INSTITUTE OF BIOLOGY

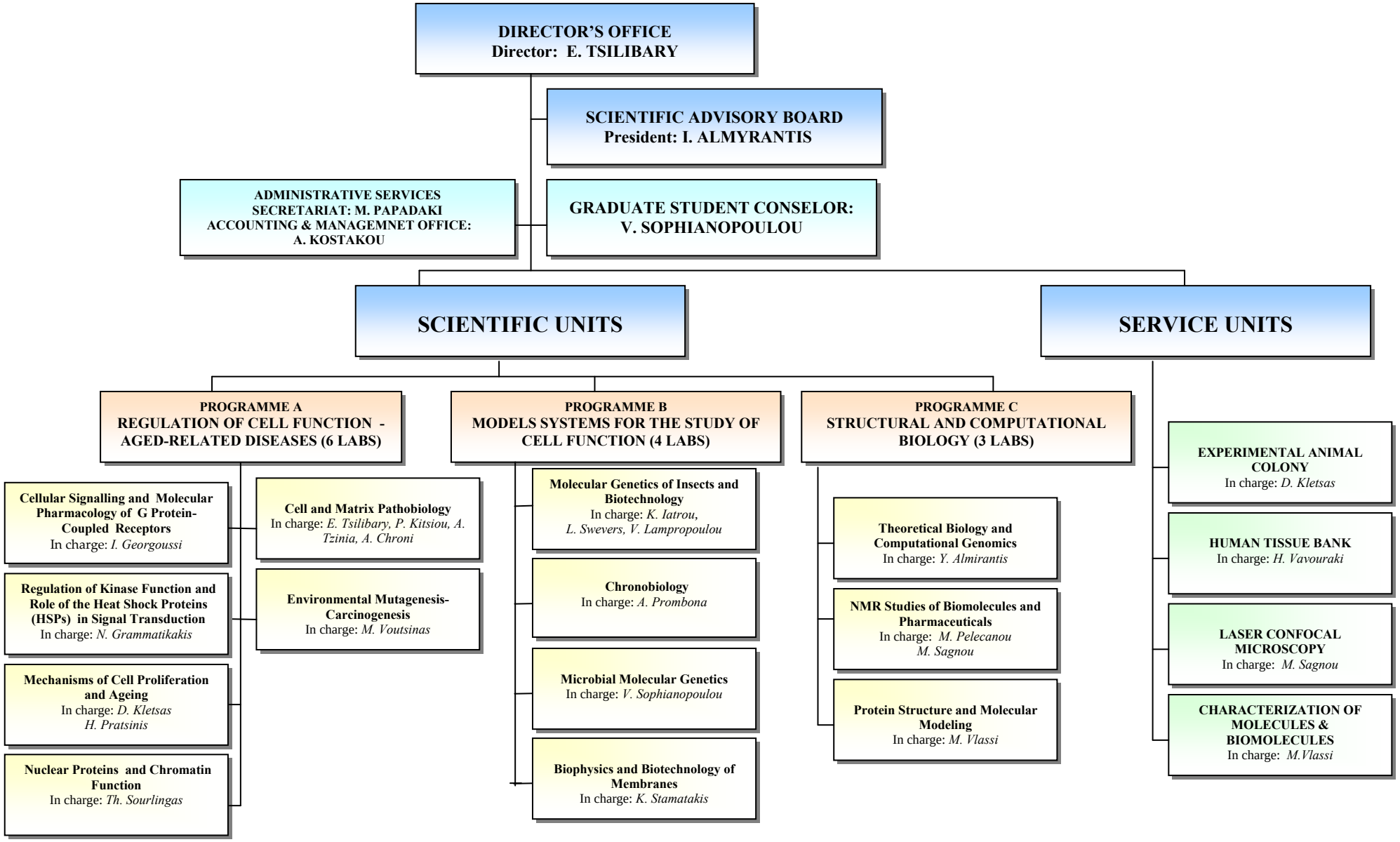
NATIONAL CENTRE FOR SCIENTIFIC RESEARCH "DEMOKRITOS"

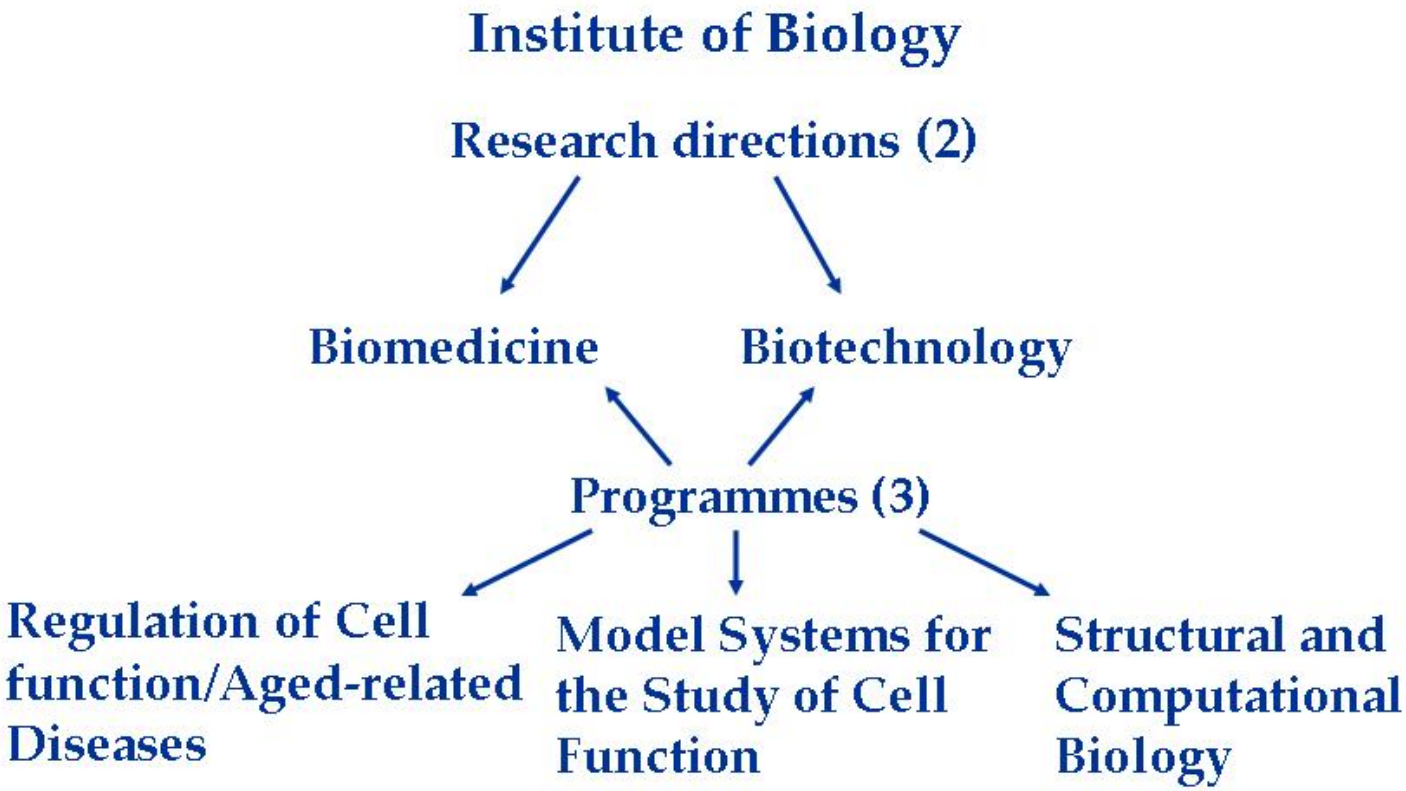
INSTITUTE OF BIOLOGY

2009
ANNUAL REPORT

TABLE OF CONTENTS

ORGANISATION CHART




Institute of Biology
Research directions (2)
Biomedicine
Biotechnology
Programmes (3)
Regulation of Cell function/Aged-related Diseases
Model Systems for the Study of Cell Function
Structural and Computational Biology

P E R S O N N E L

DIRECTOR

Tsilibary Effie	MD, Cell Biologist

ACTING DIRECTOR

Almirantis Yannis	Chemist

SCIENTIFIC STAFF

Research Directors

Almirantis Yannis	Chemist
Iatrou Kostas	Professor of Biochemistry and Molecular Biology
Kletsas Dimitris	Biologist
Pelecanou Maria	Pharmacist
Sophianopoulou Vassiliki	Biologist
Tsilibary Effie	MD, Cell Biologist
Vlassi Metaxia	Physicist-Chrystallographer

Senior Researchers

Chroni Aggelika	Biologist
Georgoussi Zafiroula-Iro	Biochemist
Grammatikakis Nikolaos	Cell Biologist
Labropoulou Vassiliki	Biochemist
Prombona Anastasia	Biologist
Stamatakis Konstantinos	Biologist
Swevers Luc	Biologist
Tzinia Athina	Biochemist
Voutsinas Gerassimos	Biologist

Researchers

Kitsiou Paraskevi	Biologist
Konstantopoulou Maria	Biologist
Sourlingas Thomae	Biologist
Vavouraki Helen	Radiopharmacist

Lecturers

Drossopoulou Garifallia	Biologist
Pratsinis Haris	Chemist
Sagnou Marina	Biologist/ Chemist

Technical Specialists

Panagiotopoulou Angeliki	Biochemist
Stefanou Dimitra	Agronomist

RESEARCH TECHNICIANS

Argyri Letta
Avgeris Socrates
Doulgeridis George
Kakkos Stilianos
Kopanelis Dimitris
Kotsopoulou Eleni
Pantazi-Mazomenou Anastassia
Stefou Vassiliki
Zafiropoulos Ioannis

ADMINISTRATIVE STAFF (2)

Kostakou Athanassia	Accountant
Papadaki Margarita	Secretary

EMERITUS & COLLABORATING SCIENTISTS

Emeritus Scientists	**Laboratory**
Ignatiadou Lydia (Dr. Hydrobiologist)- *Emeritus*	Iatrou K.
Papageorgiou George (Dr. Biochemist)- *Emeritus*	Stamatakis K.
Papageorgiou Spyros (Dr. Physicist)- *Emeritus*	Almirantis I.
Sekeri Kalliope (Dr. Biochemist) – *Emeritus*	Sourlingas Th.
Sideris Eleftherios (Dr. Geneticist)- *Emeritus*	Sophianopoulou V.
Stathakos Dimitrios (Dr. Biochemist)- *Emeritus*	Kletsas D.
Tsimilli – Michael Meropi (Dr. Biologist)	Stamatakis K.

