

## Discovery and characterisation of novel CHHs involved in the immune response of the red swamp crayfish *Procambarus clarkii*

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Crustacean hyperglycaemic hormone (CHH) has many functions to regulate carbohydrate metabolism, ecdysis and reproduction including ion transport in crustaceans. Furthermore, rapid mobilization of neuropeptides, including CHHs, from the sinus gland in the eyestalks represent the primary response to stress.

Here we report the identification of two potential transcripts pertaining to the CHH family in *Procambarus clarkii*, thanks to a whole-transcriptome sequencing approach.

These two members, named CHHip (CHH Immune-related *Procambarus*) and CHHop (CHH homologous *Procambarus*) share the typical features of the CHH (the presence of a CHH-precursor-related peptides between the signal peptide and the peptide, and 6 conserved cysteines involved in the three typical disulfide bonds of the CHH-superfamily) and they have been found up-regulated following the knock-down of the main CHH isoforms through RNA interference.

To shed light on their possible role in the immune response of *P. clarkii* two experiments have been performed to evaluate CHHip and CHHop relative expression in the eyestalk in response to a lipopolysaccharide (LPS)- and a *Staphylococcus aureus*-challenges (1 µg/animal (body weight 40 g) of LPS and *S. aureus* 3x10<sup>8</sup> CFU/100 µl per animal, respectively).

Following the LPS injection, only CHHip increased significantly (7-fold at 2 hours post injection–hpi– and 14-fold at 4 hpi, p-value ≤ 0.01). *S. aureus* injection triggered CHHip at 6 and 12 hpi (p-value ≤ 0.05), but not at 24 hpi. Conversely, CHHop resulted up-regulated exclusively at 24 hpi of *S. aureus* (p-value ≤ 0.05).

These preliminary findings suggest an early immunological response to bacterial infection driven by CHHip followed by an up-regulation of CHHop, with the nearly contemporary down-regulation of CHHip, at 24 hpi. These results seem to support the existence of a combined action of CHHip and CHHop in response to LPS and Gram - bacteria in a time-dependent manner and increase the spectrum of action of this family of hormones up to their direct involvement in the immune response.