

Final project report

Project ID	2005/1.02
Title	Evolutionary adaptation of genes, molecules, and cells involved into the immune response of Antarctic teleosts
Principal investigator	Umberto Oreste
Institution	CNR Istituto di Biochimica delle Proteine, Napoli
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Duration	3 years
Assigned funding	230.000,00 Euro

Activities and results

The overall studies performed in the framework of the project have extensively increased the knowledge of the molecules of the immune system of Antarctic teleosts. The numerous sequences deposited in the databanks (about 150), and available to the international scientific community, have been used by several groups. The papers published within the project have been largely quoted.

The project has been focused on the study of the antibody molecule of Antarctic teleost, investigating both the heavy and light chains as well as the secretory and membrane-bound form. The antibody repertoire has been investigated, amino acid sequences and molecular structures have been analyzed, some conclusions have been drawn on the evolutionary adaptation. Other molecules have been studied such as Toll-like receptors, Interleukin 1, T-cell receptor, $\Delta 9$ -desaturase, and pepsin 1 and 2.

In particular, Dr Oreste's Unit, in collaboration with Prof. Pisano, has mapped the IgH gene locus on chromosomes of 10 species of the genus *Trematomus*

Furthermore, some physiological aspects of the Ig-mediated immune response have been studied in collaboration with Prof. Abelli: the hepato-biliary transport of Ig has been demonstrated in *Trematomus bernacchii*.

The gene coding for *T. bernacchii* Immunoglobulin light chain has been studied. Three distinct isotypes have been identified and found different in the primary sequence and the molecular structure.

The *Chionodraco hamatus* Ig heavy chain membrane-bound form has been sequenced. Molecular models of the α -helix transmembrane dimer have been built. Based on these results, the evolutionary history of the immunoglobulin membrane form of Notothenioidae and, more in general, through the vertebrate evolution, has been reconstructed.

Prof. Bargelloni has determined the sequence of genes encoding Toll-like receptors TLR2 e TLR9 of *Trematomus bernacchii* and *C. hamatus*. Based on comparison with ortholog sequences from other species using computational tools, the role of natural selection during evolution of the Antarctic species TLR has been investigated.

Prof. Scapigliati has studied the pro-inflammatory cytokine Interleukin-1 β (IL-1 β) and the *C. hamatus* T-cell receptor β chain (TCR β). The mature transcript and genomic DNA coding for IL-1 β have been cloned and sequenced. Moreover, *in vivo* and *in vitro* expression of mRNA coding for IL-1 β has been studied in different organs and tissues. The gene coding for the T-cell receptor β chain (Tr β) has been cloned and sequenced and its basal expression has been analyzed in various organs and tissues. Molecular models have been built.

Prof. Maresca has cloned and sequenced the gene encoding $\Delta 9$ -desaturase of *T. bernacchii* and *C. hamatus*. Dr. Capasso has compared sequence and structural data collected on metallothioneins from *T. bernacchii* and *C. hamatus* to those from non-Antarctic species. Moreover, *T. bernacchii* pepsin A1 and A2 have been purified and recombinant enzymes have been obtained.