POSTDOCTORAL FELLOWS

Fellow	**Supervisor**
Agalou Adamantia (NCSR "Demokritos")	Georgoussi I.
Benaki Demetra (NCSR "Demokritos")	Pelekanou M.
Efrose Rodica (Programme)	Iatrou K.
Fourla Danai (Programme)	Georgoussi I.
Fragouli Apostolia (NCSR "Demokritos")	Tsilibary E.
Koussis Konstantinos (Programme)	Iatrou K.
Lagos Dimitris (NCSR "Demokritos")	Sophianopoulou S.
Martinou Kelly (Other Sources)	Konstantopoulou M.
Siskos Elias (other sources)	Konstantopoulou M.
Tsitoura Panagiota (Programme)	Iatrou K.
Vamvakas Sotirios - Spiridon (NCSR "Demokritos")	Kletsas K.
Vlachakis Dimitris (NCSR "Demokritos")	Vlassi M.

GRADUATE STUDENTS

Student	Supervisor
Aliberti Sofia (NCSR "Demokritos")	Grammatikakis N.
Bouzarelou Dimitra (NCSR "Demokritos")	Sophianopoulou V.
Daniil George (Programme, *MSc*)	Chroni A. - *MSc obtained*
Dafnis Ioannis (Programme)	Chroni A.
Dimozi Anastasia (NCSR "Demokritos")	Kletsas D.
Galeou Aggeliki (NCSR "Demokritos")	Prombona A.
Georganta Irene (Programme)	Georgoussi I.
Handris Panagiotis (NCSR "Demokritos")	Kletsas D.
Ioannidis Konstantinos (NCSR "Demokritos")	Iatrou K.
Kapodistria Katerina (NCSR "Demokritos")	Kitsiou P.
Karkoulis Panagiotis (NCSR "Demokritos")	Voutsinas G.
Konstantinou Vassos (Programme)	Kletsas D.
Koutmos Theodore (NCSR "Demokritos")	Tsilibary E.
Kostomiri Mirto (NCSR "Demokritos")	Tsilibary E.
Leontiadis Leonidas (NCSR "Demokritos")	Georgoussi I.
Magkrioti Christiana (Programme)	Iatrou K.
Mavrogonatou Eleni (NCSR "Demokritos")	Kletsas D. *–PhD obtained*
Ninios Yannis (Programme)	Sourlingas Th. *–PhD obtained*
Papadopoulou Adamadia (Programme)	Kletsas D.
Papakonstantinou Maria (NCSR "Demokritos")	Georgoussi I.
Repouskou Anastasia (NCSR "Demokritos")	Prombona A.
Roumelioti katerina (Programme)	Sophianopoulou V. *–PhD obtained*
Salpea Paraskevi (NCSR "Demokritos")	Sourlingas Th.
Tsagaraki Ioanna (NCSR "Demokritos")	Tzinia A.
Tsotakos Nikos (NCSR "Demokritos")	Tsilibary E.
Vaggelatos Ioannis (NCSR "Demokritos")	Sophianopoulou V.
Xedous Marios (NCSR "Demokritos")	Sourlingas Th.

GRADUATE RESEARCH ASSOCIATES

Fellow	Supervisor
Rapropoulos Dimitris (Dr. Biologist)	Iatrou K.
Sellis Diamadis *(MSc)*	Vlassi M.

COLLABORATING GRADUATE STUDENTS

Student (University)	Supervisor
Anastassiou Dimitra (Univ. of Athens)	Voutsinas G.
Athanassopoulou Labrini (Athens Polytechnic School)	Almyrantis I.
Armatas Andreas (Univ. of Athens, *MSc*)	Kletsas D. - *MSc obtained*
Chrissouli Stefania (Univ. of Athens, MSc)	Kletsas D. - *MSc obtained*
Kachrilas Stafanos (Univ. of Athens)	Voutsinas G.
Klimopoulos Alexandros (Univ. of Athens, *MSc*)	Almyrantis I.
Konstantakatou Evmorphia (Univ. of Athens)	Voutsinas G
Lagopati Nefeli (Athens Polytechnic School)	Tsilibary E./Tzinia A.
Oikonomopoulos Spiros (Univ. of Athens, *MSc*)	Kletsas D. - *MSc obtained*
Polichronopoulos Dimitris (Univ. of Athens, *MSc*)	Almyrantis Y.
Rapti Martina (Univ. of Athens, *MSc*)	Kletsas D.
Tartas Athanassios (Univ. of Patras)	Vlassi M. - *PhD obtained*

Tsiagas Ioannis (Univ. of Athens, *MSc*)	Almyrantis I.
Verouti Sofia (Univ. of Athens)	Tsilibary E. .
Vestaki Katerina (Univ. of Athens, *MSc*)	Voutsinas G. - *MSc obtained*

UNDERGRADUATE STUDENTS AND OTHER IN TRAINING

Student (University)	**Supervisor**
Antoniou Dafni (Univ. of Athens)	Prombona A.
Arvaniti Maria (Univ. of Athens)	Tsilibary E./Tzinia A.
Krezias George (Univ. of Athens)	Sophianopoulou V.
Faidonos Alexia (Univ. of Bath, UK)	Chroni A. - *undergraduate dissertation completed*
Hassapis Kiriakos (Univ. of Athens)	Chroni A.
Nikolos Fotis (Univ. of Athens)	Georgoussi I. - *undergraduate dissertation completed*
Pantazopoulou Vassiliki (Univ. of Athens)	Sophianopoulou V.
Papadopoulou Natalia (Univ. of Athens)	Kletsas D.
Panopoulos Andreas (Univ. of Wales,UK)	Sophianopoulou V.
Triantou Marianna (Athens Polytechnic School)	Stamatakis K.

INTRODUCTION

The Institute of Biology (IB), one of eight institutes of National Center for Scientific Research (NCSR) Demokritos. The Center has the unique feature of combining scientists with different expertise who conduct basic research and collaborate. The thrust is optimal research and technology progress in the thematic areas covered by research interests of researchers from the different institutes.

The IB with 23 faculty members, has recently obtained significant new and upgraded equipment through competitive funding for infrastructure for the total amount of over 500.000 €; Two major service laboratories, the animal colony and the human tissue bank have been certified by international standards (IS0 9000/2001); these laboratories are gradually upgraded by adding new equipment, offices and/or office space, and space for experimentation. Both laboratories have a presence in the market: in the case of the animal colony, increasing numbers of small animals bred are sold within Demokritos and also to different university departments, pharmaceutical companies, etc. In the case of the Human Tissue Bank, processed tissue and bone samples are sold through a company to private and public hospitals, etc. It is anticipated that the number of animals and samples sold will progressively increase to provide substantial income for the IB, after fulfilling the expensive upgrading which is mandated by the ISO qualifications.

The IB has significant independent and collaborating activities aiming at internationally competitive research and the achievement of excellence. During the last five years, there have been 191 research publications in peer-reviewed journals; whereas intra- and inte-rinstitutional collaborations resulted in over 30 publications in peer-reviewed journals and substantial funding, reaching over 2.5 million €. Interdisciplinary research related to life sciences and the environment is a unique and characteristic advantage of NCSR Demokritos and the IB as well. Presently, the conducted interdisciplinary research focuses on the environment, the development of innovative molecules and biomolecules for diagnostic and therapeutic use, nanomaterials for medical and imaging purposes, targeted drug delivery, etc. These activities form a dynamic research cluster which includes many IB researchers. Moreover, the main independent IB activities focus on the one hand on biomedical research with different biochemical, cellular, molecular, pharmaceutical, proteomic and other approaches; on the other hand, biotechnological research is performed related to the environment, using similar approaches. Finally, structural and theoretical approaches make up one more research direction of the IB.

During 2009, the IB Dr. Angelika Chroni, Chief Researcher, received an important distinction, the L'OREAL-UNESCO Award for young women scientists: this was one of three similar awards all over Greece. Sincere congratulations to Dr. Chroni, to whom best wishes are extended for success and continuous distinctions throughout her career.

Retired, emeritus researchers proved to be very active once again contributing publications, seminars, participation to IB research projects etc, thus being a significant part of IB productivity.

I wish to thank the members of the IB scientific advisory committee who supported the task of upgrading the IB, and also the members of the Educational Committee. I am also thankful to all researchers who participated in various committees, as well as the Vice-Director of the IB, Dr. Almyrantis who substantially contributed to a smooth operation of the IB and helped with my numerous administrative tasks.

Despite the various obstacles and difficulties, support and confidence constantly provided by the majority of researchers is a main source of optimism and confidence in successfully achieving the aim of upgrading the IB towards excellence. It is my belief that based on objective indexes such as: funding, number of publications, citations, etc, the IB progressively becomes recognized as an internationally competitive institute. I have trust in the research potential of IB researchers who on a daily basis prove their contribution and keep trying harder and getting better, and I extend to all IB members my best wishes for continuous success and recognition.

Finally, I wish to heartfully thank Ms. Athanasia Kostakou, the IB accountant, and Ms. Margarita Papadaki, the IB secretary.

