Developmental and Comparative Immunology 50 (2015) 78–86

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Rab3 is involved in cellular immune responses of the cotton bollworm, *Helicoverpa armigera*

Jie Li [1], Cai-Xia Song [1], Yu-Ping Li, Li Li, Xiu-Hong Wei, Jia-Lin Wang *, Xu-Sheng Liu **

Hubei Key Laboratory of Genetic Regulation and Integrative Biology, School of Life Sciences, Central China Normal University, 152 Luoyu Road, Wuhan 430079, China

ARTICLE INFO

Article history:
Received 28 July 2014
Revised 23 December 2014
Accepted 12 January 2015
Available online 4 February 2015

Keywords:
Rab GTPase
Phagocytosis
Nodulation
Encapsulation
Cellular immune response
Helicoverpa armigera

ABSTRACT

Rab3, a member of the Rab GTPase family, has been found to be involved in innate immunity. However, the precise function of this GTPase in innate immunity remains unknown. In this study, we identified a *Rab3* gene (*Ha-Rab3*) from the cotton bollworm, *Helicoverpa armigera* and studied its roles in innate immune responses. Expression of *Ha-Rab3* was upregulated in the hemocytes of *H. armigera* larvae after the injection of *Escherichia coli* or chromatography beads. The dsRNA-mediated knockdown of *Ha-Rab3* gene in *H. armigera* larval hemocytes led to significant reduction in the phagocytosis and nodulation activities of hemocytes against *E. coli*, significant increase in the bacterial load in larval hemolymph, and significant reduction in the encapsulation activities of hemocytes toward invading chromatography beads. Furthermore, *Ha-Rab3* knockdown significantly suppressed spreading of plasmatocytes. These results suggest that *Ha-Rab3* plays important roles in *H. armigera* cellular immune responses, possibly by mediating spreading of hemocytes.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Rab GTPases (Rab proteins), the largest family of the small GTPase superfamily (Ras/Rap/Ral, Rho/Rac/Cdc42, Arf /Sar, Rab, and Ran family), are evolutionarily conserved in all eukaryotes (Eoin et al., 2012; van Dam et al., 2011). The first Rab GTPase was discovered in yeast *Saccharomyces cerevisiae* (Novick et al., 1980; Salminen and Novick, 1987). Subsequently, its homologs have been identified in many species, particularly in mammals (Eoin et al., 2012). In humans, 63 *Rab* genes have been identified, while 11, 29 and 31 *Rab* genes have been identified in the genomes of *S. cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster*, respectively (Bock et al., 2001; Pereira-Leal and Seabra, 2001; Zhang et al., 2007).

Rab GTPases act as molecular switches by being active in the GTP-bound state and inactive in the GDP-bound state (Harris and Littleton, 2011). They take part in a vast array of basic cell processes including exocytic and endocytic membrane trafficking, cell proliferation and differentiation, and cell–matrix and cell–cell adhesion. They also contribute to cell type-specific functions such as secretion (in endocrine and exocrine cells), synaptic transmission (in neurons), and phagocytosis (in macrophages and dendritic cells) (Pfeffer, 2001; Schwartz et al., 2007; Segev, 2001).

Recently, studies have revealed the involvement of Rab GTPases in invertebrate innate immunity. For example, Rab7 of the black tiger shrimp, *Penaeus monodon*, was found to be involved in white spot syndrome virus (WSSV) infection through binding directly to VP28 protein of WSSV (Sritunyalucksana et al., 2006). A separate study showed that the infections of WSSV and yellow head virus (YHV) were inhibited in *P. monodon* when Rab7 was suppressed with dsRNA (Ongvarrasopone et al., 2008). The expression of *Rab6* mRNA was upregulated in shrimp *Penaeus japonicas* following virus infection (Wu and Zhang, 2007). Further, it has been shown that *P. japonicas* Rab6 interacts with the envelope protein VP466 of WSSV as a virus intracellular receptor and regulates the hemocytic phagocytosis of WSSV by interacting with actin (Wu and Zhang, 2007; Wu et al., 2007). In shrimp *Marsupenaeus japonicus*, siRNA-mediated silencing of *Rab6* gene inhibited the phagocytosis against bacteria, while its overexpression led to increased phagocytosis, suggesting that this Rab GTPase was involved in the regulation of hemocyte phagocytosis of shrimp (Zong et al., 2008). *M. japonicus* Rab6 could interact with actin and regulate hemocyte phagocytosis against WSSV by inducing actin cytoskeletal rearrangement (Ye et al., 2012). In *D. melanogaster*, Rab35 plays a key regulatory role in the phagocytosis of hemocytes by controlling actin rearrangement at the immune cell periphery (Shim et al., 2010). *D. melanogaster* Rab14 regulated hemocytic phagocytosis against *Staphylococcus aureus* by

