CIRCADIAN CLOCK LOCALIZATION IN THE GHOST SHRIMP, PALAEMONETES PALUDOSUS AND THE NORTHERN CRAYFISH, ORCONECTES VIRILIS

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Introduction

Circadian rhythms are daily anticipatory patterns of behavior and physiology, which are conserved across the animal kingdom. These rhythms are maintained by endogenous biological clocks, which are comprised of a network of specific genes and proteins. These genes and proteins function in various feedback loops, with circadian changes in gene expression and protein abundance (Bell-Peterson et al., 2005). In Drosophila, mutation of the period (per) gene, which is responsible for PER protein synthesis, causes alterations to the species circadian rhythm (Lin, 2005) and thus PER has been identified as a key clock regulator (Meyer et al. 2006). PER has been conserved across taxa, and localizing the protein has been used as a proxy for circadian rhythm (Lin, 2005) and thus PER has been conserved across taxa, and localizing the protein has been used as a proxy for circadian rhythm (Lin, 2005). Circadian rhythms are daily anticipatory patterns of behavior and physiology, which are conserved across the animal kingdom. These rhythms are maintained by endogenous biological clocks, which are comprised of a network of specific genes and proteins. These genes and proteins function in various feedback loops, with circadian changes in gene expression and protein abundance (Bell-Peterson et al., 2005). In Drosophila, mutation of the period (per) gene, which is responsible for PER protein synthesis, causes alterations to the species circadian rhythm (Lin, 2005) and thus PER has been identified as a key clock regulator (Meyer et al. 2006). PER has been conserved across taxa, and localizing the protein has been used as a proxy for circadian rhythm (Lin, 2005).

METHODS

Animals and Environmental Conditions

The Ghost shrimp, Palaemonetes paludosus and the northern crayfish, Orconectes virilis, were acquired from a commercial supplier. Both species were put on a reverse light cycle of 12:12 for an adequate period for circadian entrainment.

Experimental Procedure

Specimens were dissected on ice in a saline solution for circadian entrainment. Intact eyestalks, brain, and nerve cord were extracted from the animals and fixed overnight in a 4% paraformaldehyde solution at 4°C, which was buffered with 0.1M Sorenson’s buffer. The tissues were washed several times with buffer over the course of one hour, and dehydrated using ETOH dilutions from 30% up to 95%. After rehydrating, tissues were washed in PTA solution, which helps to prevent growth of microorganisms during the ensuing antibody incubations. Tissues were blocked overnight in a 6% Goat serum, which helps to prevent nonspecific background staining. The neurotransmitter, Serotonin was used as a positive control, as it is highly conserved across taxa. Two initial dilutions were used for the serotonin Ab: 1:500 and 1:250 in 6% goat serum. Tissues were incubated at 4°C for 48 hours, with constant agitation. Tissues were washed with PTA for twelve hours with several changes of solution, removing 1st Ab. The 2nd used was Alexaflour 488, which is a fluorescent tagging agent used to reveal the binding of the 1st Ab. 2nd Ab dilution was 1:100 in PTA, with an overnight incubation at 4°C. After the incubation, the tissues were rinsed with Sorenson’s buffer for twelve hours. Tissues were mounted onto slides and stored in a light sealed slide holder at 4°C.

PER localization was then performed. Areas of interest were stained with the fluorescent agent Alexafluor 488 and visualized in a fluorescent microscope.

PER staining was quantified by comparison to the negative control (2nd Ab only). A grid consisting of 50µm² squares was placed over the images, and random squares were chosen for quantification. Stained cell bodies located in each of 16 randomly selected squares were counted, a total cell count was determined and divided by total area to determine stained cells per mm².

PER was found in the retina, eyestalks and parts of the brain of P. clarkii (Escamilla-Chimal et al. 2010). The purpose of this study was to use immunohistochemistry to localize PER in two closely related crustacean species: the ghost shrimp, Palaemonetes paludosus and the northern crayfish, Orconectes virilis, thus potentially locating the species circadian clock.

PER Staining in Ventral Nerve Cord

Figure 2: PER staining in ventral nerve cord ganglia of O. virilis and P. paludosus. Arrows indicate stained cell bodies.

Figure 3: Stained serotonergic cell bodies, indicated by arrows (A) were visualized and 2nd Antibody only incubations (B), show lack of nonspecific binding.

Conclusion

• Potential clock localization to VNC ganglia and subesophageal ganglion
• PER appears to increase at night compared to day
• Limited PER staining in brain and eyestalks (not shown)
• Serotonin (5-HT) localized for first time in O. virilis and P. paludosus

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