

Final project report

<i>Project ID</i>	2005/1.01
<i>Title</i>	Genomics and Proteomics of the Antarctic ciliate <i>Euplotes focardii</i>
<i>Principal investigator</i>	Miceli Cristina
<i>Institution</i>	University of Camerino
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<i>Duration</i>	3 years
<i>Assigned funding</i>	220.000,00 Euro

Activities and results

We carried out a pilot genome project including protein analysis in *Euplotes focardii*, a ciliated protozoan endemic of Antarctic coastal sea waters, that shows a strict psychrophilic behaviour. This is the first genome project in a psychrophilic unicellular eukaryote.

Ciliates are characterized by nuclear dimorphism: the germline micronucleus contains the entire genome in the form of large chromosomes; the somatic macronucleus contains small linear DNA molecules (nanochromosomes), each of which constitutes a single genetic unit, usually producing a single transcript, flanked by regulatory regions and capped by telomeres. Each nanochromosome is amplified to thousands of copies.

The objective of the project was approached by the construction of a library starting from nanochromosomes ranging in size from 2 to 5 kb into the pJazz linear vector from LUCIGEN, corresponding to about one fourth of the total macronuclear genome. About 9000 clones corresponding to partial or entire nanochromosomes (each containing at least one telomere) were obtained and are now still under annotation. Comparative genomics with genes of *Tetrahymena thermophila* and *Paramecium tetraurelia* was performed. The comparison with homologous genes from the congeneric mesophilic *E. crassus* was also of particular help (for this species genome is under annotation in collaboration with Dr. Vadim Gladyshev, Harvard Medical School, Boston). Finally, to better predict the open reading frames of the nanochromosomes, most of them containing introns, a transcriptome analysis of RNA from cells in logarithmic phase was necessarily performed. Sequences were carried out by the pyrosequencing technique. All data analysed by BLAST are stored in the web site: <http://eufocardii.irideprogetti.it/FRM/Master/Login.aspx>.

About 15Gbases were sequenced and assembled in 7158 transcripts. The Gene Ontology annotation revealed the presence of a high percentage of transcripts encoding proteins involved in oxidoreductase activity. A similar finding emerged also from the analysis of transcriptome from the Antarctic fish *Dissostichus mawsoni* in which genes responding to oxidative stress are up regulated and duplicated, confirming that increased defenses against oxidative stress likely constituted an important aspect of evolutionary adaptation of the Antarctic organisms in their oxygen-rich environment.

Together with the gene annotation, we deeply analysed genes encoding proteins of interest for

- i) the involvement in cold adaptation, such as tubulins (showing unique substitutions increasing the hydrophobicity of the regions involved in the lateral and longitudinal contacts), putative 2-on-2 haemoglobin (found in *E. crassus*, and not in *E. focardii*)... and potential antifreeze (derived from bacterial collagen, found in *E. focardii* and not in *E. crassus*);
- ii) representing biomarkers of temperature changes, such as heat shock proteins and oxidative stress proteins regulated by inducible promoters;
- iii) potential industrial applications, such as enzymes including lipases, amylases, and permeases, more rich in Gly, Ala and Ser residues in *E. focardii*. Most likely an increased molecular flexibility facilitates the conformational changes of proteins in an energy-poor environment, ensuring an efficient metabolic activity at low temperature. Phospholipases are the most promising for industrial applications. They are easily expressed in high amount and show unexpected high stability and high activity from temperatures from 0 up to 30 °C.

