it showed 80% identity, which is slightly less than the identity between the human and *Drosophila* sequences. The sequenced cDNA of S30 showed an open reading frame that starts at position 39 and includes 131 amino acids (Fig. 4). In several eukaryotic organisms, ribosomal protein S30 was shown to be transcribed as a single mRNA that is a fusion

Received 20 June 2005

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with a ubiquitin-like sequence⁽¹⁴⁾. As a result, a fusion protein is translated, then undergoes posttranslational processing to yield the S30 protein and the ubiquitin-like protein, respectively. Similarly the *C. gigas* S30 cDNA showed a fusion sequence composed of the ribosomal protein and the ubiquitin-like protein. When compared to *C. virginica* S30, the amino-terminal ubiquitin-like protein region showed 90% identity and the calboxy-terminal ribosomal protein S30 region showed 98% identity (Fig. 5). This difference of the identities indicates that two regions (ubiquitin-like protein and ribosomal protein S30) of the fusion gene have evolved under different selective pressure. Comparison of the determined sequence of 4 ribosomal proteins of *C. gigas* with counterparts of *C. virginica* showed striking identity, indicating that *C. gigas* and *C. virginica* are highly correlated evolutionarily. This notion is consistent with the result obtained from sequence analyses of 18S rRNA and 28S rRNA in many mollusc species⁽¹⁵⁾.

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1  TGTTAACGGCACTATGACTGAGAAGTGGGATGAGCCTGCTCCGGCAGTAGAATTGCCAGA
      M T E N W D E P A P A V E L P E 16
61  AATCAAGCTCTTTGGCAATGGTCATCAGATGATGTCCAAGTCAGCGACATCAGTTTAAC
      I K L F G K W S S D D V Q V S D I S L T 36
121 TGATTACATTGCTGTCAAAGAGAAGTATGCAAAATATTTGCCCACTCCTCAGGCAGATA
      D Y I A V K E K Y A K Y L P H S S G R Y 56
181 CCAAGTAAAGAGATTTAGAAAATCACAGTGCCCAATTGTTGAACGCCTGACATGTTCACT
      Q V K R F R K S Q C P I V E R L T C S L 76
241 TATGATGCATGGAAGAAACAATGGAAAGAAACTCTTGACAACCCGATTGTGAAACATGC
      M M H G R N N G K K L L T T R I V K H A 96
301 CTTTGAAATCATTCACTTGCTCACAGGAGAAAACCTCTCCAAGTTTTGGTGAATGCCAT
      F E I I H L L T G E N P L Q V L V N A I 116
361 CATCAACAGTGGCCCCGTGAGGACTCCACTCGTATTGGTCGTGCTGGTACCGTCAGGGG
      I N S G P R E D S T R I G R A G T V R R 136
421 TCAGGCTGTGGACGTCTCTCCACTGAGGCGTGTCAACAGGCCATCTGGCTCCTGTGTAC
      Q A V D V S P L R R V N Q A I W L L C T 156
481 CGGGGCACGTGAAGCCTCTTCAGGAATATCAAGACTATTGCTGAGTGTGGCTGATGA
      G A R E A S F R N I K T I A E C L A D E 176
541 GCTGATCAATGCTGCCAAGGATCTTCAAACCCATGCCATCAAGAAGAAGGATGAATT
      L I N A A K G S S N S H A I K K K D E L 196
601 GGAACGTGTGGCAAGTCCAACCGATAAACTATTTACTGTGCTTCTGTGAACAGGAAAA
      E R V A K S N R * 204
661 TAAACTGTCAGGCA

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Fig. 1 Nucleotide and deduced amino acid sequence of ribosomal protein S5.

The nucleotide positions are indicated on the left and the amino acid positions are indicated on the right. The putative polyadenylation sequence is underlined. The stop codon is marked with an asterisk. DDBJ accession number: AB199894

