

Final project report

Project ID: 2002/1.1
Title: Molecules and genes of Antarctic marine organisms in evolutionary and cold-adaptive perspective
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Duration: two years
Assigned funding: € 109.500,00

Activities and results

The project involved seven research unities, each one from a distinct University and headed by Drs F. Dini (Univ. Pisa), P. Luporini (Univ. Camerino), M. Mazzini (Uni. La Tuscia), E. Olmo (Univ. Ancona), T. Paternello (Univ. Padova), V. Sbordoni (Univ. Roma 2) e B. M. Uva (Univ. Genova). Material of study was represented by ciliated protozoa, mollusks, krill, tunicates and fish, mostly collected from the surrounding of the Stazione Mario Zucchelli. Results of major interest (described in published articles listed below) can be summarized as follows. (1) By analysis of functional biology and molecular phylogenetics it was possible to identify two marine ciliate species, *Euplotes focardii* and *E. nobilii*, that represent new, excellent experimental models to study the molecular basis of cold-adaptation in eukaryotic microorganisms. In *E. focardii*, it was shown that: (a) the heat-shock genes have selectively lost (like icefish) any capacity to respond heat shocks, albeit having retained a canonical structure and a full transcriptional responsiveness to other chemical and physic environmental stresses; (b) the most expressed isotypes of beta-tubulin carry specific aminoacid substitutions and modifications in functionally critical sites of the molecule and their folding into microtubule structures requires specific interactions with at least one type of "tcp-1" molecular chaperones. In *E. nobilii*, it was possible to isolate, purify, and determine the primary structure of a set of water-borne signal proteins (pheromones) with mitogenic and sexual-inducing activity. (2) In the icefish *Chionodraco hamatus*, in a general context of immunological studies, it was determined the structure (as predicted on the basis of the cloned relevant gene sequences) of the IL-1 cytokine and of a major component of the T cell receptor; then, the levels expression of these molecules were quantified in different organs and tissues. (3) In *Euphausia superba* (krill), the PGI-coding nucleotide sequence was obtained by selective amplification from genomic DNA preparations and used to screen a representative set of Antarctic krill populations for assessing their degrees of gene flow in relation with their ecological distribution. (4) In mollusks, it was carried out an initial characterization of homeotic (Hox- and ParaHox-like) genes involved in the early stage of embryo development of *Yoldia eightisi* and, based on comparison of 16S and 12S mitochondrial gene sequences, new phylogenetic relationships and taxonomic positions were proposed for the common Antarctic scallop, *Adamussium colbecki*, and the chitons *Callochiton steinenii* and *Nuttallochiton mirandus*.

Products

A – papers in scientific magazines

1. Alimenti C., Orteni C., Carratore V. & Luporini P. (2002). Structural characterization of a protein pheromone from a cold-adapted (Antarctic) single-cell eukaryote, the ciliate *Euplotes nobilii*. *FEBS Letters*, 514: 329-332.
2. Alimenti C., Orteni C., Carratore V. & Luporini P. (2002). Structural characterization of En-1, a cold-adapted pheromone isolated from the Antarctic ciliate, *Euplotes nobilii*. *Bioch. Biophys. Acta*, 1621: 17-21.
3. Pucciarelli S. & Miceli C. (2002). Microtubule cold adaptation: characterization of alfa and beta tubulin from

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- the Antarctic ciliate, *Euplotes focardii*. *Extremophiles*, 6: 385-389.
4. Pucciarelli S., Miceli C., Melki R. (2002). Heterologous expression and folding analysis of the Antarctic ciliate *Euplotes focardii* beta-tubulin. *Eur. J. Biochem.* 269: 6271-6277.
 5. Pucciarelli S., Di Giuseppe G., Dini F., Luporini P. & Miceli C. (2003). *Euplotes focardii*, an Antarctic psychrophilic ciliate of presumed Gondwanan origin. *J. Euk. Microbiol.* 50: 133-135.
 6. Alimenti C., Ortenzi C., Carratore V. & Luporini P. (2003). Cold-adapted *Euplotes* pheromones. *Eur. J. Protistol.*, 39: 399-403.
 7. Barucca M., Olmo E. & Canapa A. (2003). Hox and Para-Hox genes in bivalve molluscs. *Gene*, 317: 97-102.
 8. Patarnello T. et al. (2003). Phylogeography of the *Chionodraco* genus (Perciformes, Channichthyidae) in the Southern Ocean. *Mol. Phylogenet. Evol.*, 28: 420-429.
 9. Barucca M., Olmo E., Schiaparelli S. & Canapa A. (2004). Molecular phylogeny of the family Pectinidae (Mollusca: Bivalvia) based on mitochondrial 16S and 12S rRNA genes. *Mol. Phylogenet. Evol.* 31: 89-95.
 10. La Terza A., Miceli C. & Luporini P. (2004). The gene for the heat-shock protein 70 of *Euplotes focardii*, an Antarctic psychrophilic ciliate. *Antarctic Sci.*, 16: 23-28.
 11. Pucciarelli S., Marziale F., Di Giuseppe G., Barchetta S. & Miceli C. (2005). Ribosomal coldadaptation: characterization of the acidic ribosomal P0 and P2 proteins from the Antarctic ciliate *Euplotes focardii*. *Gene*. 360: 103-111.
 12. Luporini, P., Alimenti, c., Ortenzi, C., & Vallesi, A. (2005). Ciliate mating types and their specific protein pheromones. *Acta Protozool.*, 44: 89-101.
 13. Buonocore F., Randelli E., Paderi F., Bird S., Secombes C.J., Mazzini M. & Scapigliati G. (2006). The cytokine IL-1 from the crocodile icefish *Chionodraco hamatus* (Perciformes: Channichthyidae). *Polar Biol.*, 29: 1018-1027.
 14. Luporini P., Vallesi A. Alimenti C. & Ortenzi C. (2006). The cell type-specific signal proteins (pheromones) of protozoan ciliates. *Curr. Pharm. Des.*, 12: 3015-3024.
 15. Papetti C., Zane L. & Patarnello T. (2006). Isolation and characterization of microsatellite loci in the icefish *Chionodraco rastrospinosus* (Perciformes, Notothenioidae, Channichthyidae). *Mol. Ecol. Notes*, 6: 207-209.
 16. Zane L., Marcato S., Bargelloni L., Bortolotto E., Papetti C., Simonato M., Varotto V. & Patarnello T. (2006). Demographic history and population structure of the Antarctic silverfish *Pleuragramma antarcticum*. *Mol. Ecol.*, 15: 4499-4511.
 17. La Terza A., Passini V., Barchetta S., & Luporini P. (2006). Adaptive evolution of the heat shock response in the Antarctic psychrophilic ciliate, *Euplotes focardii*: hints from the comparative determination of the *hsp70* gene structure. *Antarctic Sci.*, 19: 239-244.

Research units

Dini F. (UniPI); Luporini P. (UniCam); Mazzini M. (UniTus); Olmo E. (UniAN); Patarnello T. (UniPD); Sbordoni V. (UniRM2); Uva B.M. (UniGE)

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