Products

A – papers in scientific magazines

1. **Yu T, Barchetta S, Yang G, de Pascale D, Devaraj RR, Pucciarelli S, Gladyshev VN, Miceli C.** Cold adaptation of membrane transport proteins by comparative genomics in the ciliate *Euplotes focardii*. Manuscript submitted
2. **Devaraj RR, Pucciarelli S, Yu T, Barchetta S, Yang G, Miceli C.** Ontology analysis of the transcriptome from the cold adapted ciliate *Euplotes focardii*. Manuscript submitted
3. **Yang G, de Pascale D, Yu T, Barchetta S, Pucciarelli S, Gladyshev VN, Miceli C.** Characterization of patatin-like phospholipases in the Antarctic ciliate *Euplotes focardii*, enzymes with potential biotechnological applications. Manuscript submitted
4. **Chiappori F, Pucciarelli S, Merelli I, Ballarini P, Miceli C, Milanesi L.** Structural thermal adaptation of β - tubulins from the antarctic psychrophilic protozoan *Euplotes focardii*. *Proteins*. 2011 Dec 22. doi: 10.1002/prot.24016.
5. **Yu T, Barchetta S, Pucciarelli S, La Terza A, Miceli C.** [A Novel Robust Heat-inducible Promoter for Heterologous Gene Expression in *Tetrahymena thermophila*](#). *Protist*. 2012 Mar;163(2):284-95
6. **Giordano D, Russo R, Coppola D, di Prisco G, Verde C.** (2010) Molecular adaptations in haemoglobins of notothenioid fishes. *J Fish Biol.* Feb;76(2):301-18.
7. Parrilli E, Giuliani M, **Giordano D**, Russo R, Marino G, **Verde C**, Tutino ML. (2010) The role of a 2-on-2 haemoglobin in oxidative and nitrosative stress resistance of Antarctic *Pseudoalteromonas haloplanktis* TAC125. *Biochimie*. Aug;92(8):1003-9.
8. **Pucciarelli S, La Terza A, Ballarini P, Barchetta S, Yu T., Marziale F**, Passini V, Methé B, **Detrich HW III, Miceli C.** (2009) Molecular cold-adaptation of protein function and gene regulation: The case for comparative genomic analyses in marine ciliated protozoa. *Marine Genomics*. 2(1), pp. 57-66
9. Tutino ML, **di Prisco G**, Marino G, **de Pascale D** (2009). Cold-adapted esterases and **lipases: from fundamentals to application. Protein Pept Lett.**16(10):1172-80. **Review.**
10. **Marziale F, Pucciarelli S, Ballarini P, Melki R**, Uzun A, Ilyin VA, **Detrich HW 3rd, Miceli C.** 2008. Different roles of two gamma-tubulin isoforms in the cytoskeleton of the Antarctic ciliate *Euplotes focardii*: remodelling of interaction surfaces may enhance microtubule nucleation at low temperature. *FEBS J.* Nov;275(21):5367-82
11. **de Pascale D**, Cusano AM, Autore F, Parrilli E, **di Prisco G**, Marino G, Tutino ML. (2008) The cold-active Lip1 lipase from the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 is a member of a new bacterial lipolytic enzyme family. *Extremophiles*. 2008 May;12(3):311-23.
12. **Verde C**, Vergara A, Mazarella L, **di Prisco G.** (2008) The hemoglobins of fishes living at polar latitudes - current knowledge on structural adaptations in a changing environment. *Curr Protein Pept Sci.* Dec;9(6):578-90. **Review.**
13. **Verde C, Giordano D, di Prisco G.** (2008) The adaptation of polar fishes to climatic changes: Structure, function and phylogeny of haemoglobin. *IUBMB Life*. Jan;60(1):29-40. **Review.**
14. Dettai A, **di Prisco G**, Lecointre G, **Parisi E, Verde C.** (2008); Inferring evolution of fish proteins: the globin case study. *Methods Enzymol.* 436:539-70. **Review.**
15. Negrisola E, Bargelloni L, Patarnello T, Ozouf-Costaz C, Pisano E, **di Prisco G, Verde C.** (2008) Comparative and evolutionary genomics of globin genes in fish. *Methods Enzymol.*;436:511-38.

B - proceedings of international conferences

1. S. Pucciarelli, S. Barchetta, P. Ballarini, D. Sparvoli, C. Miceli. Ciliated protozoans as model organisms to study beta-tubulin isoforms specifically involved in cilia formation.
2. 2010 FASEB Summer Research Conferences, Biology of Cilia and Flagella, July 5-10, 2010, Saxtons River, Vermont
3. S. Pucciarelli, Raghul Rajan Devaraj, S. Barchetta, T. Yu, A. La Terza, C. Miceli Characterization of new biomarkers from the Antarctic ciliate *Euplotes focardii* by a transcriptomic approach. 27th New European Society for Comparative Physiology and Biochemistry (ESCPBnew) Congress, Alessandria, Italy - 5-9 September 2010
4. S. Pucciarelli, R. Rajan Devaraj, G. Yang, S. Barchetta, T. Yu, C. Miceli. Characterization of new biomarkers from the Antarctic ciliate *Euplotes focardii* by a transcriptomic approach. 60th Annual Meeting of the International Society of Protistologists. July 18-23, 2010 Canterbury, UK.
5. S. Pucciarelli, F. Marziale, P. Ballarini, D. Sparvoli, C. Miceli, Molecular adaptation to the cold: characterization of tubulin from the Antarctic ciliate *Euplotes focardii*. Xth SCAR international Biology symposium, Sapporo, Giappone, 26-31 Luglio 2009
6. S. Pucciarelli, S. Barchetta, P. Ballarini, D. Sparvoli, C. Miceli, Properties of diverse tubulin isoforms in *Euplotes* and *Tetrahymena*, FASEB Summer Research Conference on Molecular Biology of ciliates, Tucson, Arizona. July, 21-27 2009.

