PC-ASTAKINE mRNA LEVELS DECREASE AFTER BOTH LPS INJECTION AND HEMOLYMPH WITHDRAWALS IN THE GASTROPOD PEST *POMACEA CANALICULATA*

A Accorsi[1,2], K Gotting[1], E Ross[1,2], S Benatti[3], M Nasi[4], E Ottaviani[3], A Sánchez Alvarado[1,2], D Malagoli[3§]

[1]Stowers Institute for Medical Research, Kansas City, MO, USA

[2]Howard Hughes Medical Institute, Stowers Institute for Medical Research, Kansas City, MO, USA

[3]Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

[4]Department of Surgery, Medicine, Dentistry and Morphological Sciences, University of Modena and Reggio Emilia, Modena, Italy

The freshwater gastropod *Pomacea canaliculata* has recently been included among emerging pests in the EU. Hence, its immune system may be a promising target for pest control strategies. By analyzing a set of *P. canaliculata* transcriptomes, we have discovered an Astakine-1-like molecule (*Pc*-Astakine) in this organism. Astakine was first isolated as a hematopoietic cytokine in the crayfish *Pacifastacus leniusculus* (*Pl*-Astakine-1), and later found in arthropods and the bivalve *Crassostrea gigas*. Bioinformatic analyses show that *Pc*-Astakine is a 121 aa protein with a conserved distribution pattern of cysteines retrievable in both *Pl*-Astakine-1 and the vertebrate Prokineticin protein family. *Pc-astakine* is expressed at low levels in circulating hemocytes, but markedly in hemocyte reservoirs (*e.g.*, ampulla) and hematopoietic tissue (pericardial fluid) suggesting a conserved role in hematopoiesis. We tested this potential role of *Pc-Astakine* in two ways. First, we measured RNA levels of this gene in immune-related tissues 24 h after injection of 50 µg of *Escherichia coli*-derived lipopolysaccharide (055:B5) (LPS). We observed a sharp decrease of *Pc-astakine* mRNA in all the analyzed organs, with *Pc-astakine* mRNA levels almost undetectable in anterior kidney (a hemocyte reservoir) when compared to sham-injected controls. Second, we defined the profile of *Pc-astakine* expression after a series of 4 hemolymph withdrawals performed within 72 h. As with the LPS-treatment, a general decrease of mRNA levels was observed, with the largest decrease observed in hematopoietic tissue and circulating hemocytes (reduced to 20% of the control). Altogether, we hypothesize that the decrease of *Pc-astakine* mRNA after both LPS injection and hemolymph withdrawal may be due to an increased rate of translation, rather than a reduction of gene expression. This is in part supported by similar observations in the oyster *C. gigas* in which massive translation of *Cg-astakine* is observed as a consequence of environment stress, including immune stimuli, and in *P. leniusculus* in which increased levels of Astakine in the plasma of LPS-injected animals have been related to an increased translational rate.

To our knowledge, this is the first report of an astakine-like molecule in gastropods and our data indicate a potential involvement of *Pc*-Astakine in both the immune response and hematopoiesis of the molluscan pest *P. canaliculata*.

Keywords: mollusk; pest; hematopoiesis; transcriptome; cytokine

[§]Corresponding author. Tel.: +39 0592055538; Fax: +39 0592055548.
E-mail address: davide.malagoli@unimore.it