Effie C. Tsilibary, MD, PhD

Director of IB
March 2010

PROGRAMME A:

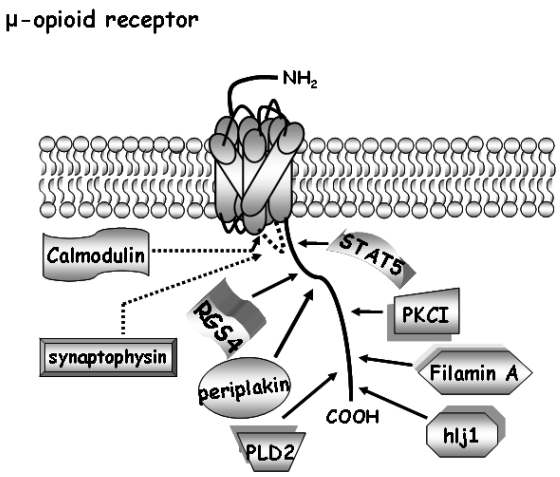
REGULATION OF CELL FUNCTION

AGED-RELATED DISEASES

Research Group: Cellular Signalling and Molecular Pharmacology of G Protein-Coupled Receptors

Research Staff
Iro Georgoussi, Senior Researcher
Adamantia Agalou, Postodoctoral Fellow
Danai Fourla, Postodoctoral Fellow
Leonidas Leontiadis, Graduate Student
Irene Georganta, Graduate Student
Maria Papakonstantinou, Graduate Student
Fotis Nicolos, Undergraduate Student

Research Interests

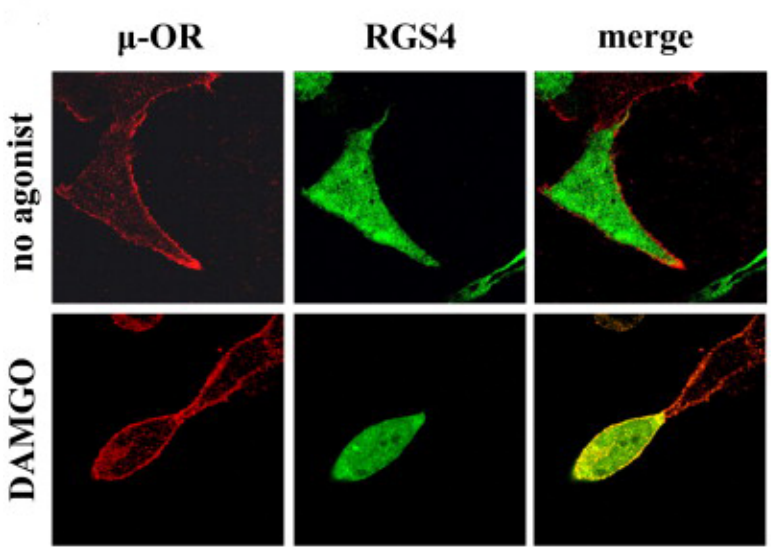


The research interests of our group are mainly focused on the elucidation of the molecular circuits that regulate G protein-coupled receptors (GPCRs) signaling. We use as a model system the opioid receptors because of their involvement in pain perception and in mechanisms related to tolerance and dependence upon chronic opioid administration. More specifically our objectives aim:

- To identify novel opioid receptor-interacting proteins and signaling pathways in an attempt to define novel pharmacological targets **(Fig. 1)**
- To identify transcription factors and genes whose action is altered upon opioid receptor activation.
- To characterize and evaluate pharmacologically novel compounds that bind to the three opioid receptor subtypes (μ, δ and κ) as potential analgesics to alleviate chronic pain.

2009 Findings

Novel interacting partners of the μ-, δ- and κ- opioid receptors.

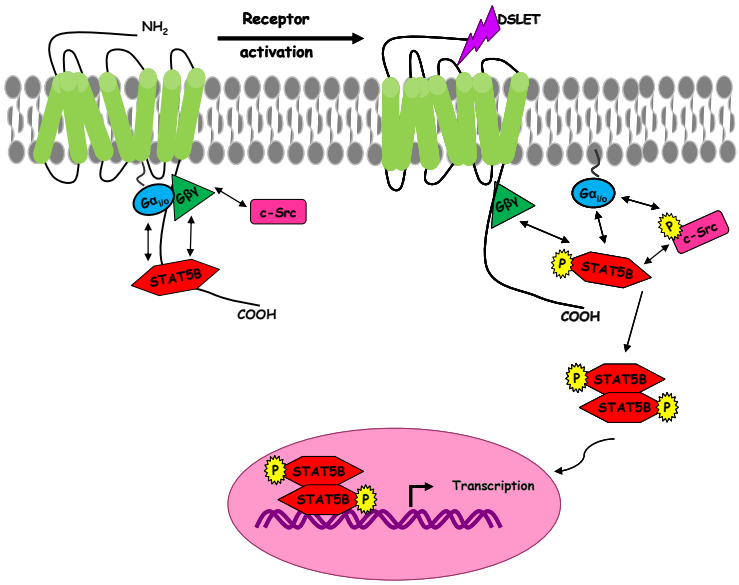


In an attempt to investigate the signaling mechanisms mediated upon activation of μ and δ opioid receptors (μ-OR, δ-OR) we demonstrated for the first time that both receptors interact with members of the B/R4 subfamily of Regulators of G protein Signaling (RGS proteins) such as RGS4 and RGS2. RGS proteins are involved in G protein activation cycle thus modulating the signaling of a given G protein coupled receptor (GPCR). Our studies showed that RGS4 confers selectivity to a specific subset of G proteins to modulate μ- and δ-opioid receptor signaling. RGS4 but not RGS2 accelerates the rate of internalization of δ-OR. Confocal microscopy showed that the activation of ORs directs the translocation of RGS4 from the cytoplasm to the cell membrane in HEK293 cells. **(Fig. 2)**.
Using truncated versions of RGS4 proteins, we also revealed the importance of the N-terminus of RGS4 for proper function. In parallel studies we also investigated the role of another protein, spinophilin, in opioid receptor (μ-OR, δ-OR) signaling. Spinophilin interacts with GPCRs and act as scaffold molecule

for the recruitment of several proteins to the receptors. Our results showed that spinophilin directly interacts with δ-OR and modifies cAMP accumulation mediated by opioid receptor activation.

Activation of transcription factors and functional analysis of genes whose action is altered upon opioid receptor stimulation.

In an attempt to elucidate the molecular mechanisms involved in the phosphorylation of transcription factors and define alterations in gene expression after opioid administration we demonstrated the formation of a multi-component functional complex (signalosome) between the δ-opioid receptor (δ-OR), Signal Transducer and Activation of Transcription 5A (STAT5A), c-src kinase and selective G protein subunits **(Fig. 3)**. Through this dynamic complex STAT5B is phosphorylated and induces transcriptional activation, revealing a novel signaling pathway through which δ-OR may regulate gene transcription. We also demonstrated that STAT5B signaling can be modulated by its coupling with a specific subset of G protein members revealing a novel signaling mechanism for the transcriptional regulation of STAT5B-dependent genes.



Pharmacological characterization of new selective compounds targeting the opioid receptors with analgesic effects.

Under the 6th Framework we participated in the EU Consortium "NORMOLIFE" (LSHC-CT2006-037733). Our group characterized new pharmacological compounds (synthesized by other members of the consortium) with potent *in vivo* analgesic activity. Our results indicated that a chimeric peptide displaying a μ-OR agonistic and neurotensin antagonistic core, activates the μ-OR. Studies in rats have shown that this chimeric compound can be considered as a novel powerful tool for treatment of chronic pain. Part of this work was awarded twice with the gold medal a) the International Federation of Inventors' Association and b) the International Warsaw Inventions Show (IWIS). Additional studies have shown that N-substitution of 6,7 benzomorphan compounds possess a μ/δ agonist profile and could represent a lead in further developing benzomorphan-biased ligands

Intracellular signaling mechanisms mediated by the olfactory receptors of the mosquito *Anopheles gambiae*.

In collaboration with the group of Insect Molecular Genetics and Biotechnology of the Institute of Biology (Prof. K. Iatrou and Dr L. Swevers) and under the 7th FP framework we participate in the research consortium "ENAROMATIC". Our group studied the olfactory mechanisms of the mosquito *Anopheles gambiae,* focusing on the orientation and topology of olfactory odorant receptors OR1 and OR2 after expression in in lepidopteran Bm5 cells. Our results demonstrated that mosquito olfactory receptors OR1 and OR2 exhibit a reverse orientation as compared to the classical GPCRs.

2009 Publications

Leontiadis L. J., Papakonstantinou M. P., Georgoussi Z. (2009). Regulator of G protein signaling 4 confers selectivity to specific G proteins to modulate μ- and δ-opioid receptor signalling, Cellular signalling. 21, 1218-1228.

Articles in Press

Georganta E-M. Agalou A., Georgoussi Z. (2010) Novel dynamic complexes between the δ-opioid receptor, STAT5B and G proteins are implicated in STAT5B phosphorylation, Neuropharmacology 59 139-148 (i.f.3. 7)

Lorella Pasquinucci, Orazio Prezzavento, Agostino Marrazzo, Emanuele Amata, Simone Ronsisvalle, Zafiroula Georgoussi, Danai-Dionysia Fourla, Giovanna M. Scotto, Carmela Parenti, Giuseppina Arico and Giuseppe Ronsisvalle (2010) Evaluation of N-substitution in 6,7 benzomorphan compounds J.Biorg and Med. Chem. *doi:10.1016/j.bmc.2010.06.005 in press* (i.f 3.4)

Georgoussi Z. and Milligan G. (2010) "The other side of the opioid receptor signaling: Regulation by protein-protein interaction". Current Drug Targets (i.f. 4.2)

2009 Proceedings to Conferences

Georganta E.M., Agalou A., Georgoussi Z. New signaling pathways induced by activation of δ-opioid receptor. In "Epitheorese Klinikes Farmakologias kai Farmakokinetikes", vol. 27 (1), pp. 16-18, 2009.

Other Publications

"Normolife ", Parliament Magazine 281, EU Parliament journal focussing on World Cancer Day, February 2009, page 28.

2009 Presentations at Scientific Conferences

E-M. Georganta, A. Agalou and Z. Georgoussi (2009). "New signaling pathways induced by activation of δ-opioid receptor". 9th Pharmacology Symposium, 8th March 2009, Athens, Greece

Z. Georgoussi (2009) "Pharmacological profile and signalling mechanisms of dimeric peptides targeting the neurotensin1 and the μ-opioid receptors". NORMOLIFE Satellite Meeting Rostock-Warnemuende, Germany, 24 April 2009 **(invited speaker)**

E-M. Georganta, A. Agalou and Z. Georgoussi (2009). "Novel dynamic complexes between the δ-opioid receptor, STAT5B and G proteins". 41st Annual General Meeting of the European Brain and Behaviour Society, 14th-18th September 2009, Rhodes Island, Greece.

