Fish & Shellfish Immunology 48 (2016) 54–61

Contents lists available at ScienceDirect

Fish & Shellfish Immunology

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

Short communication

Identification and characterization of nascent polypeptide-associated complex alpha from Chinese mitten crab (*Eriocheir sinensis*): A novel stress and immune response gene in crustaceans

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ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form
16 September 2015
Accepted 6 November 2015
Available online 11 November 2015

Keywords:
Nascent polypeptide-associated complex
NACA
Chinese mitten crab
Eriocheir sinensis
Environmental stresses
Innate immune response

ABSTRACT

Disease in aquatic animals is tightly linked to environmental challenges and their immune responses are greatly modified by their external environment. The chaperone protein nascent polypeptide-associated complex alpha (NACA) has been suggested to play important roles in the cellular response to stress and immune challenges, while the related biological functions remain largely unknown in invertebrates. In the present study we identified a *NACA* gene (termed *EsNACA*) from Chinese mitten crab *Eriocheir sinensis* and analyzed its expression changes in response to ambient (salinity and pH) stresses and immune challenges. The *EsNACA* protein is comprised of 209 amino acid residues with a conserved DNA binding domain, a C-Jun binding domain, a NAC domain and an ubiquitin-associated domain and shows the highest sequence identity (87%) with its counterpart in shrimp *Penaeus monodon*. *EsNACA* mRNA transcripts are presented in all tested normal tissues with predominant expression in hepatopancreas and lower expression in hemocytes. In addition, *EsNACA* expression was significantly altered in response to the ambient salinity (15‰ and 30‰ salinities) and pH (pH 6 and 8.5) stresses in gill, hepatopancreas, muscle, hemocytes and intestine tissues. Furthermore, *EsNACA* gene expression was substantially induced upon LPS and Poly(I:C) immune stimulations in *E. sinensis* hemocytes *in vitro*. Finally, *EsNACA* expression was up-regulated in *E. sinensis* hemocytes, gill, hepatopancreas, intestine and muscle tissues in response to *Vibrio anguillarum* challenges *in vivo*. Taken together, our findings for the first time show that *EsNACA* is an inducible gene involved in stress and immune response in crustaceans.

1. Introduction

Aquatic animals are often challenged with the changes of their external environment, such as temperature, salinity and pH alterations. Consequentially, their immunity is usually negatively impacted by the unfavorable environmental changes. The Chinese mitten crab *Eriocheir sinensis* is an important crustacean species with high economic value in China [1]. Associated with the rapid development of *E. sinensis* fishery industry, however, diseases have led to great economic loss [2]. Importantly, under the intensive farming conditions the environmental stress can even worsen the outcomes of disease progression in *E. sinensis*. Dissecting the molecular determinants that respond both to stress and immune challenges thus is of a great interest to understand the crosstalk between stress and immunity in aquatic animals. Several genes and pathways involved in *E. sinensis* stress [3] and immune response [4] have been revealed by transcriptomic analysis, while the candidates that may participate in both stress adaptation and immune response remain largely uncharacterized. We have been perusing to identify the candidates that respond to stress and immune challenges in the Chinese mitten crabs and several candidate genes have been identified [5,6].

Nascent polypeptide-associated complex (NAC) is a heterodimeric protein comprised of an alpha subunit and a beta subunit [7] and functions as an essential component of translation machinery and a translational chaperone in eukaryotic cells [8]. The alpha subunit of NAC (termed NACA) possesses several important

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http://dx.doi.org/10.1016/j.fsi.2015.11.014
1050-4648/