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1  CTTTTCCGCTGAATTATCAACAATGGCTTTGATACTGCCAGAGAAGTTTCAGCACATTCT
      M A L I L P E K F Q H I L 13
61  TCGTATCCTCAACACAAATATTGATGGACGAAGGAAAATTATGTTTCGCTATGACTGCCAT
      R I L N T N I D G R R K I M F A M T A I 33
121 CAAGGGTATCGGTAGACGATATGCTAATGTTGTCTGCAAGAAAGCTGATGTAGATATCAC
      K G I G R R Y A N V V C K K A D V D I T 53
181 AAAAAGGGCAGGGGAACCTCTCAGAAGAAGAGATTGACAAAATTGTCACAATTATGCAGAA
      K R A G E L S E E E I D K I V T I M Q N 73
241 CCCTCGTCAGTACAAGATTCTGACTGGTTCCTTAACAGGCAGAAGGACATTAAGGATGG
      P R Q Y K I P D W F L N R Q K D I K D G 93
301 TAAATTCAGCCAGGTCATGTCCAACACTGGACAACAACTCCGTGAGGATCTGGAGCG
      K F S Q V M S N T L D N K L R E D L E R 113
361 ACTAAAGAAGATCCGAGCACACAGAGGCTCCGTCACACTGGGGTCTAAGAGTGAGAGG
      L K K I R A H R G L R H Y W G L R V R G 133
421 TCAGCACACAAGACCACAGGAAGAAGAGGAAGAACTGTTGGTGTGGCCAAGAAGAAGTA
      Q H T K T T G R R G R T V G V A K K K * 152
481 AACTGTGTAATGAGGGAGGAAAAGTAAAAAAAAGTGATCCA

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Fig. 2 Nucleotide and deduced amino acid sequence of ribosomal protein S18.

The nucleotide positions are indicated on the left and the amino acid positions are indicated on the right. The stop codon is marked with an asterisk. DDBJ accession number: AB199895

To investigate the expression of 4 ribosomal proteins of *C. gigas*, Northern blot analysis was conducted using cDNA fragments as probes. The signals of 4 transcripts were stronger in the mantle than in the egg. This finding is in agreement with the fact that the cDNAs were isolated as sequences expressed highly in the mantle by the SSH method. In invertebrates, early development is relatively independent of newly synthesized transcripts, and zygotic gene activation is usually observed after the cleavage stage. Low expression of the 4 ribosomal protein genes suggests that translational activity is relatively low in the eggs of molluscs. As for S5, S18, and S30, hybridization signals were equally detected in the digestive gland, the gill, and the mantle. S27 was expressed highly in the adductor muscle. It is reported that the ribosomal protein S27 is differentially expressed in the tissues of mammals⁽¹⁶⁾. In human periodontal ligament cells, which connect the teeth and the dental alveoli, S27 expression is induced by mechanical stress⁽¹⁷⁾. Both the periodontal ligament in mammals and the adductor muscle in molluscs are fibrous tissue and are exposed to mechanical stress, suggesting that the ribosomal protein S27 is involved in the morphogenesis and maintenance of these tissues in both vertebrates and invertebrates.

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1  ATGCCTCTCGCTAAAGATTTATTGCATCCCTCTTTGGAGGAGGAGAAAAGAACATGCAAA
   M P L A K D L L H P S L E E E K R T C K      20
61  CTGAAGAGATTGGTCCAAAGTCCAAACAGTTATTTTCATGGATGTTAAATGTCCAGGATGC
   L K R L V Q S P N S Y F M D V K C P G C      60
121 TACAAGATTACCACAGTTTTTCAGCCACGCCAGACGGTGGTGTATGTGTGGGGTGCTCC
   Y K I T T V F S H A Q T V V L C V G C S      80
181 ACAGTGTCTGCCAGCCACCCGGGGGAAAGCCAGACTCACAGAGGGTGTCTCTTCCGT
   T V L C Q P T G G K A R L T E G C S F R      100
241 AAGAAGTCGCACTAGACTGACAACGTTATTTATATAGGCTGTATGAGAAAGAGAGGCTGA
   K K S H *      104
301 GATATTTGGGGTTGGCATGGCGACCGTCGTCACATTTGGACTTTGTGATACTTTCATT
361 TATTTTTTTTTCTTGGACAACACGCATAATTGTTCTTTGATTACGACCCCTGGGTGAGA
421 TCATCAATCGTCTCAGAAGATTCCACACCCAGGCTTTG

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Fig. 3 Nucleotide and deduced amino acid sequence of ribosomal protein S27.

The nucleotide positions are indicated on the left and the amino acid positions are indicated on the right. The stop codon is marked with an asterisk. DDBJ accession number: AB199896

