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Syntenin is involved in the bacteria clearance response of kuruma shrimp (*Marsupenaeus japonicus*)

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ABSTRACT

Syntenin is a multifunctional cytosolic adaptor protein that contributes to cell migration, proliferation, attachment, and apoptosis, as well as immune response to virus, in vertebrates. However, the functions of syntenin in the antibacterial response of invertebrates remain unclear. In this study, we identified a syntenin-like gene (*MjSyn*) from the kuruma shrimp (*Marsupenaeus japonicus*) and detected its function in the antibacterial immunity of shrimp. The full-length *MjSyn* was 1223 bp with a 963 bp open reading frame that encodes 320 amino acids. The deduced *MjSyn* proteins contained two atypical PDZ domains (sequence repeat that was first reported in the postsynaptic density protein or PSD-95, DlgA, and ZO-1 protein), an N-terminal domain, and a C-terminal domain. Reverse transcription (RT)-PCR results showed that *MjSyn* was expressed in all tested tissues. Quantitative real-time PCR analysis revealed that *MjSyn* transcripts in the hemocyte, gill, and intestine were significantly induced at various time points after infection with *Staphylococcus aureus* and *Vibrio anguillarum*. The knockdown of the expression of *MjSyn* by RNA interference resulted in a significant decrease in the phagocytic ability and increased bacteria number *in vivo* of shrimp. Moreover, the expression of *Mj*Cnx, a cytoplasma and membrane location lectin chaperone protein, was inhibited in the *MjSyn*-knocked down shrimp, which indicated a possible calnexin-related way. Thus, the *MjSyn* participates in the bacterial clearance response of kuruma shrimp, thereby providing new insight into the function of this kind of important adaptor protein.

1. Introduction

Adaptor proteins are physiologically pertinent molecules that are responsible for the regulation and integrity of signaling pathways by interacting with relevant proteins via specific conserved domains [1]. The PDZ (previously known as Discs-Large homology repeats [DHRs] or GLGF repeats)-domain-containing protein is an important and well-known family of adaptor proteins. These proteins are abundant (more than 400 copies in the human genome) in metazoan genomes and have also been identified in bacteria, plants, and yeast [2]. The PDZ domain is an 80–100 amino acid sequence homolog that was named by the three proteins in which these domains have been identified: the brain synaptic protein PSD-95, the Drosophila septate junction protein Discs-Large, and the epithelial tight junction protein ZO1 [3–5]. Crystallographic and/or nuclear maginatic resonance (NMR) results show that the PDZ domains consist of six β-strands and two α-helices, which are folded into a compact globular with an extended groove between the β-strand B and α-helix B for peptide ligand binding [6–8]. Functionally, the PDZ domain recognizes and binds the cytoplasmic tails of the transmembrane receptors and channels or mediates the interaction with internal motif of other proteins. This behavior contributes to the two main functions of PDZ-containing proteins, namely, the organization of multi-protein signaling complexes and the establishment, as well as maintenance, of cell polarity [9,10].

Syntenin is a PDZ-domain-containing protein that binds to the cytoplasmic domains of the syndecans [11]. This protein is also known as an interferon γ-induced potential melanoma differentiation-associated gene (mda-9) [12]. Structurally, syntenin contains an N-terminal domain (NTD), a C-terminal domain (CTD), and two PDZ domains [13]. Syntenin binds with the PDZ binding motif (PBM) of more than 20 proteins through its PDZ domains [14]. This protein also participates in various important physiological processes, such as cancer metastasis [15], early development [16,17], axonal outgrowth [18], synaptic transmission, intracellular trafficking, and signaling transduction [19], through the formation of macromolecular complexes with other proteins.

Syntenin has been recently implicated in several viral infections. For example, syntenin regulates actin polymerization,

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1050-4648/

Table 1
Sequence of primers used in this research.

