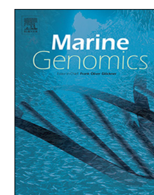
ARTICLE IN PRESS

Marine Genomics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Marine Genomics

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



Method paper

Circadian signaling in *Homarus americanus*: Region-specific *de novo* assembled transcriptomes show that both the brain and eyestalk ganglia possess the molecular components of a putative clock system

Andrew E. Christie[a,*], Andy Yu[a], Micah G. Pascual[a], Vittoria Roncalli[a], Matthew C. Cieslak[a], Amanda N. Warner[b], Tess J. Lameyer[c], Meredith E. Stanhope[c], Patsy S. Dickinson[c], J. Joe Hull[b]

[a] Békésy Laboratory of Neurobiology, Pacific Biosciences Research Center, School of Ocean and Earth Science and Technology, University of Hawaii at Manoa, 1993 East-West Road, Honolulu, HI 96822, USA

[b] Pest Management and Biocontrol Research Unit, US Arid Land Agricultural Research Center, USDA Agricultural Research Services, Maricopa, AZ 85138, USA

[c] Department of Biology, Bowdoin College, 6500 College Station, Brunswick, ME 04672, USA

ARTICLE INFO

Keywords:
Biological rhythm
Circadian rhythm
Transcriptomics
Crustacea
Decapoda

ABSTRACT

Essentially all organisms exhibit recurring patterns of physiology/behavior that oscillate with a period of ~24-h and are synchronized to the solar day. Crustaceans are no exception, with robust circadian rhythms having been documented in many members of this arthropod subphylum. However, little is known about the molecular underpinnings of their circadian rhythmicity. Moreover, the location of the crustacean central clock has not been firmly established, although both the brain and eyestalk ganglia have been hypothesized as loci. The American lobster, *Homarus americanus*, is known to exhibit multiple circadian rhythms, and immunodetection data suggest that its central clock is located within the eyestalk ganglia rather than in the brain. Here, brain- and eyestalk ganglia-specific transcriptomes were generated and used to assess the presence/absence of transcripts encoding the commonly recognized protein components of arthropod circadian signaling systems in these two regions of the lobster central nervous system. Transcripts encoding putative homologs of the core clock proteins clock, cryptochrome 2, cycle, period and timeless were found in both the brain and eyestalk ganglia assemblies, as were transcripts encoding similar complements of putative clock-associated, clock input pathway and clock output pathway proteins. The presence and identity of transcripts encoding core clock proteins in both regions were confirmed using PCR. These findings suggest that both the brain and eyestalk ganglia possess all of the molecular components needed for the establishment of a circadian signaling system. Whether the brain and eyestalk clocks are independent of one another or represent a single timekeeping system remains to be determined. Interestingly, while most of the proteins deduced from the identified transcripts are shared by both the brain and eyestalk ganglia, assembly-specific isoforms were also identified, *e.g.*, several period variants, suggesting the possibility of region-specific variation in clock function, especially if the brain and eyestalk clocks represent independent oscillators.

1. Introduction

Coordination of physiology and behavior to recurring changes in the environment is a requirement for all living organisms. In many cases, this coordination is achieved via the action of intrinsic, genetically-encoded timekeeping systems, so called "biological clocks", which operate on a wide range of time scales, from sub-second to seasonal and even longer (Golombek et al., 2014). One of the best known clock systems is the circadian pacemaker, which is responsible for the timing of recurring patterns of physiology and behavior that oscillate with a period of ~24-h and are synchronized to the solar day. The molecular cascade responsible for the establishment of circadian rhythmicity has been well characterized for several species, for example the fruit fly, *Drosophila melanogaster* (Allada and Chung, 2010; Hardin, 2011; Mendoza-Viveros et al., 2017; Ozkaya and Rosato, 2012; Yoshii et al., 2015). In all species, circadian pacemakers involve interacting feedback loops of transcriptional activation and repression, as well as modulation of the feedback loops via processes such as phosphorylation and degradation of key protein components (see the abovementioned references for a complete description of the *D. melanogaster* cascade).