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1  GTAGCTGCCATTTGTGATTGCACAAAACGTAAGACAATGCAGTTGTTGTACGAGGTA
   M Q L F V R G S      8
61  GCGAGACTCATGCCCTTGCAGTTGGCAGGAAACGAAACTGTGTCGACATCAAGAATTTGA
   E T H A L Q L A G N E T V S D I K N L I      28
121 TTAGCAAGAGAGAGGGGTTTCCAGTTGAGGAACAGATTATTCTGTATGCCGGCAAACCCAC
   S K R E G F P V E E Q I I L Y A G K P L      48
181 TTCAGGATGAATATGAGTTGACCAAATTAATGACTTGTCCACCCTGGACATTGAAGTCA
   Q D E Y E L T K L N D L S T L D I E V R      68
241 GAATGCTTGGAGGTAAAGTCCATGGCTCTCTTGTCTGTCGCGGAAAAGTCAAGGGACAGA
   M L G G K V H G S L A R A G K V K G Q T      88
301 CCCCAAAGGTTGAGAAACAAGAGAAGAAGAAACAGAGGACAGGAAGGGCCAAGAGACGCA
   P K V E K Q E K K K Q R T G R A K R R M      108
361 TGCAGTACAACAGAAGATTTGGAGTTGTCGTCTCTACATTGCGGTCGAGAAAGGGACCCA
   Q Y N R R F G V V V S T F G R R K G P N      128
421 ATGCTAACTCCTAAAATGTGTTTCTATCAAAAACGGATTAATAATTCGTTGTAGCTGCA
   A N S *      131

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Fig. 4 Nucleotide and deduced amino acid sequence of ribosomal protein S30.

The ubiquitin-like protein region is boxed. The nucleotide positions are indicated on the left and the amino acid positions are indicated on the right. The stop codon is marked with an asterisk. DDBJ accession number: AB199897

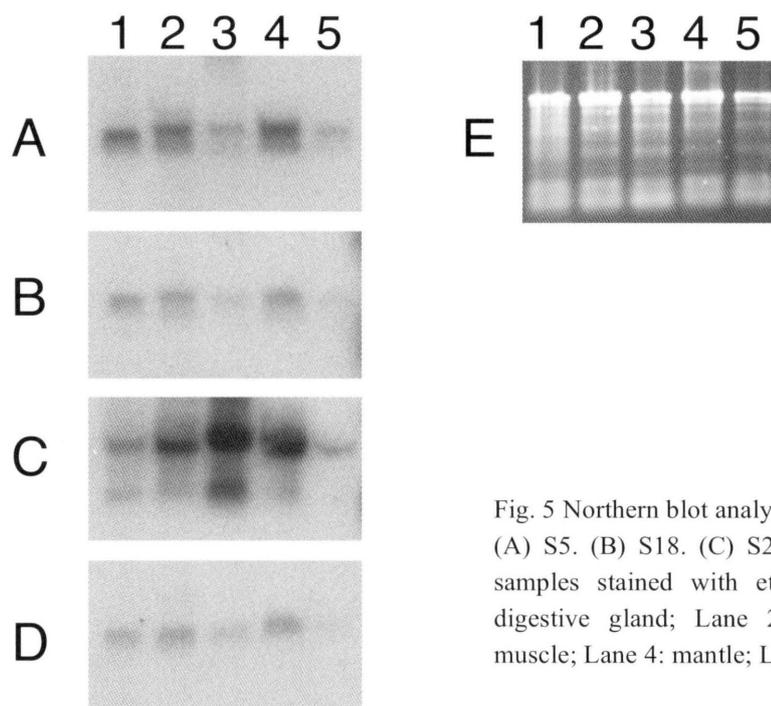


Fig. 5 Northern blot analysis of 4 ribosomal proteins. (A) S5. (B) S18. (C) S27. (D) S30. (E) The same samples stained with ethidium bromide. Lane 1: digestive gland; Lane 2: gill; Lane 3: adductor muscle; Lane 4: mantle; Lane 5: egg.

3. Acknowledgements

This work was supported by grants from Wakayama Prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence of JST (Japan science and technology agency) and the ministry of Education, Culture, Sports, Science and Technology of Japan.

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和文抄録

マガキ *Crassostrea gigas* と *Crassostrea virginica* において高度に保存された リボソームタンパク質 S5, S18, S27, S30

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マガキ *Crassostrea gigas* の 40S リボソームタンパク質 S5, S18, S27, S30 に関して、cDNA を単離し塩基配列を決定した。予想されるアミノ酸配列は、いずれも *Crassostrea virginica* 種において明らかにされている配列と高い相同性を示した。S30 は、ユビキチン様ドメインと融合遺伝子として転写されており、*Crassostrea gigas* と *Crassostrea virginica* においてひとつの転写ユニットであるにもかかわらず、ユビキチン様ドメインと S30 領域では、相同性において明らかな違いがみられ、モザイク的な進化が生じた考えられる。発現様式は、それぞれのサブユニットごとに違いがみられ、S27 では、閉殻筋での強い発現が認められた。

