Subtractive cloning of hypo-osmotic responsive gene in *Mytilus*

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INTRODUCTION

Most of marine invertebrates are osmoconformers, and the osmolality of their body fluids is influenced by the environmental osmolality. Thus, they must adapt to it at a cellular level when they experience change in environmental osmolality. This suggests that marine invertebrate cells are equipped with a specific osmoconforming mechanism that is lacking in most of vertebrate cells.

Presently, there has been no reports of the molecular mechanism of cellular adaptation to osmotic stress in marine invertebrates, except studies on amino acid transporters induced by change in environmental osmolality in the marine mussel *Mytilus galloprovincialis*¹⁾ and the pacific oyster *Crassostrea gigas*²⁾.

In this study, to acquire more information of the osmotic responsive mechanism in marine invertebrate cells, we proposed to clone genes specifically induced by change in osmolality, by suppression subtractive hybridization in *M. galloprovincialis*.

MATERIALS AND METHODS

We obtained the specimens of *M. galloprovincialis* cultured in Matoya bay, Japan. Mussels were kept in 3‰ artificial seawater for acclimation for at least 5 days. After the acclimation, mussels (n=3) were exposed to 1.5‰ artificial seawater (hypo-osmotic stress) for 24 h following which gill tissue samples were excised from stressed and control (24 h in 3‰ artificial seawater) and stored at -80°C. Total RNA was extracted from the tissue by guanidine isothiocyanate/cesium chloride method, and poly(A)⁺ RNA was isolated from the total RNA by oligo(dT) - latex beads (OligotexTM - dT30 (Super), TaKaRa).

Poly(A)⁺ RNA isolated from the control and hypo-

osmotic stressed gills were analyzed by suppression subtractive hybridization according to the manufacturer's instructions (PCR-Select cDNA Subtractions kit, CLONTECH Laboratories, Inc.).

RESULTS AND DISCUSSION

As a result of suppressive subtractive hybridization, we obtained a cDNA fragment of 216-base pairs containing a part of open reading frame, the transcript of which was markedly induced in the hypo-osmotic stressed gills, while no signal was detected in the control and hyper-osmotic stressed gills by northern blot analysis (Fig. 1). The fragment was subcloned into pGEM-T Easy vector (Promega Corporation) and tentatively named as hypo-osmotic responsive (HPOR) gene.

Fig. 1 Exclusive induction of the transcripts of HPOR gene by hypo-osmotic stress. Twenty micrograms of total RNA in each lane was extracted from gill of the control (1), exposed to hypo-osmotic stress (2), and hyper-osmotic stress (3) for 24 h. The cDNA fragment used as a probe is shown in Fig. 2.

For the identification of the full-length cDNA, we performed the 5'- and 3'- RACE by SMARTTM RACE cDNA Amplification Kit (CLONTECH Laboratories, Inc.) and obtained 233 base pairs 5'-fragment and 282 base pairs 3' fragment. The all nucleotide sequences of the cDNA fragments were determined with CEQ2000 (BECKMAN COULTER, Inc.).

cDNA and deduced amino acid sequence of HPOR gene were shown in Fig.2. It consists of 451 base pairs and contains a single open reading frame of 240 base pairs starting an ATG codon at position 47. This ATG (GCAATGG) strongly matches with the Kozak consensus sequence for translational initiation³. The cDNA encodes a protein with a calculated molecular mass of 9.6 kDa. The 3'-end of the sequence contains a poly(A) stretch, preceded by putative polyadenylation signals (AATAAA) at position 406 to 411.

The deduced amino acid sequence is not homologous to any reported protein in SWISSPROT, PIR, PDB and DAD searched by FASTA. The hydrophobicity plot and analysis by SOSUI indicate this protein is soluble, and has no signal peptide.

This is the first report of the gene specifically induced by hypo-osmotic stress in a marine invertebrate. According to the deduced amino acid sequence and northern blot analysis, HPOR gene encodes a novel cytosolic soluble protein that may play an important role in the cellular adaptation to hypo-osmotic stress in *M. galloprovincialis*. Further studies are required to elucidate the biological function of the translated product of HPOR gene.

REFERENCES

1. Hosoi M, Goto C, Takeuchi K, Kubota S, Sakaguchi M, Hayashi I, Yokoyama Y and Toyohara H. Amino acid transporter of mussel. *Comp. Biochem. Physiol.* 2000; 126A Suppl. 1: S70.
2. Goto C, Hosoi M, Takeuchi K, Sakaguchi M, Hayashi I, Yokoyama Y and Toyohara H. Amino acid transporter of oyster. *Comp. Biochem. Physiol.* 2000; 126A Suppl. 1: S63.
3. Kozak M. An analysis of 5'-noncoding sequence from 699 vertebrate messenger RNAs. *Nucleic Acids Res.* 1987; 15: 8125-8148.

Fig. 2 cDNA schematic representation and deduced amino acid sequence of HPOR gene. Kozak consensus sequence (44-50), polyadenylation signal (406-411) and poly(A) stretch (427-451) are indicated by the black boxes. The line (position 64-252) shows the probe used for the northern blot.