Molecular cloning of transducer gene \textit{hjtB} from extremely halophilic archaeon \textit{Haloarcula japonica}

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\textbf{ABSTRACT}

A transducer gene, \textit{hjtB}, was cloned from genomic DNA of extremely halophilic archaeon \textit{Haloarcula japonica}. The structural gene consisted of an open reading frame of 984 nucleotides encoding 328 amino acids. RT-PCR analysis revealed that this gene was transcribed in \textit{Ha. japonica}.

\textbf{INTRODUCTION}

Migration of microbes toward more favorable environments is called taxis. In tactic responses, it has been proposed that the information of environmental stimuli is transmitted to flagellar motors via transducer proteins. In bacteria and archaea, many studies have shown that tactic transducers have highly conserved signalling domains which relay stimulus information to signal transduction proteins.

\textit{Escherichia coli} has 5 transducer genes\textsuperscript{1}. On the other hand, 18 transducer genes have been found in extremely halophilic archaeon \textit{Halobacterium salinarum} (http://www.halolex.mpg.de/). It can be interpreted that extremely halophilic archaea have complex tactic systems.

\textit{Haloarcula japonica} strain TR-1 is a triangular disc-shaped extremely halophilic archaeon\textsuperscript{2}. Transducer genes \textit{hjtA} and \textit{hjtC} have been cloned from \textit{Ha. japonica} previously\textsuperscript{3}. Here, we describe molecular cloning and sequencing of another tactic transducer gene \textit{hjtB} from \textit{Ha. japonica}.

\textbf{RESULTS AND DISCUSSION}

For cloning of a transducer gene from \textit{Ha. japonica}, an FITC-labelled oligonucleotide probe of 27 mer, 5'-CAGACGAAACATGCTGGCGTTGAACGC-3', was synthesized and used. It corresponds to the amino acid sequence QTNMLALNA which is highly conserved in the known transducers\textsuperscript{4,5}. Genomic DNA of \textit{Ha. japonica} was digested with \textit{Pst} I, and analysed by Southern hybridization using the probe mentioned above. A hybridized band was detected around 2.4 kb. The 2.4-kb fragment was cloned and sequenced to identify a possible transducer gene consisting of 984 nucleotides (Fig. 1). An AT-rich region was found around 30 bp upstream of the initiation codon of \textit{hjtB}, suggesting that it might be the TATA box (boxA). The gene product of 328 amino acids contains the highly conserved sequence in signalling domains of transducers, although the molecular mass of the product seemed lower compared to other functionally-known transducers. These data suggested that \textit{HjtB} could be a novel transducer. In the 2.4-kb fragment, it was also revealed that partial sequence of genes encoding a putative carboxypeptidase and a molybdenum cofactor biosynthesis protein existed upstream and downstream of \textit{hjtB}, respectively.

Total RNA was extracted from \textit{Ha. japonica} and RT-PCR was performed to detect the transcript of \textit{hjtB}. An antisense primer B2-R1 (5'-TTGGCTGCGTGCGTGAC-TG-3') was used for reverse transcription; RT-products were then amplified with primers B3-F2 (5'- ATGGCTGAGAGCCGCAGGGAAAAC-3') and B2-R1. The result revealed that \textit{hjtB} was transcribed in \textit{Ha. japonica}.

\textbf{CONCLUSION}

The putative transducer gene \textit{hjtB} was cloned. Transcription of \textit{hjtB} was confirmed by RT-PCR, although the function of HjtB remained unknown. We recently developed gene disruption methodology of \textit{Ha. japonica}. In the near future, \textit{hjtB}-disrupted strain should tell us novel functions of the transducer.

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\textbf{REFERENCES}

Fig. 1 Nucleotide sequence and deduced amino acid sequence of hptB. Possible TATA box (boxA) is underlined; grey boxes indicate the amino acid sequence corresponding to the highly conserved sequence in signalling domains of transducers.