Z. Georgoussi (2009). "From G protein-coupled receptor signaling to novel therapeutic targets: The opioid receptor paradigm". In symposium III "Intracellular signaling" In the 23rd Hellenic Society for Neuroscience Meeting, Rhodes 13-18 September, Rhodes Island **(invited speaker)**

E-M. Georganta, A. Agalou and Z. Georgoussi. (2009). "Novel dynamic complexes between the δ-opioid receptor, STAT5B and G proteins". 23d Annual Meeting of the Hellenic Society for Neuroscience, 13th-14th September 2009, Rhodes Island, Greece

Z. Georgoussi (2009). "Novel opioid compounds with mixed δ- inverse agonist /μ-agonist activities modulate opioid receptor signaling". Normolife Working Research Meeting, Free University of Brussels, Belgium, 6th November (**invited speaker**)

E-M. Georganta, A. Agalou and Z. Georgoussi. (2009) "STAT5B transcription factor forms functional complexes with the δ-opioid receptor and selective G protein subunits". 60th Greek Conference of Biochemistry and Molecular Biology, 20th–22nd November 2009, Athens, Greece.

M-P. Papakonstantinou, M. Sarris, F. Nikolos, L.J. Leontiadis, D.D. Fourla and Z. Georgoussi (2009) "RGS2: A new modulator of opioid receptor signalling". 60th Greek Conference of Biochemistry and Molecular Biology, 20th–22nd November, Athens, Greece.

Educational Activities

Fotis Nikolos (graduate student of the Molecular Biology and Genetics Department, Demokritio University of Alexandroupolis) completed his Diploma entitled «*The role of RGS2 protein in the regulation of δ-opioid receptor signaling*» Demokitos University of Thrace, April 2009 (Supervisor: Dr Z. Georgoussi)

Coordinator of the postgraduate lecture course of the Institute of Biology in 'Signal Transduction Mechanisms'

Member of Recruitment Committee of postdoctoral fellows in the I.B.

Advisor of the postdoctoral researchers Dr A. Agalou (N.C.S.R. «D») and D. Fourla (European Consortium "Normolife")

Theses Advisor of the postgraduate students E. Georganta, M. Papakonstantinou and L. Leontiadis

Member of the evaluation and examination Committee of the postgraduate students' exams

Speaker in the Summer School of N.C.S.R. "Demokritos" on «*Novel drugs and heptahelical receptors: the present and the future*» July2009

Teaching in the Postgraduate course of the University of Athens on «Cellular signaling mechanisms of the heptahelical receptors for the development of novel therapeutic analogs» University of Athens, Department of Biochemistry and Molecular Biology, December and January 2009 (6 hours, 15 students)

Advisor of women under the auspices of the Hellenic Association of Women Scientists AMALIA FLEMING

Participation in the Educational exchange program of the University of Catania, Sicily, Italy

Participation in the postgraduate course of the Polish Academy of Sciences, Warsaw, Poland

E. Georganta, M. Papakonstantinou: Research seminars under the postgraduate program of the Institute of Biology

Other Scientific/Research Activities

Z. Georgoussi:
Co-founder together with Prof. K. Iatrou and Dr L. Swevers of the spin-off company GENEXPA

Reviewer of grant proposals of the General Secretary for Research of Cyprus (RPF),Cyprus

Reviewer of MRC grant proposals, Scotland, UK

Reviewer in: Molecular Pharmacology, Journal of Neurochemistry, Journal of Pharmacology and Experimental Therapeutics, Cellular Signaling, Neurophychopharmacology, Neuropharmacology, Journal of Biotechnology

Representative of the N.C.S.R. "D" in the Greek Women Scientists Network "Periktioni"

Member of the International Research Consortium "Normolife" for palliative care, related with the design, development and assessment of new analgesic compounds

Member of the WISE (Working group of Women in Science) of FEBS

Chair of the Organizing Ccommittee for "Neuroscience Days 2010" of the Hellenic Society for Neurosciences (1-2 October 2010)

Member of the Scientific Organizing Committee of the International Neuroscience conference (August 2011)

Other Distinctions

Invited speaker at the University of Rostock entitled "*Novel therapeutic targets of the opioid receptors*" Rostock, Germany, May 2009

Invited speaker in the 23rd Hellenic Society for Neuroscience Meeting in Symposium III entitled "*From G protein-coupled receptor signaling to novel therapeutic targets: the opioid receptor example*" Rhodes Island, September 2009

Invited speaker at the University Zhejiung entitled "*Novel therapeutic targets of G protein-coupled receptors: the opioid receptor paradigm*" Hongzhou, China, October 2009

Invited speaker in the Department of Organic Chemistry, Free University of Brussels, entitled "*Novel opioid compounds with mixed δ- inverse agonist /μ-agonist activities modulate opioid receptor signaling*" November 2009, Brussels, Belgium.

Honorary Medal "Prof. Miroslaw J. Mossakowski" of the Polish Government for the contribution in the research for the «Development of novel therapeutic substances and strategies for the alleviation of pain in cancer patients» November 2009, Polish Embassy, Brussels, Belgium.

Wikipedia (RGS4-wikipedia) (http://en.wikipedia.org/wiki/CCG-4986) for the contribution of RGS4 work in opioid action

Other Activities for the Institute of Biology

Z. Georgoussi:

Member of the evaluation committee of the postdoctoral researchers in the Institute of Biology

Member of the selection committee of the postgraduate students (scholarship) in the Institute of Biology

Scientific responsible for special equipment of the Institute of Biology

Impact factor (for 1 publication): 4,3

Citations for 2009 (without self citations): 21

Citations for 2006-2009 (without self citations): 74

h-factor: 12

Current External Funding

Research Program entitled: Normolife-*Development of new therapeutic substances and strategies for treatment of pain patients with advanced stages of cancer,* funded by the EU with Responsible Scientist Dr Z. Georgoussi
Duration: 12/2006-12/2009
Total funding of the program (*for the whole duration)*: 2.039.925 €
Total funding (lab): 541.331 €
Funding of the lab for 2009: 88.985,86 €.

Participation in the Research Program entitled ENAROMaTIC- *European Network for Advanced Research on Olfaction for Malaria Transmitting Insect Control,* funded by the EU with responsible Scientist and program coordinator Dr K. Iatrou
Duration: 12/2008-12/2012
Total Funding of the program (*payment in advance*): 1.208.333 €
Funding of the lab for the year 2009: 17.550 €

Pending proposals:

- Proposal EU 7FP Marie Curie Mobility Actions-Industry Academia Partnerships and Pathways (IAPP) entitled "Neurotensin and opioid receptor heterodimers and multivalent ligands: A strategy for developing new analgesics devoid of undesired side effects" with the Polish Academy of Sciences (Prof. A. Lipkowski, Polish Academy of Sciences) and the German company NeuroProof GmbH (Dr Olaf Schroeder), Brussels July 27, 2009 (Coordinator of the consortium: Dr Z. Georgoussi)
- Bilateral joint proposal cooperation between Hungary-Greece (ΕΣΠΑ 2007-13) entitled «Selective opioid-neurotensin mosaic peptide analogs targeting heterodimerization between respective receptors» Prof. G. Toth, Hungarian Academy of Science, GSRT, September 25, 2009 (Scientific Responsible: Dr Z. Georgoussi)

- Participation in the proposal of the consortium entitled NIMVeCTA (New and Improved Malaria Vector Control in Africa) submitted to the European Union, HEALTH.2010.2.3.2-4: Controlling Malaria by Hitting the Vector: New or Improved Vector Control Tools. FP7–CALL–FOR-AFRICA-2010. Coordinator: Fulvio Esposito, University of Camerino, Italy (Scientific Responsible for the Greek part: Prof. K. Iatrou in cooperation with Dr Z. Georgoussi's group)

Moreover, Dr Georgoussi's group participates in three proposals as scientific responsible scientist in the call of «THALIS» grant application of the General Secretary of Research and Technology on:

- Title «*Structure and function of CRF_1: design and synthesis of novel CRF_1-selective anxiolytic and anti-depressant drugs*» Coordinator: Prof. G. Liapakis, University of Crete (Scientific Responsible of the Institute of Biology: Dr Z. Georgoussi)
- Title: «*Prostanoid receptors in the development of insects – study of the signal transduction mechanisms and detection of agonists and antagonists in natural products*». Coordinator: Dr D. Skarlatos, University of Athens (Z. Georgoussi)
- *Title «Interdisciplinary approach and study of the activation mechanisms, intracellular signaling and biological-pharmacological actions of transmembrane receptors»* Coordinator: Dr M. Mangoura, BRFAA (Scientific Responsible of the Institute of Biology: Z. Georgoussi)

Research Group: Regulation of Kinase Function and Role of the Heat Shock Proteins (HSPs) in Signal Transduction

Research Staff

Nikos Grammatikakis, Senior Researcher

Sofia Aliberti, Graduate Student

Research Interests

A) Cell Signaling

- Mechanisms of mammalian kinase regulation during normal differentiation and disease
- Chemotherapeutical inhibition of oncogenic kinase activity

B) Cellular Responses to Stress and Nutrition

- Regulation of Chaperone Protein Activity
- Identification of Signaling Mediators (including kinases and transcriptional factors) which are modulated by the Chaperone Machinery in response to Stress and Dietary Factors

C) Cell Cycle Regulation

- The Chaperone Machinery as an effector of cellular Stress in cell cycle progression

D) Novel Molecular Chaperones

- Characterization and study of a group of novel Molecular Chaperones identified in our lab and their potential role as mediators of the assembly and activity of ErbB2, Raf, Akt, Cdk4 and I-kappaB kinases (IKK) in cell proliferation and cell cycle progression. Our study extends to learning how the activity of these novel signal modulators is regulated by Growth conditions and Stress (Radiation and chemotherapeutic drugs).