* Corresponding author at: Békésy Laboratory of Neurobiology, Pacific Biosciences Research Center, School of Ocean and Earth Science and Technology, University of Hawaii at Manoa, 1993 East-West Road, Honolulu, HI 96822, USA.

E-mail address: crabman@pbrc.hawaii.edu (A.E. Christie).

https://doi.org/10.1016/j.margen.2018.03.002
Received 23 January 2018; Received in revised form 5 March 2018; Accepted 6 March 2018
1874-7787/ © 2018 Elsevier B.V. All rights reserved.

One group of animals for which numerous circadian rhythms of physiology and behavior have been documented is the crustaceans. As reviewed by Strauss and Dircksen (2010), known crustacean physiological/behavioral systems that are under circadian control include, but are not limited to, feeding, locomotion, molting, pigment dispersion and reproduction. Despite the diversity of documented circadian rhythms in crustaceans, little is known about the molecular underpinnings of circadian signaling in these animals (Chen et al., 2017; Christie et al., 2013, Christie et al., 2018; Nesbit and Christie, 2014; Mazzotta et al., 2010; O'Grady et al., 2016; Roncalli et al., 2017; Sbragaglia et al., 2015; Tilden et al., 2011; Yang et al., 2006; Zhang et al., 2013). In fact, large collections of putative circadian genes/proteins have been identified and characterized for only a handful of crustacean species (Christie et al., 2013, 2018; Nesbit and Christie, 2014; Roncalli et al., 2017; Tilden et al., 2011; O'Grady et al., 2016; Sbragaglia et al., 2015). Moreover, the location of the crustacean central circadian pacemaker remains unclear, although several sites in the central nervous system (CNS) have been proposed as the locus of this clock system (Strauss and Dircksen, 2010). Proposed sites include both the eyestalk ganglia and the supraoesophageal ganglion, commonly referred to as the brain (Strauss and Dircksen, 2010).

One decapod crustacean for which multiple circadian rhythms have been documented is the American lobster, *Homarus americanus*. In this species, which has significance both for its commercial value (Overton, 2017) and as a biomedical model for investigating the basic principles governing the generation, maintenance and modulation of rhythmically active motor behavior (Blitz and Nusbaum, 2011; Hooper and DiCaprio, 2004; Marder and Bucher, 2007; Marder et al., 2017; Nusbaum et al., 2001; Otopalik et al., 2017; Schulz and Lane, 2017; Stein, 2009), circadian rhythms have been shown or are hypothesized to play roles in the control of locomotor activity (Jury et al., 2005), neurogenesis (Goergen et al., 2002), brain serotonin levels (Wildt et al., 2004), and heartbeat frequency (Chabot and Webb, 2008). Western blots of *H. americanus* eyestalk ganglia- and brain-derived protein extracts using an antibody generated against period (PER) protein, a key component of circadian signaling systems (*e.g.*, Allada and Chung, 2010), revealed immunoreactivity in the eyestalk ganglia but not in the brain (Grabek and Chabot, 2012). These data suggested that the central circadian clock of the lobster is located in the eyestalk ganglia rather than in the brain. However, the size of the protein detected in the eyestalk ganglia was significantly smaller than most other arthropod PERs, *i.e.*, ~70 *vs.* ~100^{+} kDa (Grabek and Chabot, 2012), raising the question of whether or not the immunoreactive protein was a true member of the PER family.

In the study presented here, a transcriptomics approach was used to assess the presence/absence of circadian signaling system transcripts and, by proxy, proteins, including PER, in the *H. americanus* brain and eyestalk ganglia. Specifically, region-specific transcriptomes were generated and searched for sequences encoding putative homologs of known arthropod circadian proteins. Transcripts encoding putative homologs of all of the circadian proteins searched for, including PER, were identified in both the brain and eyestalk ganglia assemblies, with a number of the identified sequences confirmed using PCR and traditional Sanger sequencing. These findings suggest that both the brain and eyestalk ganglia are likely to possess intrinsic circadian signaling systems. Interestingly, while most of the proteins deduced from the identified transcripts are shared by both the brain and eyestalk ganglia, there is also evidence for assembly-specific isoforms of some proteins, including PER, suggesting the possibility of region-specific functional variation in the brain and eyestalk clocks, especially if they represent distinct timekeeping systems rather than a single distributed one. Collectively, the data presented here provide a foundation for future investigations of circadian signaling in *H. americanus*, including studies designed to determine the diel cycling patterns of clock gene expression in the brain and eyestalk ganglia, as well as studies examining the location and identity of clock neurons in the lobster brain and eyestalk ganglia and whether these cells represent two distinct or one distributed timekeeping system.

