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Short communication

The first evidence of positive selection in peptidoglycan recognition protein (PGRP) genes of *Crassostrea gigas*

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ABSTRACT

The oyster *Crassostrea gigas* is thought to have developed effective immunity to potentially harmful pathogens while under continuous exposure to marine microorganisms; however, the evolutionary mechanisms by which such immunity developed has not been understood. To understand the evolution of immunity, we characterized the family of peptidoglycan recognition proteins in the oyster (CgPGRPs). These proteins are crucial pattern recognition receptors for peptidoglycans (PGNs) and thereby, for activating the innate immune response of host. Herein, we identify seven new CgPGRP genes. Phylogenetic analysis of the seven new and five previously reported CgPRGP genes reveals that the CgPRGP gene family can be clustered into two groups, CgPRGPS and CgPRGPL. Moreover, the CgPRGPS group can be further divided into five subgroups. A codon-substitution model and three likelihood ratio tests (LRTs) suggest that seven sites in the CgPGRP family of genes have been subjected to strong positive selection (ω = 3.035–4.143), Three dimensional modeling revealed that these sites are found primarily at the periphery of coils and α-helices rather than in β-strands, perhaps allowing PGRP to adapt to, and recognize, variability of PGN structure. In conclusion, our studies provide the first evidence of positive Darwinian selection in the CgPGRP family, contributing to a better understanding of the adaptive mechanism of host-pathogens interaction in marine mollusks.

1. Introduction

Innate immunity provides the first line of defense against invading pathogens, and is the only line of immune defense in invertebrates. By recognizing constitutively expressed pathogen-associated molecular patterns (PAMPs) that are exclusively expressed in bacteria and viruses, hosts have evolved function-specific pattern recognition receptors (PRRs) that can provide the earliest detection of a pathogen and initiation of an immune response [1]. Peptidoglycans (PGNs), integral components of the cell wall in both gram-positive and gram-negative bacteria, consist of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) in β-1,4 linkage, cross-linked by short peptide stems composed of alternating L- and D-amino acids [2]. Metazoans from insects to mammals have evolved one type of PRR, known as peptidoglycan recognition proteins (PGRPs), that can specifically bind to PGNs [3].

As an important PRR of the innate immunity system, all PGRPs function in immune defense and are structurally related to bacterial amidases. Accordingly the host immune responses raised by PGNs can be terminated by mean of PGRP hydrolytic activity. Several PGRPs have lost this enzymatic activity in *Drosophila*, including PGRP-SA and PGRP-SD; these proteins still serve as sensors of gram-positive bacteria via recognition of PGNs and they activate the Toll receptor pathway [4] [5]. Other PGRP members with amidase activity, such as PGRP-SC1, have been shown to control the IMD signaling pathway after bacterial challenge [6]. These examples demonstrate the diversification of PGRP function.

Under continuous exposure to marine water rich in microorganisms, the oyster *Crassostrea gigas* is thought to have evolved an effective immune system used to discriminate beneficial microorganisms or commensals from potentially harmful pathogens [7]. Therefore, rapid evolution of host PRRs should be required for survival during combat with pathogens. Five PRGPs were previously cloned in *C. gigas*, named CgPRGPs; these genes were confirmed as being involved in immune defense [8,9]. We here identify and characterize seven new genes encoding amidase/PGRP domains in *C. gigas*. These CgPRGP genes show a high level of diversity, and the following codon-substitution analysis reveals a strong effect of positive selection on the promotion of rapid divergence within the CgPGRP gene family.

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2. Materials and methods

2.1. Database search and gene discovery

Homologs of PGRP were found by searching the Pacific oyster EST database using TBLASTN (http://www.ncbi.nlm.nih.gov/blast) with previously published PRGP sequence [8,9] as a query. Seven new PRGPs gene sequences were obtained and assembled using the ContigExpress program of the Vector NTI advance 10 software package (Invitrogen, USA). Domain structure was identified with the Simple Modular Architecture Research Tool (SMART) version 4.0 programs on line (http://smart.embl-heidelberg.de/).

2.2. Sequence analysis

2.2.1. Sequence alignment and phylogenetic analysis

Alignment of nucleotide and amino acids sequences of the 12 genes were performed using the ClustalX 1.81 software. An unrooted phylogenetic tree of the proteins was constructed using the neighbor-joining method of the MEGA 4.1 software package [10] with Poisson correction for all sites under pairwise deletion. The bootstrap values were estimated by 1000 replications and are given at each branch point.