Citations 2009 (without self- citations): 87

Total Citations 2006-2009 (without self- citations): 355

h-factor: 16

Research Group: Mechanisms of Cell Proliferation and Ageing

Research Staff
Dimitris Kletsas, Research Director
Haris Pratsinis, Lecturer
Dimitrios Stathakos, Emeritus Scientist
Sotirios-Spyridon Vamvakas, Postdoctoral Fellow
Eleni Mavrogonatou, Graduate student – *Phd Thesis completed in 2009*
Panagiotis Handris, Graduate Student
Adamantia Papadopoulou, Graduate Student
Anastasia Dimozi, Graduate Student
Vassos Constantinou**,** Graduate Student
Stafania Chrissouli, Collaborating Graduate Student *(MSc)- MSc obtained in 2009*
Spiros Econopopoulos, Collaborating Graduate Student *(MSc)- MSc obtained in 2009*
Andreas Armatas, Collaborating Graduate Student *(MSc)- MSc obtained in 2009*
Martina Rapti**,** Collaborating Graduate Student *(MSc)*
Natalia Papadopoulou, Undergraduate Student

Research Interests

The Laboratory is focusing on tissue repair during development and ageing with an emphasis on the role of growth factors, and especially that of TGF-β. The action of growth factors on cell proliferation and extracellular matrix production, as well as the responsible signaling pathways are investigated. Alternative mechanisms of cell proliferation and differentiation, such as autocrine regulation, cell-matrix interactions, exogenous stresses and the effect of mechanical forces are also studied.

Main goal of the Laboratory is the investigation of the mechanisms of ageing and longevity. The structural and functional characteristics of the senescent cell - as a result of successive duplications or of exogenous stresses - in comparison to that of the young or the cancer cell are investigated. Especially, we are interested on the role of the senescent – somatic and stem - cell in the process of ageing and the development of age-related diseases, including cancer. In this direction, we study the interaction between the senescent stromal fibroblasts and adjacent cancer cells. Emphasis is given in tissues, such as the intervertebral disc, the degeneration of which provokes severe dysfunctions during ageing.

Aim of our studies is the elucidation of the mechanisms underlying the regulation of tissue homeostasis, especially during ageing, and furthermore the contribution, through research networks, in the development of cell replacement therapies. Finally, we study natural products and new synthetic compounds with putative anti-cancer, anti-ageing/anti-oxidant and wound healing action, as well as their mode of action.

2009 Findings

In the context of our studies on the role of growth factors in tissue repair, and having in mind the different repair strategies between fetuses and adults, the role of the amniotic fluid – i.e. the physiological environment of embryonic cells – in tissue repair was examined. Amniotic fluid was found to regulate central parameters of the repair process, and specifically to stimulate cell proliferation, migration, contraction and collagen synthesis in fetal and adult fibroblasts. Furthermore, we have shown that the most important growth factor of human amniotic fluid for the stimulation of cell proliferation is bFGF. In parallel, TGF-β was studied and it was discovered that, in contrast to its differential regulation of human fetal and adult skin fibroblast-proliferation (inhibition of the former vs. stimulation of the latter), human lung fibroblasts are inhibited by this growth factor, regardless of their developmental origin; hence, diverse roles of TGF-β in homeostasis of skin and lung are indicated.

Main goal of our laboratory is the investigation of the structural and functional feature of the senescent cell and its role in the development of age-related diseases, including cancer. Tumor stroma

is an important factor in cancer development. We have shown that repeated therapeutic doses of ionizing radiation provoke premature senescence in stroma fibroblasts, a process strictly regulated by the oncosuppressor p53. Moreover, we showed that senescent cells promote the growth of adjacent cancer cells in vitro and in immunocompromised mice (SCID) in vivo. In contrast to studies from other laboratories, we have found that this phenomenon is not related to an epithelial-to-mesenchymal transdifferentiation (EMT) of cancer cells and is, at least in part, provoked by matrix metalloprotease (MMP) secretion by senescent fibroblasts. In parallel, the premature senescence of stromal fibroblasts after treatment with certain antitumour compounds is under investigation, as well as the role of these senescent cells in the growth of adjacent cancer cells.

Stem cells are currently studied in several biomedical applications. Our Laboratory participates in a large EU-funded network aiming at the use of stem cells in the generation of autologous surrogate tissues. More specifically, we are studying the characteristics of human mesenchymal stem cells that have reached in vitro senescence, and the consequences of their use in vivo. Our preliminary data suggest that senescent stem cells are expressing a pro-inflammatory phenotype, they possess a limited differentiation potential, and exhibit a diminished capacity to colonize three-dimensional scaffolds (Figure 1), compared to their early-passage counterparts. This indicates the necessity of examining any stem cell population regarding the percentage of senescent cells present therein before their use in cell replacement therapies.

Figure 1. Growth of young and senescent stem cells on three-dimensional scaffolds.

One of the tissues being severely affected by ageing is the intervertebral disc. We have studied intervertebral disc cells' proliferation, based on the hypothesis that proliferation in the disc is inhibited under normal conditions (due to the adverse nutritional environment) and it is stimulated during disc degeneration aiming at tissue repair. We have shown that one of the stresses to which disc cells are subjected during daily activities, i.e. hyperosmolality, inhibits cell proliferation by activating the G2 and G1 cell cycle checkpoints. G2 arrest under these conditions is largely due to p38 MAPK activation. In parallel, high osmolality activates also the ATM-p53-$p21^{WAF1}$ pathway leading to the observed G1 arrest. As we have shown with siRNA experiments, this arrest is controlled by p53. Furthermore, hyperosmotic stress induces DNA damage, as shown by histone H2A.X phosphorylation among others, and activates a DNA damage response, similar to the one observed after ionizing radiation (Figure 2). Even more, we have shown – in contrast to previous studies – that disc cells residing within a hyperosmotic environment retain their ability to respond to newly introduced DNA damage, and possess an increased DNA repair efficiency.

On the other hand, we have shown that various autocrine growth factors secreted during intervertebral disc damage, such as, PDGF, bFGF and IGF-I, stimulate cell proliferation via the activation of the MEK/ERK and the PI3K/Akt pathways. Performing immunohistochemical staining of patients' samples, we have observed, especially in cell clusters (characterized by enhanced proliferation), an increased activation of these signaling pathways. Furthermore, we have shown that the response of the intervertebral disc cells to the above growth factors is attenuated under hyperosmotic conditions, while it is intensified under the hypo-osmotic conditions prevailing in degenerated discs, possibly leading to more efficient cell proliferation and tissue regeneration.

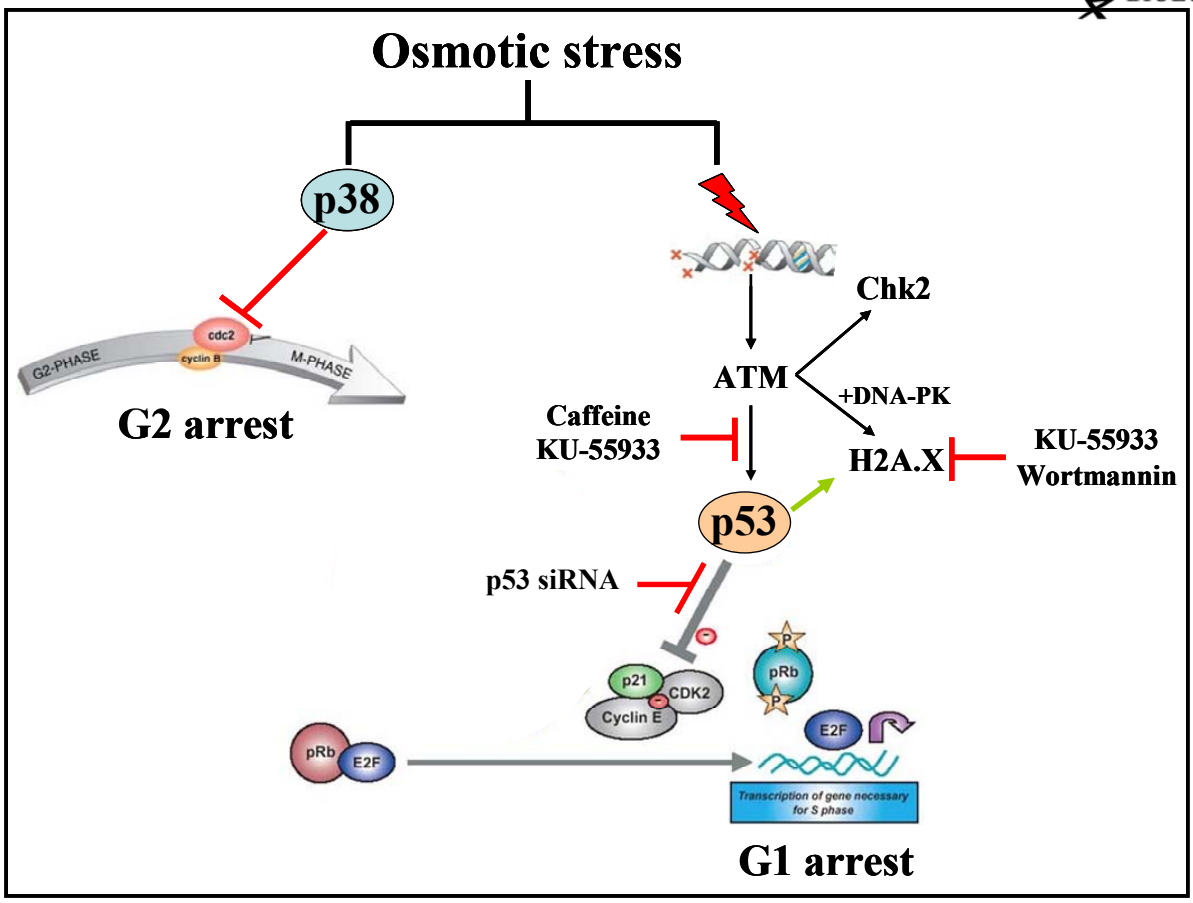


Figure 2. Proposed mechanism of the regulation of intervertebral disc cell proliferation in the presence of hyperosmotic stress.

In addition, the roles of various stresses to which disc cells are subjected (osmotic, oxidative, mechanical) in the regulation of cell proliferation (see also previous paragraph), as well as, in the process of cellular ageing are objects of ongoing studies.