Programma Nazionale di Ricerche in Antartide (PNRA)

7. S. Barchetta, T. Yu, S. Pucciarelli, P. Ballarini, A. La Terza, H.W. Detrich, C. Miceli, *Euplotes focardii*, a model organism for the characterization of enzymes with potential industrial value, SCAR/IASC IPY Open Science Conference St. Petersburg, Russia, July 8th – 11th 2008
8. S. Pucciarelli, F. Marziale, P. Ballarini, H.W. Detrich, C. Miceli, Molecular adaptation to the cold: characterization of tubulin from the Antarctic ciliate *Euplotes focardii* SCAR/IASC IPY Open Science Conference St. Petersburg, Russia, July 8th – 11th 2008
9. Pucciarelli S., Marziale F., Ballarini P., Melki R., Miceli C. Localization in microtubule organizing centers and aberrant folding of a beta-tubulin isotype of *Euplotes focardii*. FASEB Summer Research Conference on Molecular Biology of ciliates, Tucson, Arizona. July, 21-27 2007.

D – proceedings of national meetings and conferences

1. S. Pucciarelli, R. Rajan Devaraj, G. Yang, S. Barchetta, T. Yu, C. Miceli. Characterization of new biomarkers from the Antarctic ciliate *Euplotes focardii* by a transcriptomic approach. XX congresso SitE (Società Italiana di Ecologia). Roma, 27-30 Settembre 2010
2. S. Pucciarelli, R. Rajan Devaraj, G. Yang, S. Barchetta, T. Yu, C. Miceli. Transcriptomics to identify new biomarkers from the Antarctic ciliate *Euplotes focardii*. Italian Society of Protozoology 2-4 settembre 2010
3. S. Pucciarelli, P. Ballarini, S. Barchetta, D. Sparvoli, M. Montani, C. Miceli. I ciliati come organismi modello per studiare le diverse funzioni dei microtubuli mediante la genomica funzionale. 70° Congresso Nazionale dell'Unione Zoologica Italiana Rapallo (GE), 21-24 Settembre 2009
4. S. Barchetta, T. Yu, S. Pucciarelli, P. Ballarini, A. La Terza, C. Miceli. *Euplotes focardii*, un organismo modello per la caratterizzazione di proteine di potenziale interesse industriale. 70° Congresso Nazionale dell'Unione Zoologica Italiana Rapallo (GE), 21-24 Settembre 2009
5. S. Barchetta, T. Yu, S. Pucciarelli, P. Ballarini, A. La Terza, H.W. Detrich, C. Miceli. Genomic studies and characterization of enzymes with potential industrial value in *Euplotes focardii*. Italian Society of Protozoology April 2008-J.Euk.Microbiol. in press
6. S. Pucciarelli, F. Marziale, M. Montani, P. Ballarini, R. Melki, C. Miceli
7. Molecular adaptation to the cold: a study on tubulin polymerization and folding in the Antarctic ciliate *Euplotes focardii*. Italian Society of Protozoology April 2008- J.Euk. Microbiol. in press
8. Pucciarelli S., Marziale F., Ballarini P., Melki R., Miceli C. Molecular adaptation to the cold environment: a study on tubulin polymerisation and folding in the Antarctic marine ciliate *Euplotes focardii*. SIBMA 2007. Biochimica Marina e dell'Ambiente. San Benedetto del Tronto (AP). 31 maggio-1° giugno 2007.