2.2.2. Maximum likelihood analysis

The nucleotide sequences encoding the PGRP domain of the 12 genes were aligned according to their amino acid alignments, and all sequences were manually adjusted to remove the gaps and regions of misalignment prior to maximum likelihood analysis. The ratio of non-synonymous and synonymous substitutions, also named ω, was calculated with codon-substitution models by using the CODEML program of the PAML package [11,12]. Values of $\omega > 1$ indicate positive selection, while $\omega = 1$ and $\omega < 1$ indicate neutral evolution and purifying selection. Six codon-substitution models form three likelihood ratio tests (LRTs): M0/M3, M1a/M2a and M7/M8. M0 (one-ratio) assumes that all sites have the same ω ratio. LRTS were also implemented to verify the positive selection of each model by comparing M0/M3, M1a/M2a and M7/M8.

2.2.3. Structural modeling

Three dimensional structures were modeled using SWISS-MODEL, a fully automated protein structure homology modeling server (http://swissmodel.expasy.org), and *Drosophila* PGRP (PDB code: 2F2LX) was used as the template [13]. Model quality was evaluated by Anolea and QMEAN; the structure of model was visualized by using PyMol (http://www. pymol.org).

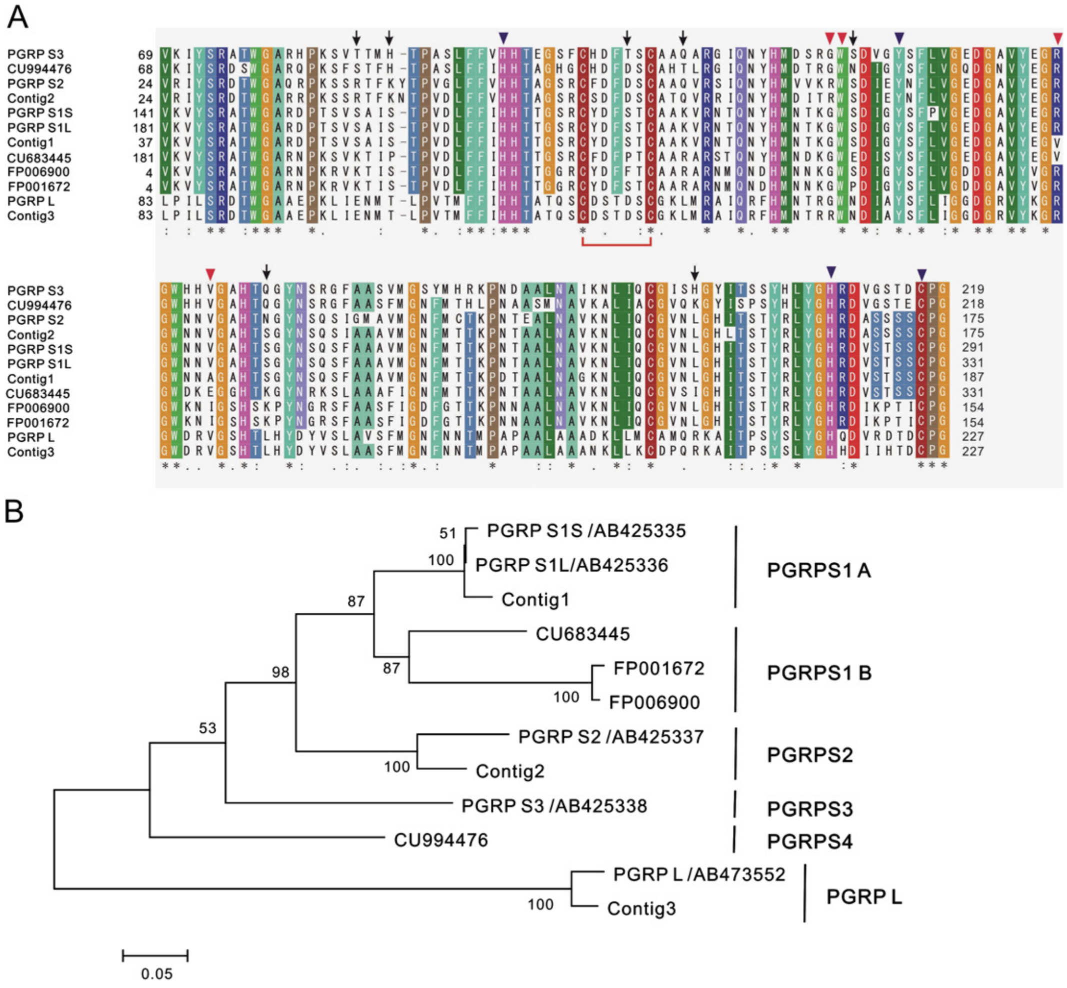

Fig. 1. The PGRP family of *C. gigas*. (A) Multiple sequence alignment of amidase/PGRP domains. Catalytic residues for amidase activity and specific PGN recognition were indicated by blue and red triangles, respectively. Disulfide bridges are shown with red lines. (B) Phylogeny of 12 PGRP genes from *C. gigas*. An unrooted neighbor-joining distance tree was constructed from the alignment of amino acid sequences presented in Fig. 1A. Numbers on interior branches represent bootstrap values.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)