2. Materials and methods

2.1. De novo transcriptome assembly

Two transcriptomes, one for the eyestalk ganglia and the other for the brain, were generated for use in determining the presence/absence of circadian signaling systems in these two regions of the lobster CNS. The production of the eyestalk ganglia-specific transcriptome is described in an earlier study (Christie et al., 2017); production of the brain-specific assembly is described below. Tissues from the same set of lobsters were used to produce both transcriptomes, with all dissection, RNA isolation, cDNA library production and Illumina sequencing conducted simultaneously and using identical methods.

2.1.1. Animals and tissue dissection

American lobsters, *H. americanus*, (n = 4) were purchased commercially from seafood retailers in Brunswick, Maine. Lobsters were maintained in recirculating natural seawater aquaria at 10–12 °C and were fed a diet of chopped shrimp approximately weekly. For the isolation of the brain, animals were anesthetized by packing in ice for approximately 30 min, after which the anterior portion of the thorax and its underlying tissue were isolated. The brain was dissected from this preparation in chilled (~4 °C) physiological saline (composition in mM/l: 479.12 NaCl, 12.74 KCl, 13.67 $CaCl_2$, 20.00 $MgSO_4$, 3.91 Na_2SO_4, 11.45 Trizma base, and 4.82 maleic acid [pH = 7.45]).

2.1.2. RNA isolation

Freshly dissected individual brains (n = 4) were placed into sterile RNase-free 1.5 ml microfuge tubes containing 300 µl of TRIzol Reagent (catalog no. 15596018; Thermo Fisher Scientific Inc., Waltham, MA, USA) and manually homogenized using a sterile RNase-free disposable pestle (catalog no. 9950–901; Argos Technologies Inc., Elgin, IL, USA). RNA was isolated from the resulting homogenate using a Direct-zol RNA MiniPrep spin column system (catalog no. R2052; Zymo Research, Irvine, CA, USA) according to the manufacturer-supplied protocol. RNA quality was assessed using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). All RNA samples were stored at −80 °C until being shipped on dry ice to the Georgia Genomics Facility (University of Georgia, Athens, GA, USA) for library preparation and sequencing.

2.1.3. cDNA library production and Illumina sequencing

Double-stranded cDNA libraries were prepared from total RNA using a KAPA Stranded mRNA-Seq kit (catalog No. KK8420; KAPA Biosystems, Wilmington, MA, USA) following the manufacturer's instructions; 3 µg of total RNA/sample was used for library generation. In brief, total RNA samples were purified with two oligo-dT selection (poly (A) enrichment using oligo-dT beads). Samples were then fragmented and reverse transcribed into double-stranded complementary cDNA using random primers, with second strand synthesis marked using dUPT. Each brain library was tagged with a unique indexed adapter. A Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA), an AATI Fragment Analyzer (Advanced Analytical Technologies, Ankeny, IA, USA), and KAPA qPCR assays were used to determine the quality and quantity of the final pool of libraries. Paired-end Illumina sequencing (150 base pairs [bp]) was performed on a NextSeq 500 system (Illumina, San Diego, CA, USA) using the high output kit v2 with 300 cycles.

2.1.4. Transcriptome assembly

Prior to transcriptome assembly, raw sequencing reads were assessed for quality using FASTQC (v1.0.0) software (Illumina Basespace Labs). Specifically, each RNA-Seq brain library was quality filtered using FASTQ Toolkit (v.2.0.0) by trimming the first 9 bp of each read,

Download English Version:

https://daneshyari.com/en/article/10157246

Download Persian Version:

https://daneshyari.com/article/10157246

Daneshyari.com