F – patents, prototypes and data bases

1. <http://eufocardii.irideprogetti.it/FRM/Master/Login.aspx> In this data bank are stored all genomic and transcriptomic sequences.

G – exhibits, organization of conferences, editing and similar:

Organization of one day meeting at the University of Camerino (The relevance of this meeting appears in the notes at the bottom):

Tuesday 14 September 2010 Cycle of Seminars on MOLECULAR BIOLOGY OF CILIATES

"New roles for maternally-inherited noncoding RNA in *Oxytricha*"

Dr. Mariusz Nowacki, Institut für Zellbiologie, Universität Bern Switzerland

"RNA-directed DNA elimination in *Tetrahymena*"

Dr. Kazufumi Mochizuki, IMBA (Institute of Molecular Biotechnology of the Austrian Academy of Sciences) Vienna, Austria

"The molecular basis for maternal inheritance of mating-type determination in *P. tetraurelia*"

Dr. Eric Meyer, CNRS, Paris, France

"Epigenetic control of macronuclear differentiation in the ciliate *Stylonychia lemnae*"

Dr. Franziska Jönsson, Universität Witten/Herdecke gGmbH, Institut für Zellbiologie Witten

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

1. **PhD thesis of** Francesca Marziale in Environmental Sciences and Public Health at the School of Advanced Studies- Università di Camerino, June 13th 2007. Title: Molecular mechanisms of cold adaptation in the Antarctic Ciliate *Euplotes focardii*
2. **PhD thesis of** Ting Yu in Environmental Sciences and Public Health at the School of Advanced Studies- Università di Camerino, 12 March 2010. Title: Genomic studies and stress genes in Ciliates
3. **Degree Thesis of** Daniela Sparvoli in Biomolecular Sciences ("laurea magistrale"). Title: Cell localization and function of beta-tubulin isotypes in the Antarctic ciliate *Euplotes focardii*. February 19th 2009.

Research units

n. 1

Name	Institution
Cristina Miceli (coordinator)	Dip. di Biologia MCA Camerino
Antionietta La Terza	Dip. di Biologia MCA Camerino
Sandra Pucciarelli	Dip. di Biologia MCA Camerino
Patrizia Ballarini	Dip. di Biologia MCA Camerino
Sabrina Barchetta	Dip. di Biologia MCA Camerino
Francesca Marziale	Dip. di Biologia MCA Camerino
Ting Yu	Dip. di Biologia MCA Camerino

n. 2

Name	Institution
Donatella de Pascale	IBP/CNR-Napoli
Ennio Cocca	IBP/CNR-Napoli
Guido di Prisco	IBP/CNR-Napoli
Katia Marino	IBP/CNR-Napoli
Vito Carratore	IBP/CNR-Napoli

n.3

Name	Institution
Cinzia Verde	IBP/CNR-Napoli
Guido Di Prisco	IBP/CNR-Napoli
Marco Balestrieri	IBP/CNR-Napoli
Daniela Giordano	IBP/CNR-Napoli
Vito Carratore	IBP/CNR-Napoli

Date: The project started in 2007 and closed in 2010

Notes

The main value of this project is the fact that the obtained results are prospective: time is still necessary to finish the genome annotation of *Euplotes focardii* and other research products are going to appear. The collaboration with the laboratory of Dr. Vadim Gladyshev of the Harvard Medical School (Boston, MA, USA), where the genome of a not-cold adapted *Euplotes* species is under annotation, is very promising for comparative genomics. More characteristics of cold adaptation at level of regulation of gene expression and at level of protein structure will appear soon. Furthermore, the analysis of *E. focardii* transcriptome provides evidence that also epigenetic phenomena are involved in the adaptation to the cold environment. The correlation of epigenetic variations to the response of environmental changes is a very new and important concept. The meeting of September 14th 2010 at the University of Camerino highlighted this idea and stimulated the proposal, followed by approval and activation of a COST (European Cooperation in Science and Technology) action for the following 4 years. The action is based on a European network at present involving members of 11 countries. The title of the action is "Ciliates as model systems to study genome evolution, mechanisms of non-Mendelian inheritance, and their roles in environmental adaptation". Cristina Miceli, coordinator of this PNRA project is now the Chair of the action (please see the web site: http://www.cost.esf.org/domains_actions/bmbs/Actions/BM1102).