Products

A – papers in scientific magazines

1. Abelli, L., Coscia, M.R., De Santis, A., Zeni, C., Oreste, U., 2005. Evidence for hepato-biliary transport of immunoglobulin in the Antarctic teleost fish *Trematomus bernacchii*. *Dev. Comp. Immunol.* 29, 431-42.
2. Pisano, E., Coscia, M. R., Mazzei, F., Ghigliotti, L., Coutanceau, J., Ozouf-Costaz, C., Oreste, U., 2007. Cytogenetic mapping of immunoglobulin heavy chain genes in Antarctic fish. *Genetica* 130, 9-17.
3. Brier S, Maria G, Carginale V, Capasso A, Wu Y, Taylor RM, Borotto NB, Capasso C, 2007. Engen JR. Purification and characterization of pepsins A1 and A2 from the Antarctic rock cod *Trematomus bernacchii*. *FEBS J.* 274:6152-66.
4. Buonocore F., Randelli E., Paderi F., Bird S., Secombes C.J., Mazzini M., Scapigliati G., 2006. The cytokine IL-1 β from the Crocodile icefish *Chionodraco hamatus* (Perciformes: Channichthyidae). *Pol. Biol.* 29: 1018-1027.
5. Coscia M.R., Giacomelli S., De Santi C., Varriale S., Oreste U., 2008. Immunoglobulin light chain isotypes in the teleost *Trematomus bernacchii*. *Mol. Immunol.* 45:3096-3106.
6. Merlino A., Varriale S., Coscia M.R., Mazzarella L., Oreste U., 2008. Structure and association of the transmembrane immunoglobulin domain: insights from molecular dynamics studies on the B Cell Receptor of the teleost *Chionodraco hamatus*. *J. Mol. Graph. Mod.* 27:401-407.
7. Coscia M. R. Oreste U., 2009. Exploring Antarctic teleost immunoglobulin genes. *Mar. Genom.* 2: 19-25.
8. Coscia M. R., Varriale S., De Santi C., Giacomelli S., Oreste U., 2010. Evolution of the Antarctic teleost immunoglobulin heavy chain gene locus. *Mol. Phylogenet. Evol.* 55:226-233.
9. Varriale S., Merlino A., Coscia M.R., Mazzarella L., Oreste U., 2010. An evolutionary conserved motif is responsible for Immunoglobulin heavy chain packing in the B cell membrane. *Mol. Phylogenet. Evol.* 57: 1238-1244.
10. Coscia M. R., Varriale S., Giacomelli S., Oreste U., 2010. Antarctic Teleost Immunoglobulins: more extreme, more interesting. *Fish & Shellf. Immunol.* in press (doi10.1016/j.fsi.2010.10.018).

B – book chapters

1. Oreste U., Maglione M., Giacomelli S., Coscia M.R., 2005. Evolutionary adaptation of membrane-bound immunoglobulins of Antarctic teleost. In *Proceeding of the fifth PNRA meeting on Antarctic Biology* (P. Luporini & M. Morbidoni eds). Published by the Polarnet coordinating unit., pp 121-126.
2. Oreste U., Coscia M.R., 2007. Evoluzione adattativa delle molecole anticorpali dei teleostei antartici. In: *Clima e cambiamenti climatici*. A cura di B. Carli, G. Cavarretta, M. Colacino, S. Fuzzi. CNR. Pag 283-286.
3. Coscia M.R., Oreste U., 2010. Fish parasites in polar seas In "Rassegna delle attività di ricerca scientifica e tecnologica del CNR nell'ambito del mare e delle sue risorse" edited by Daniela Beatrice e Luigi Mazari Villanova, Dipartimento Terra e Ambiente, CNR.

C - proceedings of international conferences

1. Abelli L. Coscia M.R., Zeni C., Bertoni F., Oreste U.: Peculiar immune mechanisms in the liver of the Antarctic teleost fish *Trematomus bernacchii*. 10th Congress of the International Society of Developmental and Comparative Immunology (ISDCI), Charleston, South Carolina (USA), 2-6 July 2006. Abstract 191, pag. 59.
2. Coscia M.R.: Unexpected features of the *Chionodraco hamatus* immunoglobulin heavy-chain gene locus. SCAR, IPY, Evolution and Biodiversity in the Antarctic. The Response of Life to Change (EBA). International Workshop: The polar and alpine environments: molecular and evolutionary adaptations in prokaryotic and eukaryotic organisms, Naples 29-30 May 2008.
3. Coscia M. R., Varriale S., De Santi C., Giacomelli S., Oreste U.: Evolution of the Antarctic teleost immunoglobulin heavy chain gene. 11th Congress of the International Society of Developmental and Comparative Immunology, Prague June 28 – July 3, 2009. Proc. Pag. 112
4. Coscia M.R., Varriale S., Giacomelli S., Oreste U. New insights into the evolution of the antarctic teleost IgM heavy chain gene. First EOEFI Symposium. Viterbo, 23-27 May 2010. Proc. P.48.
5. Oreste U.: A unique mRNA splicing generating the membrane form of the immunoglobulin heavy chain of Antarctic teleosts. SCAR, IPY Workshop, Polar Marine and Lacustrine Organism:Gene and Protein Evolution in a Changing Environment. Naples, 24-25 May 2010.

