

Final project report

Project ID	2005/1.04
Title	Circadian oscillators and light synchronization in the antarctic krill <i>Euphausia superba</i>
Principal investigator	Rodolfo Costa
Institution	University of Padova
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Duration	2 years
Assigned funding	80.000,00 Euro

Activities and results

Aim of the research was the identification and characterization of clock genes in the antarctic krill *Euphausia superba*. By using a homology-based approach we have identified a *cryptochrome* gene, which is a cardinal component of the clockwork machinery in several organisms. The *EsCRY* gene appears to be closely related to *Apis mellifera cry2* (*Amcry2*), with regard to number and position of introns, and to *Tribolium castaneum cry2* (*Tccry2*). The *EsCRY* open reading frame encodes a 545 aa protein that shares 69% sequence identity to both *A. mellifera* and *T. castaneum* CRY2, with the same overall domain arrangement and a divergent C-terminus. The phylogeny of the cryptochrome/photolyase proteins reveals that *E. superba* CRY is an ortholog of the mammalian-like cryptochromes and clusters with the insect CRY2 subfamily. Therefore, we suggest that we have identified *E. superba cry2*.

The temporal expression of *EsCRY* was evaluated in krill samples caught in the Ross Sea in January 2004 (Antarctic summer) at different times throughout the 24 h cycle (local times: 01:00, 06:00, 10:00, 15:00, and 18:00 h). At the transcriptional level, we observed by cross-correlation a significant daily fluctuation for *EsCRY* mRNA in the head, with a peak around 06:00 h. Conversely, *EsCRY* appears to be expressed at relatively high levels in the head throughout the day, and its expression profiles show considerable inter-individual variability. Nevertheless, we did detect a temporal fluctuation by cross-correlation in *EsCRY*, which may hint at an underlying cycle within our inevitably noisy data set.

By using the same approach we have identified a fragment of another clock gene, namely the krill's orthologs for *Drosophila clock* (*Clk*, circadian locomotor output cycles protein kaput), a transcription factor playing a key role in the generation of the circadian rhythmicity. We have cloned 1,113 bp in the ORF, corresponding to about 70% of the coding region and 462 bp at 5'UTR. The amino acid sequence shows high similarity (>75%) with the orthologs of arthropods and the presence of one HLH (helix-loop-helix) and two PAS (Per-Arnt-Sim) domains, fundamentals for the functioning of the protein.

In summary, we have obtained the first evidence about the presence of an endogenous circadian time-keeping mechanism in *E. superba*, that might allow this organism to synchronize its physiology and behavior to the Antarctic light regimes.

Products

A – papers in scientific magazines

C. De Pittà, C. Bertolucci, G.M. Mazzotta, F. Bernante, G. Rizzo, B. De Nardi, A. Pallavicini, G. Lanfranchi, R. Costa (2008). Systematic sequencing of mRNA from the Antarctic krill (*Euphausia superba*) and first tissue specific transcriptional signature. *BMC Genomics* 2008, 9:45.

G.M. Mazzotta, C. De Pittà, C. Benna, S. Tosatto, G. Lanfranchi, C. Bertolucci, R. Costa (2010) A *cry* from the krill. *Chronobiology International*, 27, 425-445.

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B – book chapters

C - proceedings of international conferences

Cristiano Bertolucci, Gabriella Mazzotta, Cristiano De Pittà, Elena Frigato, Silvio Tosatto, Gerolamo Lanfranchi, Rodolfo Costa (2009). Molecular circadian clock in the Antarctic krill *Euphausia superba*. XI Congress of European Biological Rhythms Society; Strasbourg 22-28 August.

De Pittà C., Biscontin A., Albiero A., Mazzotta G.M., Bertolucci C., Sales G., Romualdi C., Lanfranchi G., Costa R. – (2011) Gene expression profiling around the clock in the antarctic krill (*Euphausia superba*). Joint Meeting AGI-SIBV-SIGA, Cittadella di Assisi 19–22 settembre.