Primer name	Sequence (5′-3′)
Smart F	TACGGCTGCGAGAAGACGACAGAAGGG
Oligo anchor R	GACCACGCGTATCGATGTCGACTTTTTTTTTTTTTTV
*MjSyn*RTF	GTGGGGTTGAAGGAAGT
*MjSyn*RTR	TTACAGGTCGGGAATGGAGTGGT
*Mjβ-actin*F	CAGCCTTCCTTCCTGGGTATGG
*Mjβ-actin*R	GAGGGAGCGAGGGCAGTGATT
*MjSyn*iF	GCGTAATACGACTCACTATAGGGGGCAAGGTAGGCATGCGT
*MjSyn*iR	GCGTAATACGACTCACTATAGGGGAAGGACGGCATGATCGT
*GFP*iF	GCGTAATACGACTCACTATAGGGTGGTCCCAATTCTCGTGGAAC
*GFP*iR	GCGTAATACGACTCACTATAGGGCTTGAAGTTGACCTTGATGCC
*Rab*5F	TTCCTCCCGTCACTCCAAG
*Rab*5R	AGCCGTGTCCCAAATCTCA
*Arp*2-3F	GTCGTCGTCCGTCTCTTCCC
*Arp*2-3R	CTTTGGTGGTTGGCAGTCG
*Ran*F	GATTGCCTCACAAAAACAGC
*Ran*R	ACCAGCATTTCTCGTCCA
*Calnexin*F	TTCAAGGGCAAGTGGGG
*Calnexin*R	AACGCCCGCCTGTCC
*Myosin*F	GTTGAGGTCGGACTTGG
*Myosin*R	TGACAACCAGGACACCC

The T7 promoter sequences are underlined.
The sequences underlined are T7 promoter sequences.

phosphatidylinositol 4,5-bisphosphate (PIP2) production, and human immunodeficiency virus type 1 (HIV-1) contact to target $CD4^+$ T-cells [20]. During severe acute respiratory syndrome coronavirus (SARS-CoV) infection, syntenin binds to the E protein PDZ-binding motif (PBM) of SARS-CoV, activates the p38 MAPK, and leads to the over-expression of inflammatory cytokinesis [21]. The involvement of syntenin in the response to white spot syndrome virus (WSSV) infection in shrimp has also been reported [22]. However, whether syntenin functions in the bacterial infection process of animals remains unclear.

In this study, a syntenin-like gene (*MjSyn*) was cloned from *Marsupenaeus japonicus*, and its expression profile to the bacterial infection was investigated. Furthermore, RNA interference (RNAi), combined with bacterial clearance and phagocytic activity assays, was conducted to disclose the function of *MjSyn* in the antibacterial immunity of shrimp.

2. Materials and methods

2.1. Animal challenge and tissue collection

Kuruma shrimp (*M. japonicus*, 10 g in average) were purchased from an aquaculture market in Jinan City, Shandong Province,