D – proceedings of national meetings and conferences

1. Abelli L. Coscia M.R., Zeni C., Bertoni F., Oreste U.: Peculiar immune mechanisms in the liver of the Antarctic teleost fish *Trematomus bernacchii*. 10th Congress of the International Society of Developmental and Comparative Immunology (ISDCI), Charleston, South Carolina (USA), 2-6 July 2006. Abstract 191, pag. 59.
2. Coscia M.R.: Unexpected features of the *Chionodraco hamatus* immunoglobulin heavy-chain gene locus. SCAR, IPY, Evolution and Biodiversity in the Antarctic. The Response of Life to Change (EBA). International Workshop: The polar and alpine environments: molecular and evolutionary adaptations in prokaryotic and eukaryotic

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organisms, Naples 29-30 May 2008.

3. Coscia M. R., Varriale S., De Santi C., Giacomelli S., Oreste U.: Evolution of the Antarctic teleost immunoglobulin heavy chain gene. 11th Congress of the International Society of Developmental and Comparative Immunology, Prague June 28 – July 3, 2009. Proc. Pag. 112
4. Coscia M.R., Varriale S., Giacomelli S., Oreste U. New insights into the evolution of the antarctic teleost IgM heavy chain gene. First EOOFI Symposium. Viterbo, 23-27 May 2010. Proc. P.48.
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E – thematic maps

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F – patents, prototypes and data bases

G – exhibits, organization of conferences, editing and similar

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

Thesis Degree in Biological Sciences Degree:

1. Rosa De Feo, Polimorfismo allelico dei domini CH delle IgM del teleosteo antartico *Trematomus bernacchii*.
2. Vera Avagliano, Analisi dei geni codificanti la catena pesante dell'immunoglobulina della rezza antartica *Bathyrja eatonii*.
3. Stefano Giacomelli Analisi di sequenze geniche codificanti la forma secreta della catena pesante delle immunoglobuline in teleostei antartici
4. Rosa De Feo Polimorfismo allelico dei domini CH delle IgM del teleosteo antartico *Trematomus bernacchii*

Thesis Degree in Chemistry

1. Concetta De Santi: Studio delle catene leggere delle immunoglobuline del teleosteo antartico *Trematomus bernacchii*

PhD thesis in Chemical Sciences:

1. Sonia Varriale, Macromolecole secrete e di membrana coinvolte nella risposta immunitaria dei teleostei antartici

Fellowships:

1. Serena Ferraresso, Recettori Toll-like in teleostei antartici
2. Stefano Giacomelli, Studio della molecola immunoglobulinica e dei geni che la codificano nei teleostei antartici

Research units

Unità Operativa Oreste

Umberto Oreste, IBP, CNR, Napoli
Luigi Abelli, Dip. Biologia, Università di Ferrara
Fabrizio Bertoni, Dip. Biologia, Università di Ferrara
Elio Parisi, IBP, CNR, Napoli
Maria Rosaria Coscia, IBP, CNR, Napoli
Sonia Varriale, IBP, CNR, Napoli
Concetta De Santi, IBP, CNR, Napoli
Monica Maglione, IBP, CNR, Napoli
Stefano Giacomelli, IBP, CNR, Napoli
Chiara Motta, Dip. Scienze Biologiche, Università Federico II, Napoli

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Valentina Zappulli, Dip. Sanità Pubblica Veterinaria, Università di Padova
Barbara Cardazzo, Dip. Sanità Pubblica Veterinaria, Università di Padova
Raffaella Franch, Dip. Sanità Pubblica Veterinaria, Università di Padova
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Sergio Colonna, Dip. Scienze Farmaceutiche, Università di Salerno

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Lucia Mastrolia, Dip. Scienze Ambientali, Università della Tuscia, Viterbo
Francesco Buonocore, Dip. Scienze Ambientali, Università della Tuscia, Viterbo
Nicla Romano, Dip. Scienze Ambientali, Università della Tuscia, Viterbo
Simona Picchietti, Dip. Scienze Ambientali, Università della Tuscia, Viterbo
Randelli Elisa, Dip. Scienze Ambientali, Università della Tuscia, Viterbo
Romolo Fochetti, Dip. Scienze Ambientali, Università della Tuscia, Viterbo

Unità Operativa Capasso

Clemente Capasso, IBP, CNR, Napoli
Vincenzo Carginale, IBP, CNR, Napoli

Date: 09/02/2010

Notes