* Corresponding author. Hubei Key Laboratory of Genetic Regulation and Integrative Biology, School of Life Sciences, Central China Normal University, 152 Luoyu Road, Wuhan 430079, China. Tel.: +86 27 67867704; fax: +86 27 67861147.
E-mail address: jlwang@mail.ccnu.edu.cn (J-L. Wang).
** Corresponding author. Hubei Key Laboratory of Genetic Regulation and Integrative Biology, School of Life Sciences, Central China Normal University, 152 Luoyu Road, Wuhan 430079, China. Tel.: +86 27 67867704; fax: +86 27 67861147.
E-mail address: xsliu@mail.ccnu.edu.cn (X-S. Liu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.dci.2015.01.008
0145-305X/© 2015 Elsevier Ltd. All rights reserved.

controlling the maturation of phagosomes containing bacteria and the fusion of phagosomes with lysosomes (Garg and Wu, 2014).

In a recent transcriptome analysis of the cotton bollworm, *Helicoverpa armigera*, hemocytes identified a putative *Rab3* gene (*Ha-Rab3*). Rab3 is best known for its role in regulating synaptic vesicle trafficking in neurons (Fischer von Mollard et al., 1990; Graf et al., 2009; Nonet et al., 1997). Moreover, studies have shown that this GTPase may be involved in innate immunity. For example, the expression of *Rab3* in the Chinese mitten crab, *Eriocheir sinensis* hemocytes was significantly upregulated after the injection of bacteria *Vibrio anguillarum* (Wang et al., 2013). The expression levels of *Rab3a* mRNA in the channel catfish, *Ictalurus punctatus* were found to be upregulated after bacterial infection (Wang et al., 2014b). However, the precise function of this GTPase in innate immunity remains unknown. In this study, we investigated the role of *Ha-Rab3* in immune responses of *H. armigera*.

2. Materials and methods

2.1. Insect rearing

H. armigera larvae were reared on an artificial diet at 28 ± 1 °C under a 14-h light/10-h dark photoperiod, as described by Li et al. (2009).

2.2. Identification and sequence analysis of Ha-Rab3 gene

The immune transcriptome of *H. armigera* hemocytes was sequenced as described (Yang et al., 2013). A cDNA encoding protein homologous to Rab3 was identified and named *Ha-Rab3* (GenBank No.: KM035414). The domain prediction was performed using SMART (http://smart.embl-heidelberg.de/). The deduced amino acid sequences were aligned using MEGA 4.0 (http://www.megasoftware.net/) and GENDOC (http://www.nrbsc.org/downloads/gd322700.exe).

2.3. Recombinant protein expression and production of antibody

Full-length *Ha-Rab3* was amplified using a pair of gene specific primers (Table 1). After cutting with *Eco*RI and *Xho*I, the DNA fragments were inserted into expression vector pET-32a (Novagen, Madison, WI, USA) and then transformed into *Escherichia coli* BL21 (DE3). The transformants obtained were grown in LB medium at 37 °C under shaking conditions (200 rpm) until the optical density (OD) at 600 nm reached 0.6. IPTG was added at a final concentration of 0.1 mM, and the medium was shaken overnight at room temperature. Bacterial cells were harvested by centrifugation, resuspended in PBS, and sonicated on ice. Soluble protein fractions of bacterial cells were applied on High-Affinity Ni–NTA Resin (GenScript, Nanjing, China) to purify recombinant protein according to the manufacturer's instruction. Purified recombinant protein was used as an antigen for producing polyclonal rabbit antiserum according to the method of Wang et al. (2014a).