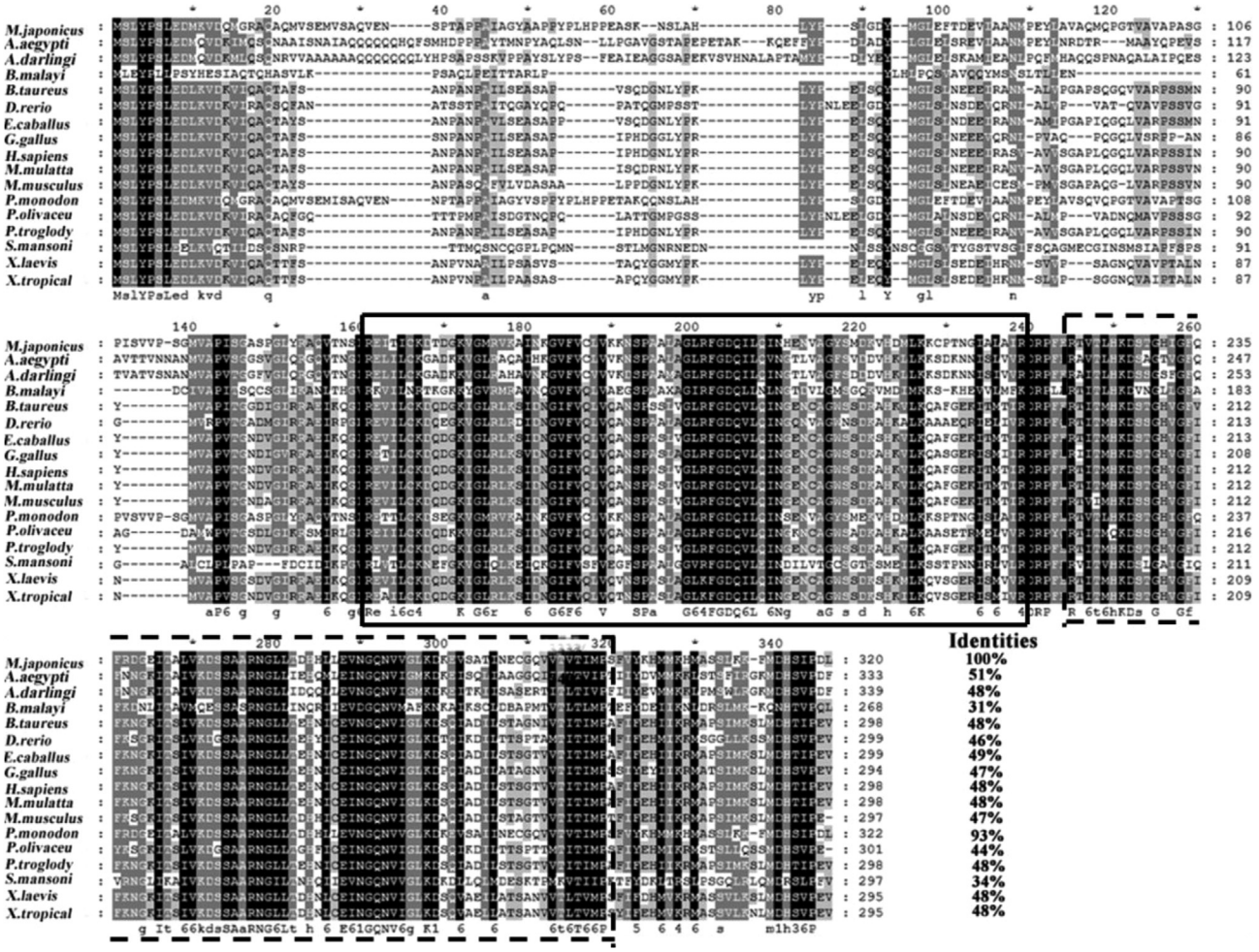

Fig. 1. Amino acid sequence alignment of syntenins from different species (*Aedes aegypti*, DQ440369.1; *Anopheles darling*, ETN58158.1; *Brugia malayi*, XP001901745.1; *Bos taurus*, BT030535.1; *Danio rerio*, BC044454.1; *Equus caballus*, NM001242477.1; *Gallus gallus*, NM_001031024.1; *Homo sapiens*, AF000652.1; *Macacamulatta*, NM_001257532.1; *Mus musculus*, AF077527.1; *Penaeus monodon*, HM210772.1; *Paralichthys olivaceus*, GU808360.1; *Pan troglodytes*, NM001246446.1; *Schistoso mamansoni*, XM002578027.1; *Xenopus laevis*, NM001086943.1; *Xenopus (Silurana) tropicalis*, M001006800.1). The multiple sequence alignment was performed using Clustal X 2.0. Conserved amino acid residues are highlighted with shaded background and shown below the alignments. The PDZ1 domain is circled in black solid line, and the PDZ2 domain is circled in dash lines.