Finally, we have continued our studies on the cytostatic/cytotoxic, anti-ageing and the wound healing activity of natural products and new synthetic compounds, as well as, studies regarding the effects of materials used in medical and/or dental practice on the homeostasis of adjacent cell types.

2009 Publications

Mavrogonatou, E., Kletsas, D. (2009). High osmolality activates the G1 and G2 cell cycle checkpoints and affects the DNA integrity of nucleus pulposus intervertebral disc cells triggering an enhanced DNA repair response. DNA Repair (Amst) 8, 930-943.

Kletsas, D. (2009). Senescent cells in the intervertebral disc: numbers and mechanisms. Spine J. 9, 677-678.

Eliades, T., Pratsinis, H., Athanasiou, A.E., Eliades, G., Kletsas, D. (2009). Cytotoxicity and estrogenicity of Invisalign appliances. Am. J. Orthod. Dentofacial Orthop. 136, 100-103.

Gioka, C., Eliades, T., Zinelis, S., Pratsinis, H., Athanasiou, A.E., Eliades, G., Kletsas, D. (2009). Characterization and in vitro estrogenicity of orthodontic adhesive particulates produced by simulated debonding. Dent. Mater. 25, 376-382.

Gialeli, Ch., Kletsas, D., Mavroudis, D., Kalofonos, H.P., Tzanakakis, G.N., Karamanos, N.K. (2009). Targeting epidermal growth factor receptor in solid tumors: critical evaluation of the biological importance of therapeutic monoclonal antibodies. Curr. Med. Chem. 16, 3797-3804.

Trougakos, I.P., Lourda, M., Antonelou, M.H., Kletsas, D., Gorgoulis, V.G., Papassideri, I.S., Zou, Y., Margaritis, L.H., Boothman, D.A., Gonos, E.S. (2009). Intracellular clusterin inhibits mitochondrial

apoptosis by suppressing p53-activating stress signals and stabilizing the cytosolic Ku70-Bax protein complex. Clin. Cancer Res. 15, 48-59.

Kassi, E., Sourlingas, T.G., Spiliotaki, M., Papoutsi, Z., Pratsinis, H., Aligiannis, N., Moutsatsou, P. (2009). Ursolic acid triggers apoptosis and Bcl-2 downregulation in MCF-7 breast cancer cells. Cancer Invest. 27, 723-733.

Pasi, S., Aligiannis, N., Pratsinis, H., Skaltsounis, A.L., Chinou, I.B. (2009). Biologically active triterpenoids from Cephalaria ambrosioides. Planta Med. 75, 163-167.

Articles in Press

Pratsinis, H., Kletsas, D., Melliou, E., Chinou, I. (2009). Antiproliferative activity of Greek Propolis. J. Med. Food (in press). [I.F. 1,288]

Anastasiadi, M., Pratsinis, H., Kletsas, D., Skaltsounis, A.L., Haroutounian, S.A. (2009). Bioactive non-coloured polyphenols content of grapes, wines and vinification by-products: Evaluation of the antioxidant activities of their extracts. Food Res. Int. (in press). [I.F. 2,073]

Mavrogonatou, E., Kletsas, D. (2010). Effect of Varying Osmotic Conditions on the Response of Bovine Nucleus Pulposus Cells to Growth Factors and the Activation of the ERK and Akt Pathways. J. Orthop. Res. (in press). [I.F. 2,963]

Chandris, P., Giannouli, C.C., Panayotou, G., Kletsas, D. (2010). Compromise in mRNA processing machinery in senescent human fibroblasts: implications for a novel potential role of phospho-ATR (ser428). Biogerontology (in press). [I.F. 3,000]

Maffeo, D., Lampropoulou, M., Fardis, M., Lazarou, Y.G., Mavridis, I.M., Mavridou, D.A.I., Urso, E., Pratsinis, H., Kletsas, D., Yannakopoulou, K. (2010). Novel Polycarboxylated EDTA-Type Cyclodextrins as Ligands for Lanthanide Binding: Study of Their Luminescence, Relaxivity Properties of Gd(III) Complexes, and PM3 Theoretical Calculations. Org. Biomol. Chem. (in press). [I.F. 3,550]

Levidou, G., Saetta, A.A., Karlou, M., Thymara, I., Pratsinis, H., Pavlopoulos, P., Isaiadis, D., Diamantopoulou, K., Patsouris, E., Korkolopoulou, P. (2010). D-type cyclins in superficial and muscle-invasive bladder urothelial carcinoma: correlation with clinicopathological data and prognostic significance. J. Cancer Res. Clin. Oncol. (in press). [I.F. 2,217]

Metwally, K.A., Khalil, A.A., Pratsinis, H., Kletsas, D. (2010). Synthesis, in vitro cytotoxicity, and a preliminary structure-activity relationship investigation of pyrimido[4,5-c]quinolin-1(2H)-ones. Arch Pharm (in press). [I.F. 1,429]

Presentations at Scientific Conferences

D. Kletsas (2009). Cellular Senescence and Tissue Homeostasis. 2nd FEBS Advanced Lecture Course "Matrix Pathobiology, Signaling and Molecular Targets", July 11-16, 2009, Patras, Greece. **Invited speaker.**

D. Kletsas (2009). The Role of Cellular Senescence on Tissue Homeostasis. 5th Joint Meeting of ETRS and WHS, August 25-25, 2009, Limoges, France. **Invited speaker.**

D. Kletsas (2009). (Common lecture with H. Beele and R. Mani). Basic and Clinical Science: Common difficult Wounds - The Role of Team Work with Clinical and Basic Researchers. EWMA2009 Meeting, 20-22 May, 2009, Helsinki, Finland. **Invited speaker.**

H. Pratsinis, V. Constantinou, K. Pavlakis, G. Sapkas, D. Kletsas (2009). Proliferative Response of Human Intervertebral Disc Cells to Exogenous and Autocrine Growth Factors: Involvement of Pivotal Signaling Pathways. 2nd FEBS Advanced Lecture Course "Matrix Pathobiology, Signaling and Molecular Targets", July 11-16, 2009, Patras, Greece.

V. Gioni, T. Karabinas, G. Voutsinas, A.E. Roussidis, S. Papadopoulos, N.K. Karamanos, D. Kletsas (2009). Imatinib Mesylate Inhibits the Proliferation and Collagen Synthesis in Human Breast Stromal Fibroblasts. 2nd FEBS Advanced Lecture Course "Matrix Pathobiology, Signaling and Molecular Targets", July 11-16, 2009, Patras, Greece.

P.G. Dedes, A.I. Tsonis, D. Kletsas, N.K. Karamanos (2009). The effects of zoledronate on tumour growth and the multistep process of metastasis in human breast cancer cells. 2nd FEBS Advanced Lecture Course "Matrix Pathobiology, Signaling and Molecular Targets", July 11-16, 2009, Patras, Greece.

Ch. Gialeli, D. Kletsas, N.K. Karamanos (2009). Implication of Epidermal Growth Factor Receptor Signaling in Growth and Metastasis Potential of Human Colon Cancer Cells. 2nd FEBS Advanced Lecture Course "Matrix Pathobiology, Signaling and Molecular Targets", July 11-16, 2009, Patras, Greece.

O.Ch. Kousidou, A. Berdiaki, A. Zafiropoulos, D. Kletsas, A. Theocharis, G.N. Tzanakakis, N.K. Karamanos (2009) Estradiol and Genistein as Key Factors Affecting the Expression of Matrix Molecules through Estrogen Receptors in Breast Cancer. 2nd FEBS Advanced Lecture Course "Matrix Pathobiology, Signaling and Molecular Targets", July 11-16, 2009, Patras, Greece.

A. Papadopoulou, D. Kletsas (2009) Human Lung Fibroblasts Prematurely Senescent after Exposure to Ionizing Radiation Enhance the Growth of Malignant Epithelial Cells *in vitro* and *in vivo*. 5th International Conference on Tumor Microenvironment: Progression, Therapy and Prevention. October 20-24, 2009, Versailles, France.

E. Mavrogonatou, D. Kletsas (2009) High osmolality activates the G1 and G2 cell cycle checkpoints and stimulates a DNA damage response mediated by p53 in nucleus pulposus intervertebral disc cells. The 34th FEBS Congress. July 4-9, 2009, Prague, Czech Republic.

H. Pratsinis, V. Constantinou, K. Pavlakis, G. Sapkas, D. Kletsas (2009). Exogenous and autocrine growth factors in the proliferation of intervertebral disc cells: Involvement of the MEK/ERK and PI 3-K/Akt signaling pathways. 10th Annual Meeting of the Hellenic Research Club for Connective Tissue & Matrix Biology, July 10-11, 2009, Patras.

S. Chrysouli, H. Pratsinis, A. Dimozi, V. Gioni, V. Anastassiou, V. Gorgoulis, D. Kletsas (2009). Effects of amniotic fluid on fetal and adult human skin fibroblasts in vitro: implications for wound healing. 10th Annual Meeting of the Hellenic Research Club for Connective Tissue & Matrix Biology, July 10-11, 2009, Patras.

E. Mavrogonatou, D. Kletsas (2009). High osmolality inhibits intervertebral disc-nuclear pulposus cells through blocking at the G1 and G2 phases of the cell cycle and induces a DNA damage-response. 60th National Conference of Biochemistry and Molecular Biology, November 20-22, 2009, Athens.

Ch. Gialeli, D. Kletsas, N.K. Karamanos (2009). Implication of Epidermal Growth Factor receptor activation in metalloproteinases expression, growth and migration of human colon cancer cells. 60th National Conference of Biochemistry and Molecular Biology, November 20-22, 2009, Athens.

P.G. Dedes, A.I. Tsonis, D. Kletsas, N.K. Karamanos (2009). The effects of zoledronate on tumour growth and the multistep process of metastasis in human breast cancer cells. 60th National Conference of Biochemistry and Molecular Biology, November 20-22, 2009, Athens.

A.K. Meligova, N. Chantzi, H. Pratsinis, D.J. Mitsiou, M.N. Alexis (2009). Estrogen receptor beta isoforms 1 and 2 differentially modulate growth and invasiveness of breast cancer cells in response to estrogens and antiestrogens. 60th National Conference of Biochemistry and Molecular Biology, November 20-22, 2009, Athens.

