Programma Nazionale di Ricerche in Antartide (PNRA)

Final project report

Project ID:	2003/1.3
Title:	Molecular neurogenetics of circadian rhythmicity in *Euphausia superba*
Principal investigator:	Rodolfo Costa
Institution:	University of Padova
Email:	rodolfo.costa@unipd.it
Duration:	2 years
Assigned funding:	€ 30.000

Activities and results

Aim of the research activity was the identification and characterization of clock genes in *Euphausia superba*. Antarctic krill were collected in the Ross Sea in January 2004 during the XIX Italian Antarctic Expedition at different time of the day (01:00, 06:00, 10:00, 15:00, 18:00, 23:00), over a complete 24-hour cycle. DNA, RNA and proteins were extracted from dissected organs (head including brain and compound eyes, abdomen, thoracopods and photophores).
Three paralles approaches have been followed: 1) Construction of a genomic library; 2) Construction and sequencing of five cDNA libraries from different tissues; 3) Amplification of regions coding for proteic domains highly conserved in other organisms' homologs, by using degenerate primers.
The construction and the systematic sequencing of EST libraries allowed us to obtain a catalogue of *E. superba* transcripts, providing a powerful genomic tool to investigate the biology of the Antarctic krill.
In particular we have identified 1.770 high-quality ESTs, which were assembled into 216 overlapping clusters and 801 singletons resulting in a total of 1.017 non-redundant sequences. Quantitative RT-PCR analysis was performed to quantify and validate the expression levels of ten genes presenting different EST countings in krill tissues.
We have identified the gene *cryptochrome*, a cardinal component of the clockwork machinery in several organisms, which leads us to hypothesize the existence of an endogenous time-keeping mechanism in this crustacean. We have evaluated the temporal expression of *Euphausia superba cryptochrome* both at mRNA and protein levels in the central nervous system (where the master clock is) as well as in peripheral organs.

Products

A – papers in scientific magazines

1. 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.

B – book chapters

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C - proceedings of international conferences

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D – proceedings of national meetings and conferences

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E – thematic maps

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F – patents, prototypes and data bases

1. http://krill.cribi.unipd.it/ is a dedicated *E. superba* database were clusters, consensuses and related similarity and gene ontology searches have been organized.

G – exhibits, organization of conferences, editing and similar

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

1. Caterina Da Rè – University of Padova, "L'orologio circadiano del krill antartico *Euphausia superba*: identificazione del gene *Cryptocrome*"
2. Dr. Belgini Michela, research fellow (01/01/2004-31/03/2005), "Neurogenetica molecolare e ritmicità circadiana in *Euphausia superba*".
3. Dr. Montelli Stefano, research fellow (01/05/2006-31/12/2006), "Caratterizzazione e screening di una libreria di cDNA di *Euphausia superba* per la ricerca di geni orologio".

Research unit

- Rodolfo Costa (PI) - Professore ordinario
- Mauro Zordan – Professore associato
- Gabriella Mazzotta - Ricercatore
- Federica Sandrelli - Ricercatore
- Lorenzo Zane - Ricercatore
- Paola Cisotto – Assistente tecnico
- Cristiano Bertolucci - Ricercatore

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