D – proceedings of national meetings and conferences

Cristiano Bertolucci (2007) Evoluzione e adattamento dell'orologio circadiano ad ambienti estremi. 68° congresso UZI, Lecce 24-27 settembre.

E – thematic maps

F – patents, prototypes and data bases

<http://krill.cribi.unipd.it/>


We have performed the first low-throughput Expressed Sequence Tag (EST) project in *E. superba*, and the systematic sequencing of four cDNA libraries prepared from different tissues has allowed us to establish an EST database containing 1,017 unique sequences. The database allows the visualization of the consensus sequences, and gives information about the expressed sequence tag (EST) assembled. Moreover it is also possible the search in the database by using key words contained in the putative description assigned to each consensus and by the ID associated to each cluster and EST.

The database is continuously updated with the identification of new sequences. At present, 6,142 ESTs, 777,544 nucleotide sequences produced by 454-next generation sequencing, 694 amino-acids sequences and the nearly complete DNA sequence of the mitochondrial genome (14,606 bp) are available for *E. superba* from public databases (taxonomy browser at NCBI, June 2011).

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Euphausia superba EST database

DATABASE
PEOPLE
RESEARCH



(<http://en.wikipedia.org/wiki/Image:Krill.jpg>)

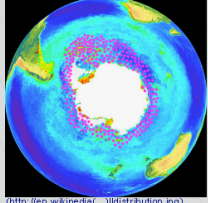
Krill are shrimp-like marine invertebrate animals. These small crustaceans are important organisms of the zooplankton, particularly as food for whales, manatees, baleen whales, seals, seabirds, fishes and squid. Krill are members of taxonomic order Euphausiacea. They are distinguished among the eucarids by the absence of maxillipeds, the exposure of the thoracic gills external to the carapace, and the possession of biramous thoracopods. These thoracic legs include the feeding legs and the grooming legs. Additionally all species have five swimming legs called pleopods or "swimmerets".

Most of them have photophores that are able to emit light on the eyestalks, the bases of the second and seventh thoracopods, and between the first 4 pairs of abdominal limbs. The precise function of these organs is as yet unknown; they might have a purpose in mating, social interaction, orientation and counter-illumination camouflage. Krill are pelagic and known from all oceanic environments to depths of 5,000 m.

Commercial fishing of krill is done in the Southern Ocean and in the waters around Japan. The total global production amounts to 150 - 200,000 tonnes annually, most of this from the Scotia Sea. Most krill is used for aquaculture, sport fishing, and in the pharmaceutical industry.

The Antarctic krill *Euphausia superba* have a circumglobal distribution, with the highest concentrations in the Atlantic sector of the Southern Ocean. It is a key species of the Antarctic ecosystem and plays an important role both as feeder of algae, bacteria and microzooplankton, and as a prey of vertebrates. *E. superba* displays a large daily vertical migration that occurs generally within the upper 200 m water column, making a significant amount of biomass available as food for predators near the surface at night and in deeper waters during the day. Antarctic krill makes up a biomass of over 500 million tons, roughly twice that of humans.

(http://en.wikipedia.org/wiki/Antarctic_krill)



(http://en.wikipedia.org/wiki/File:Krill_distribution.jpg)

PNRA

Università di Padova Università di Ferrara Università di Trieste

G – exhibits, organization of conferences, editing and similar

H - formation (PhD thesis, research fellowships, etc.)

Research units

Unit #1-PD

Rodolfo Costa (PI) – Full Professor
Gabiella Mazzotta – Assistant Professor
Federica Sandrelli – Associate Professor
Cristiano De Pittà – Assistant Professor
Clara Benna – Post-doc
Paola Cisotto – Research Assistant

Unit #2-FE

Cristiano Bertolucci – Assistant Professor
Dezfuli S. Bahram – Assistant Professor
Barbara Pavan – Research Assistant

Date: 29 September 2009

Notes