V. Gioni, T. Karabinas, G. Voutsinas, A.E. Roussidis, S. Papadopoulos, N.K. Karamanos, D. Kletsas (2009) Imatinib Mesylate Inhibits the Proliferation and Collagen Synthesis in Human Breast Stromal Fibroblasts. 15th Hellenic Congress of Clinical Oncology, March 26-28, 2009, Athens.

A. Papadopoulou, D. Kletsas (2009). Ionizing radiation induces premature cell senescence of human lung fibroblasts, that accelerates the growth of human lung cancer cells in vitro και in vivo. 15th Hellenic Congress of Clinical Oncology, March 26-28, 2009, Athens.

D. Kletsas (2009). Cell Senescence and Tissue Homeostasis. Hellenic National Research Foundation, March 15, 2009, Athens. **Invited speaker.**

D. Kletsas (2009). Proliferation and Ageing of Intervertebral Disc Cells and Impact towards Cell Therapies. Spine Workshop of "Ygeia" Hospital, November 28-29, 2009, Athens. **Invited speaker.**

D. Kletsas (2009). Cell Senescence and Carcinogenesis: Competition or Co-operation ? 3rd Congress of Oncology in Primary Care Praxis, December 4-5, 2009, Athens. **Invited speaker.**

D. Kletsas (2009). Ageing of Mesenchymal Stem Cells and Impact on Cell Therapy. 15th Seminar of Spine Biomechanics and Biotechnology, December 11-13, 2009, Athens. **Invited speaker.**

Other Scientific/Research Activities

Substitute member of the Biology & Biotechnology Sector of the Hellenic National Council for Research and Technology (D. Kletsas)

Member (Secretary General) of the Board of the Hellenic Society for Biochemistry and Molecular Biology (D. Kletsas)

Member (Special Secretary) of the Board of the Hellenic Society of Free Radicals and Oxidative Stress (D. Kletsas)

Member (Secretary) of the Research Club for Connective Tissue and Matrix Biology of the Hellenic Society for Biochemistry and Molecular Biology (D. Kletsas)

Member of the Board of the European Tissue Repair Society (D. Kletsas)

Editorial board member for the journals "Biogerontology", "Fibrogenesis and Tissue Repair", "Open Longevity Science" and "Dental Biomechanics". Advisory editorial board member for "European Spine Journal". (D. Kletsas)

Reviewing of research project-proposals submitted to the Greek General Secretary for Research & Technology and to the Swiss National Science Foundation. (D. Kletsas)

Reviewing of manuscripts submitted to European Spine Journal, Current Signal Transduction Therapy, Osteoarthritis and Cartilage, Journal of Biological Chemistry, Arteriosclerosis Thrombosis and Vascular Biology, Am. J. Orthodont. Dentof. Orthoped., The International Journal of Biochemistry & Cell Biology, Brain Behaviour and Immunity, Biogerontology, Cancer Letters, Journal of Biomedical Science and Engineering. (D. Kletsas)

Other Distinctions

The presentation entitled "Ionizing radiation induces premature cell senescence of human lung fibroblasts, that accelerates the growth of human lung cancer cells in vitro και in vivo" by A. Papadopoulou and D. Kletsas was awarded the 2nd Prize for Basic Research Studies of the 15th Hellenic Congress of Clinical Oncology, March 26-28, 2009, Athens.

Educational Activities

"Cell Senescence and Tissue Homeostasis", NCSR "Demokritos" Summer School, 2 hours, 30 students. (D. Kletsas)

"Cell Senescence and Tissue Homeostasis", NCSR "Demokritos" Summer School, 2 hours, 30 students. (D. Kletsas)

"Cell Senescence and Tissue Homeostasis", Post-graduate Master's Degree in Biochemistry, Chemistry Department of the Univarsity of Athens, 2 hours, 15 students. (D. Kletsas)

"Cell Senescence and Tissue Homeostasis", Post-graduate Master's Degree in Physiology, Medical School of the University of Athens, 2 hours, 30 students. (D. Kletsas)

"Growth Factors", Master Degree "Composition and Metabolism of Mineralized Tissues", Dental School of the University of Athens, 2 hours, 30 students. (D. Kletsas)

"Cell Culture-Tissue Culture", Post-graduate Master's Degree "Applications of Biology in Medicine", Department of Biology of the University of Athens, 6 hours, 20 students. (D. Kletsas and H. Pratsinis)

Member of four examination committees for Ph.D. theses in the University of Athens (Department of Biology, Department of Chemistry, and Dental School) and in the University of Patras. (D. Kletsas)

E. Mavrogonatou completed her Ph.D. thesis entitled "Study of ageing and regulation of cell proliferation in intervertebral disc cells" in the Department of Biology of the University of Athens, which was unanimously accepted and awarded the degree "Excellent". (Scientific Supervisor D. Kletsas).

S. Chrissouli completed her M.Sc. thesis entitled "Response of fetal and adult fibroblasts to mitogenic stimuli" in the framework of the Master's Degree Programme of the Chemistry Department of the University of Athens; the thesis was unanimously accepted and awarded the degree "Excellent". (Scientific Supervisor D. Kletsas).

S. Economopoulos completed his M.Sc. thesis entitled "Effect of the ex vivo mechanical stretching on the proliferation of intervertebral disc-annulus fibrosus cells" in the framework of the Master's Degree Programme "Applications of Biology in Medicine" of the Biology Department of the University of Athens; the thesis was unanimously accepted and awarded the degree "Excellent". (Scientific Supervisor D. Kletsas).

A. Armatas completed his M.Sc. thesis entitled "Study of the expression of Transforming Growth Factor-β-receptor in mammalian cells" in the framework of the Master's Degree Programme "Applications of Biology in Medicine" of the Biology Department of the University of Athens; the thesis was unanimously accepted and awarded the degree "Excellent". (Scientific Supervisor D. Kletsas).

Other Activities for the Institute of Biology

D. Kletsas:
Member of the Scientific Consultative Board of the Institute of Biology
Scientific Supervisor of the Experimental Animal Colony
Supervisor of the Fluorescence Activated Cell Sorting Facility

H. Pratsinis:
Seminar Supervisor of the post-graduate Ph.D. fellows of the Institute of Biology
Member of the examination committee for the selection of the post-graduate Ph.D. fellows of the Institute of Biology (October 2009)
Member of the advisory committee of the post-graduate Ph.D. fellow A. Dimozi

Impact Factors:
D. Kletsas (for 5 publications): 20,789
H. Pratsinis (for 4 publications): 8,319

Citations 2009 (without self- citations):
D. Kletsas: 381
H. Pratsinis: 110

Total Citations 2006-2009 (without self- citations):
D. Kletsas: 1366
H. Pratsinis: 274

h-factor:
D. Kletsas: 21
H. Pratsinis: 12

Current External Funding

Project entitled *Disc-degeneration linked pathologies: novel biomarkers and diagnostics for targeting treatment and repair (GENODISC),* funded by EE with Coordinator Dr. J. Urban (Greek Coordinator: D. Kletsas)
Duration: 2008-2010
Total programme funding: 2.997.144€

Funding of the lab for 2009: 44.000,03€.

Project entitled *MYJOINT: Growing a new joint in a human back,* funded by EE with Coordinator Dr. P. Warnke (Greek Coordinator: D. Kletsas)
Duration: 2007-2009
Total programme funding: 2.344.478€
Total funding (lab): 140.000 €
Funding of the lab for 2009: 47.145,43 €.

Project entitled *Senescence of intervertebral disc cells*, funded by AO Foundation with Coordinator D. Kletsas.
Duration: 2006-2007
Total funding (lab): 60.000 €
Funding of the lab for 2009: 12.000 €.

Project entitled *Tumor-stroma interactions: The role of the stromal fibroblast-premature senescence after exposure to γ-radiation*, funded by Hellenic National Health Council (KESY) with Coordinator D. Kletsas.
Duration: 1/3/2007- 31/3/2008
Total funding (lab): 12.000 €
Funding of the lab for 2009: 5.985,50 €.

Note:

- The project "Investigation of the effect of neonatal fibroblasts on the pro-inflammatory phenotype of senescent cells" funded by Organogenesis has been already launched.
- On April 2010 the launching of a European funded project under the acronym AgroCos is due.
- Project proposals under review: one to the European Union (FP7), seven to the Hellenic General Secretary for Research and Technology (four under the call "Synergasia" and three under the call "Thalis"). Another proposal to the European Union has been selected after the first screening (two-stage) for final submission in May 2010.

Research Group: Nuclear Proteins and Chromatin Function

Research Staff

Thomais Sourlingas, Researcher
Kalliope Sekeri, Emeritus Researcher
Marios Xidous, Graduate Student
Paraskevi Salpea, Graduate Student
Yiannis Ninios, Graduate Student – *PhD obtained in 2009*

Research Interests

The research interests of our group are focused on studying the functional role of the histone subtypes and their epigenetic modifications, mainly acetylation, phosphorylation and methylation, of numerous biological processes (see below). We are also studying the effects that histone deacetylase inhibitors have on the acetylation status of histone and non histone proteins and the consequences that these changes have on gene expression and cellular function. The cell systems used are ageing cell systems (fibroblasts and lymphocytes), peripheral blood leucocytes and leukemic cell lines. The specific ongoing projects are:

(1) Study of the expression of histone subtypes and more specifically, linker histone subtypes, as well as epigenetic histone modifications and their role in chromatin conformational changes during cellular ageing and apoptosis in the cell systems of human fibroblasts, human peripheral blood lymphocytes and human cancer cell lines. The central focus of these studies is the investigation of the potential involvement of the somatic H1 linker histones and of the H1^{o} linker histone and their phosphorylation states, as well as the role of acetylation-deacetylation and methylation of nucleosomal histones in the formation of heterochromatic regions and/or in the reorganization of euchromatic/heterochromatin regions of chromatin during ageing and apoptosis.

(2) Study of changes that are brought about by epigenetic modifications in the expression levels of age-related genes in leucocytes (lymphocytes, monocytes and dendritic cells). More specifically, we are studying the acetylation and methylation status of certain gene regions during ageing.

