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A novel isoform of actin in the pacific oyster

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SUMMARY

A cDNA encoding a novel isoform of the actin gene was isolated from the pacific oyster *Crassostrea gigas*. Sequencing of the cDNA showed that the cDNA had 57-bp and 476-bp (excluding poly A) noncoding regions at the 5 ' and 3 ' ends, respectively. The cDNA could encode a 367 amino acid polypeptide that revealed significant similarity to *Placopecten magellanicus* actin rather than the previously identified actin of *C. gigas*, whereas the noncoding regions seem not to be relevant among them.

INTRODUCTION

Actin is a major contractile and structural protein present in virtually all eukaryotic cells and plays an important role in the generation of contractile force in muscle and motility in nonmuscle cells [1]. The actin genes are encoded by members of multigene families [2,3], exceptions being a yeast [4] and a protozoan. These multigene families show tissue-specific expression at different stages of development, suggesting that this various actin proteins may have a different functional basis. In vertebrate, actin isoforms are distinguished into three types: α actin for muscle, β actin for nonmuscle and γ actin that is present in both types of tissue. Muscle and nonmuscle actins of vertebrates can be characterized by the sequence of amino acids [5].

In actins, the rate of amino acid replacement is very low, and there is a high degree of similarity of coding region among actin genes. The conservation of the actin gene family provides an opportunity to investigate its evolution.

Three actin genes have been reported in the mollusc shellfish: *C. gigas* [6], *C. verginica, P. magellanicus* [7], and *Dreisssena polymorpha* [8]. The pacific oyster *C. gigas* is an important commercial species that inhabits intertidal rocks, exposes themselves to the periodically recurring tidal cycle that involves emergence and submergence. The oysters attach on hard substances, and as a consequence, the morphology of their valves is strongly influenced by the irregularity of the rock surface to which they are attached [9]. I am interested in calcification in *C. gigas* and have been investigating genes that are expressed in the mantle that is positively involved in regulation and maintenance of the extrapallial fluid, where calcium carbonate is crystallized. In this process, we have isolated a new isotype of the actin gene in *C. gigas*. We report here the nucleotide sequence of the new isotype and the comparison with the known actin gene in *C. gigas*.

MATERIALS AND METHODS

Sequencing. The nucleotide sequences of cDNAs cloned into pT7Blue vector 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.

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5'- and 3'-RACE. RACE methods are performed according to the manufactures instructions (Clontech) using 2 μg of mantle poly (A)+RNA. As specific primers in the nucleotide sequence of No.64 cDNA, an antisense primer (5'-TTCGTAGATGGGGACAGTGTGGGTG-3') for 5'-RACE and a sense primer (5'-CACTTACAATTCCATCATGAAGTGC-3') for 3'-RACE were used. The amplified products after PCR were cloned into pT7Blue TA cloning vectors.

RESULTS AND DISCUSSION

347 cDNAs were isolated from the mantle of *C. gigas* and their nucleotide sequences were determined. Comparing the sequences to the protein database, I found that No.64 cDNA was a part of an actin gene and more similar to actin of *Placopecten magellanicus* than that of *C. gigas*. To know the whole structure, we isolated the 5'- and 3'-end cDNAs by the RACE method using oligonucleotides specific to the sequence of No.64 cDNA as primers. In full length cDNA, we found an open reading frame encoding a polypeptide of 376 amino acids (Fig. 1) which showed 99% identity to *P. magellanicus* actin, while 96% identity to *C. gigas* actin (Table 1), indicating that No.64 is thought to be a new isotype of actin genes of *C. gigas*. Hereafter, we will use actin 1 and actin 2 to refer to the previously identified actin in *C. gigas* and No.64 actin, respectively. As shown in Table 1, in spite of the high identity in the nucleotide sequences of coding regions of actin 2 and *P. magellanicus* actin, the noncoding region of actin 2 exhibited low identities to both actin 1 and *P. magellanicus* actin. From the difference of the nucleotide sequences, it is evident that actin 1 and actin 2 are separate genes on chromosome. It is considered that actin 1 and 2 are derived from a common ancestral gene in *C. gigas*, and that they may function redundantly; alternatively, it seems possible that they are implicated in the same process in different tissues.

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MGDEEVAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQ  50
KDSYVGDEAQSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPE 100
EHPVLLTEAPLNPKANREKMTQIMFETFNAPAMYVAIQAVLSLYASGRTT 150
GIVLDSGDGVTHTVPIYEGYALPHAILRLDLAGRDLTDYLMKILTERGYS 200
FTTTAEREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVIT 250
IGNERFRCPESLFQPSFLGMESAGIHETTYNSIMKCDVDIRKDLYANTVL 300
SGGTTMFPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASL 350
STFQQMWISKQEYDESGPSIVHRKCF                          376
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Fig. 1 Deduced amino acid sequence of actin 2. The amino acid positions are indicated on the right.

Table 1

Nucleotide and amino acid sequence identity of actin 2 with actin 1 and *P. magellanicus* actin

	Protein coding region		5'-untranslated region	3'-untranslated region
	Nucleotides	Amino acids		
C. gigas actin 1	86	96	51	36
P. magellanicus actin	91	99	62	55

Note. Numbers are given as percentages of identical nucleotides or amino acid residues.

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和文抄録

牡蛎（*Crassostrea gigas*）の新しいアクチン遺伝子

宮本裕史

牡蛎（*Crassostrea gigas*）の外套膜で発現する遺伝子を解析する過程で、新しいアクチン遺伝子に由来するcDNAを単離した。5'RACEおよび3'RACEにより全長のcDNAを単離し、その塩基配列を決定したところ、367アミノ酸に翻訳される領域を確認することができた。5'端には57塩基、3'端には476塩基の非翻訳領域が存在していた。予想される367アミノ酸は、すでに報告されているマガキアクチンよりも、マゼランツキヒガイ *Placopecten magellanicus* において同定されているアクチンに対して、より高い類似性を示した。