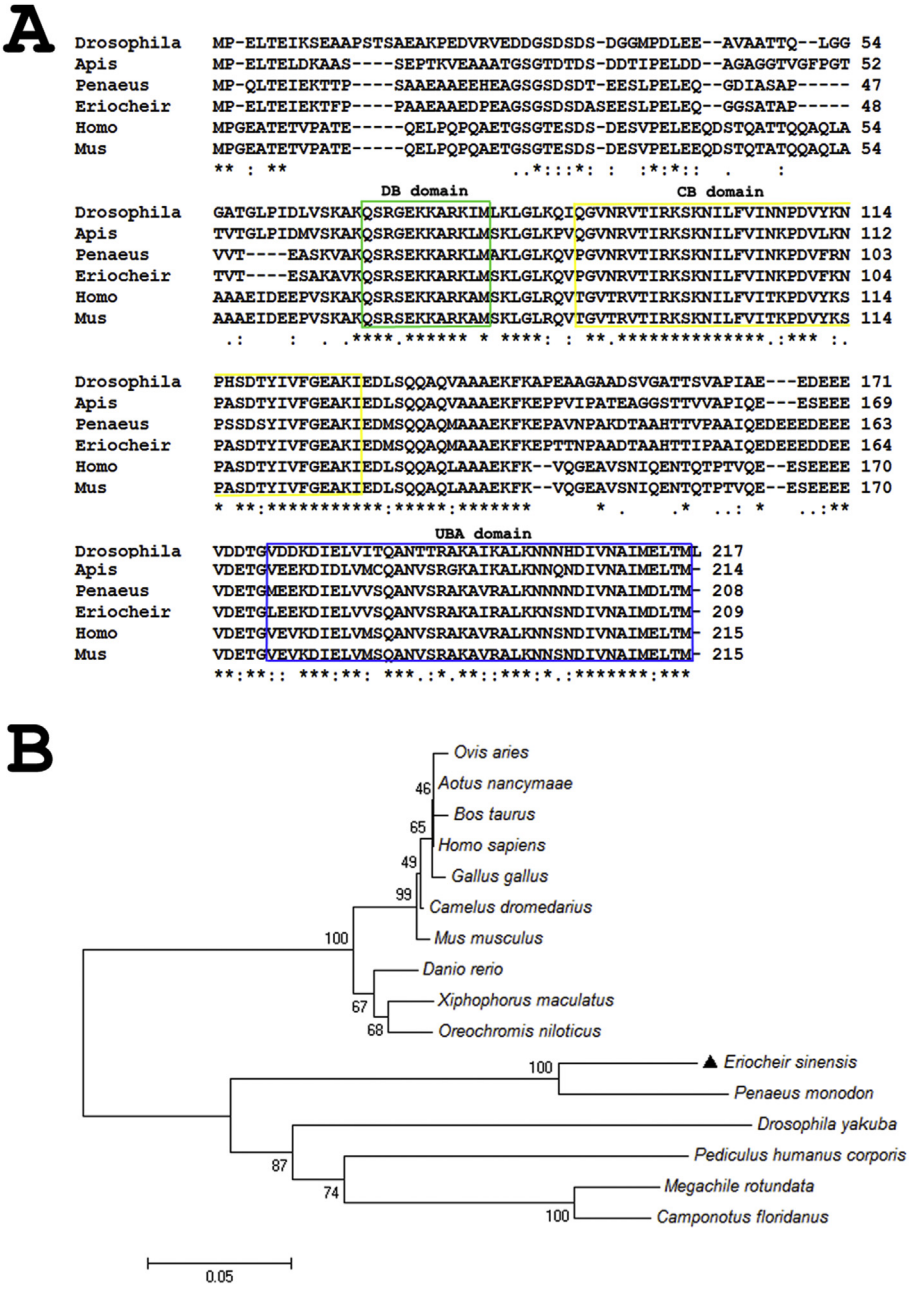

Fig. 1. Sequence alignment and phylogenetic analysis of NACA proteins from *E. sinensis* and other species. (A) Multiple sequence alignment of the amino acid sequences of EsNACA and other selected NACA proteins. The conserved DNA binding domain (DB domain), C-Jun binding domain (CB domain) and ubiquitin-associated domain (UBA domain) are boxed in green, yellow and blue, respectively. Highly conserved (:), less conserved (.) and identical (*) amino acid residues identified in all the proteins are indicated. (B) Evolutionary analysis of EsNACA protein. The NACA phylogenetic tree was constructed by the NJ-method with MEGA 5.0 program. GenBank accession numbers for the selected NACA proteins are *Drosophila yakuba* NACA (XP_002090997.1), *Penaeus monodon* NACA (ACJ47904.1), *Camelus dromedaries* (XP_010975137.1), *Mus musculus* NACA isoform b (NP_038636.2), *Homo sapiens* NACA isoform b (NP_001106673.1), *Ovis aries* NACA-like (XP_004013962.1), *Aotus nancymaae* NACA transcript variant X3 (XM_012450508.1), *Bos Taurus* (NP_001014916.1), *Gallus gallus* NACA (NP_001263232.1), *Camponotus floridanus* NACA isoform 2 (XP_011253042.1), *Danio rerio* NACA isoform 2 (NP_775371.1), *Xiphophorus maculatus* NACA-like (XP_005801810.1), *Oreochromis niloticus* NACA isoform X1 (XP_003439022.1), *Pediculus humanus corporis* NACA (XP_002426422.1), *Megachile rotundata* NACA (XP_003705050.1) and *E. sinensis* NACA (KR818702). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)