2.4. Tissue distribution and immunocytochemistry

Reverse transcription-polymerase chain reaction (RT-PCR) was used to compare the abundance of *Ha-Rab3* transcript in different tissues of *H. armigera*. Total RNA was isolated from epidermis, midgut, fat body, and hemocytes of sixth instar day 1 larvae by using Total RNA Purification System (Omega, Norcross, GA, USA) combined with On-Column DNase (Qiagen, Hilden, Germany) digestion to remove any genomic DNA contamination. The first-strand cDNA synthesis was performed using M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). The primers used for RT-PCR are listed in Table 1. The *rpS3* gene from *H. armigera* (*Ha-rpS3*, GenBank No.: KM064630) was used as the control. The annealing temperature and the number of cycles for *Ha-Rab3* and *Ha-rpS3* were 53.3 °C/35 cycles and 55 °C/28 cycles, respectively. The PCR products (3 µl each) were electrophoresed on 1% agarose gel. The DNA was stained using ethidium bromide and imaged.

Immunocytochemistry was used to analyze the localization of Ha-Rab3 protein in the hemocytes of *H. armigera* larvae. Ten microliters of hemolymph was collected from sixth instar day 1 larvae, mixed immediately with 40 µl of ice-chilled anticoagulant (62 mM NaCl, 100 mM glucose, 20 mM EDTA, 26 mM citric acid, 30 mM sodium citrate, pH 4.6), and centrifuged at 4000 rpm for 6 min to separate hemocytes from the plasma. The hemocytes were resuspended in 100 µl of phosphate-buffered saline (PBS; 138 mM NaCl, 2.7 mM KCl, 7.3 mM Na_2HPO_4, 1.47 M KH_2PO_4, pH 7.4). Twenty microliters of hemocyte suspensions were dropped onto a slide. The hemocytes were allowed to settle down for 20 min and then fixed with 4% paraformaldehyde in PBS for 20 min. After washing 3 times with PBS, the hemocytes were incubated with 0.2% Triton X-100 for 15 min. After washing another 3 times with PBS, the hemocytes were blocked with 3% BSA in PBS for 1 h. Subsequently, the hemocytes were incubated with anti-Ha-Rab3 antiserum (diluted 1:100 in 3% BSA) for 1.5 h, washed 3 times with PBS, and incubated with fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit immunoglobulin G (IgG) (diluted 1:2000 in 3% BSA) for 1.5 h. Finally, 4′-6-diamidino-2-phenylindole dihydrochloride (DAPI) was used to stain the nuclei. Rabbit preimmune serum instead of anti-Ha-Rab3 antiserum was used as negative control. A fluorescence microscope was used to detect fluorescence.

Table 1
Primer sequences used in RT-PCR (*Gene*-1), qRT-PCR (*Gene*-2), recombinant expression (*Gene*-3), and RNAi (*Gene*-4) in this study.

Gene	Primer sequence (5′→3′)	Fragment length (bp)
Ha-Rab3-1	ATGACTGGAGATGCAAAATGG	678
	GTGCAATACTGCTGGGTATAG	
Ha-rpS3-1	GTGCGCGTCACTCCGACTC	358
	TCATGAGGCCGTCCACGAAC	
Ha-Rab3-2	GAACGCTACCGCACGATCACC	145
	CTTGCGCGTTGTCCCATGAGT	
Ha-rpS3-2	CGGCGTGGAGGTGCGCGTC	194
	CGATGGCGCACAGACCGCG	
Ha-Rab3-3	CCGGAATTCATGACTGGAGATGCAAAATGGC	675
	CCGCTCGAGTTAACAGTTACAGTTGGTGG	
Ha-Rab3-4	TAATACGACTCACTATAGGGAGATCAGCCTTCGTCTCCACCG	455
	TAATACGACTCACTATAGGGAGACGCAGATTATGTCCACCAGTCG	
GFP-4	TAATACGACTCACTATAGGGAGAAGGGCGAGGGCGATGCCACC	382
	TAATACGACTCACTATAGGGAGATGTACTCCAGCTTGTGCCCC	

Note. Restriction enzyme sites in primers for recombinant expression are underlined.

Download English Version:

https://daneshyari.com/en/article/2428985

Download Persian Version:

https://daneshyari.com/article/2428985

Daneshyari.com