(3) Study of the acetylation status of the promoter regions of mammalian circadian clock genes and the role of acetylation in the regulation of their expression levels and how the products of these circadian genes affect cell cycle-related gene expression and carcinogenesis.

(4) The effects of histone deacetylase inhibitors on the acetylation of histones and non histone target molecules are also being studied. The aim of these studies is to find molecules and/or factors which may have a functionally active involvement during the course of apoptosis.

(5) Another line of research involves the study of the role of the linker histone H1 subtype composition, as well as their epigenetic changes and changes in the mRNA levels of the H1 histone subtypes in peripheral blood leucocytes from patients with schizophrenia.

2009 Findings

(1) The activation of the DNA fragmentation factor (DFF) was studied after the induction of apoptosis by histone deacetylase inhibitors in six leukemic cell lines. Activation of the heterodimeric complex DFF which consists of DFF40/DFF45 is initiated by the proteolytic degradation of DFF45 by the activated caspase 3. Our results showed that the apoptotic levels as well as the degree of DFF45 proteolysis (1) are time-dependent, (2) differ in the different cell lines studied and (3) that this proteolytic degradation is proportional to the degree of induced apoptosis. Based on these results:

- Two cell lines were selected which had the greatest response to the induction of apoptosis by the histone deacetylase inhibitor, trichostatin A (TSA), for co-immunoprecipitation experiments of whole cell lysates. The goal of these experiments was to ascertain the contribution of histone H1 in DFF40 activation. Previous work of others, using recombinant DFF in an *in vitro* cell-free system, showed that the presence of histone H1 is necessary for maximum DFF40 activation. From our work, we found that histone H1 co-

immunoprecipitates with DFF40 in human leukemic cell lines. These results indicate that histone H1 is associated with DFF40 and may be a contributing factor to DFF40 activation under apoptotic conditions in human cells.

- In continuation, chromatin was isolated in one of the two leukemic cell lines selected and Western analysis showed that DFF40 and DFF45 were localized in the chromatin fraction of the nucleus. So as to ascertain whether these two molecules were localized on chromatin and not on the nuclear matrix, which is co-isolated with chromatin, micrococcal nuclease was used, which is an enzyme that cuts only DNA. This line of work showed that DFF40 and DFF45 are found in the supernatant along with the digested DNA. These results demonstrate for the first time that in a leukemic cell line, the DFF40/45 heterodimer is associated with chromatin, even under non apoptotic conditions. In the case where TSA was used (apoptotic conditions) DFF45 is proteolyzed and only DFF40 is detected after Western analysis.
- Finally, co-immunoprecipitation experiments were undertaken exclusively with the nuclear chromatin fraction, using antibodies against DFF40 and H1 (total). The co-immunoprecipitated products after IP with DFF40 were analyzed using antibodies against the H1.1, H1.3, H1.5 and H1^{o} subtypes of the histone H1 class. Quantitative analyses of the ratios of each co-immunoprecipated subtype in the presence or absence of TSA showed that in the absence of TSA, the ratio of all four subtypes that associated with DFF40 was the same as that which was found after Western blot analysis of the subtype composition of the chromatin fraction (without co-IP), whereas after induction of apoptosis with TSA, an increased association of the H1.5 and H1^{o} subtypes was observed with respect to the H1.1 and H1.3 subtypes. In fact, H1.3 shows a distinct decrease in its binding interactions with DFF40 under these specific apoptotic conditions. From this line of work, it can be concluded that under non apoptotic conditions, DFF40 interacts with all the H1 subtypes *in vivo*. However, under the apoptotic conditions induced by trichostatin A, DFF40 shows preferential binding associations with the H1 subtypes. This result could not be previously determined with *in vitro* experiments.

With the above results, the project "Reinforcement Programme of Human Research Manpower" (PENED) co-financed by National and Community Funds (25% from the Greek Ministry of Development-General Secretariat of Research and Technology and 75% from E.U.-European Social Fund) was completed, along with the doctoral thesis work of I. Ninios, which was presented and accepted (with the grade of Excellent) at the Department of Biological Chemistry, Medical School, University of Athens, November, 2009.

(2) Study of the epigenetic changes of chromatin that occur in age-related genes. Results from this line of work will be compared to the expression levels of these genes. The genes that are under study are *H1^{o}* and *dfna5*. H1^{o} is a differentiation-associated histone subtype since its levels increase in cells and tissues during terminal differentiation. From previous studies undertaken in our lab, H1^{o} was also found to increase in aging cell systems. Dfna5 is also associated with differentiation and microarray experiments showed that the expression levels of *dfna5* changes during cellular aging. The cell systems used in this study are lymphocytes (for *H1^{o}*) and monocytes as well as monocytes that have differentiated into dendritic cells *in vitro* (for *dfna5*) of peripheral blood from donors of different age groups. Results have so far shown that:

- Histone acetylation levels increase in the *dfna5* gene region. Histone acetylation is associated with transcriptional activation, which explains the observed increase in the expression levels of this gene in dendritic cells from the same donors.
- In the *dfna5* gene region, methylation levels of lysine 4 of histone H3 were found to be higher in samples from newborns in relation to those in samples from young donors (20-30 years old). Histone H3 lysine 4 methylation is a histone modification associated with transcriptional activation. This increase agrees with the results obtained for *dfna5* expression levels, which were also found to be increased in newborns in relation to those of young donors.
- Histone acetylation in the *H1^{o}* gene region in both activated and non activated lymphocytes were found to be higher in samples from elderly donors versus those from young donors.

These results were expected since histone acetylation is associated with transcriptional activation and *H1°* expression levels increase in aging cell systems.

This study is being carried out by P. Salpea within the framework of her doctoral thesis and is also part of a collaboration with the laboratory of Dr. Bruce Howard, Head of the Laboratory of Molecular Growth Regulation of the National Institute of Child Health and Human Development; National Institutes of Health (NIH).

(3) We are also studying the effects that chemical substances which change histone acetylation levels may have on the expression levels of genes of the mammalian biological clock and cell cycle genes that may be regulated by clock genes. It is known that the acetylation levels of histones H3 and H4 in the promoter regions of the clock genes, *per1, per2,* and *cry* show circadian rhythm which follows the rhythmic changes of their mRNA levels. This strongly indicates that there is a direct relationship amongst histone acetylation and the regulation of the biological clock. Moreover, it has also been shown that the expression levels of certain cell cycle/proliferation genes are influenced by clock genes. Therefore the focus of this study is to investigate whether histone deacetylase inhibitors can change the expression levels of clock genes and whether these changes can also affect the expression levels of cell cycle/proliferation genes.

The cellular system used in our lab's investigation is mouse NIH3T3 immortalized fibroblast cell cultures whose circadian clock rhythm has been synchronized for 48 hours. The agents that were used to change histone acetylation levels at specific circadian times are trichostatin A, a histone deacetylase inhibitor (HDAC) of class I and II HDACs and nicotinamide an inhibitor of class III HDACs (sirtuins). With qPCR, we found that the expression levels of the clock genes *per1, per2,* but not *cry1,* increase after trichostatin A application. This induced increase in the expression levels of the *per* genes is not a result of *de novo* synthesis, as shown by experiments where cycloheximide (protein synthesis inhibitor) was applied in conjunction with trichostatin A. Moreover, application of nicotinamide in combination with trichostatin A lowers these levels. In order to ascertain whether changes in the acetylation status of histones in the promoter region of the *per1* gene are responsible for the observed changes in its expression levels by these two agents, chromatin immunoprecipation (ChIP) experiments were undertaken. Initial results showed that there is an increase in histone acetylation in the glucocorticoid response element (GRE) region and in the region of the transcription start site (TSS).

This study is being carried out by M. Xidous within the framework of his doctoral thesis.

The general aims of this project are being carried out within the framework of a research collaboration with the laboratory of "Chronobiology" (Group Leader, Dr. Anastasia Prombona) of the Institute of Biology N.C.S.R. "D".

(4) We are studying the composition, as well as the mRNA levels of the H1 subtypes in neutrophils and lymphocytes from the peripheral blood of patients with schizophrenia. Recent work has shown that each H1 subtype is specifically localized in either heterochromatin or euchromatin regions and may possibly have specific functional roles in chromatin conformational changes. Moreover, EM studies have shown that the chromatin from cells of the immune system from patients with schizophrenia is less condensed (euchromatin, active chromatin) in relation to that from normal individuals. In light of the above, the aim of this study is to correlate the results obtained from the biochemical analysis of the H1 subtypes with the histochemical observations of chromatin conformational changes. Experimental results have shown a decrease in the H1.5, an H1 subtype associated with heterochromatin regions (condensed chromatin, inactive chromatin) in leucocytes from patients with schizophrenia in relation to those from normal individuals. This result may possibly indicate a decrease in inactive heterochromatin in cells from patients with schizophrenia. Also, there are distinct changes in the mRNA levels of the H1 subtypes (H1.1, H1.3, H1.5, H1°) in the cells from patients versus normal individuals. In parallel to the above, this investigation also is being conducted at the morphological level.

This project is being carried out within the framework of a research collaboration with the Neurobiology Research Institute of the Th. Th. Cozzika Foundation (laboratory funding by the Foundation: 9.000 E annually).

2009 Publications

Kassi, E., Sourlingas, T.G., Spiliotaki, M., Papoutsi, Z., Pratsinis, H., Aligiannis, N., Moutsatsou, P. (2009). Ursolic acid triggers apoptosis and downregulates bcl-2 downregulation in MCF-7 breast cancer cells. *Cancer Invest.,* 27(7): 723-733. 2009.

Ninios, Y.P., Sekeri-Pataryas, K.E., Sourlingas, T.G. (2009). Differential sensitivity of human leukemic cell lines to the histone deacetylase inhibitor, trichostatin A. *Leuk. Res.,* doi: 10.1016/j.leukres.2009.09.004.

Ninios, Y.P., Sekeri-Pataryas, K.E., Sourlingas, T.G. (2009). Histone H1 subtype preferences of DFF40 and possible nuclear localization of DFF40/45 in normal and trichostatin A-treated NB4 leukemic cells. *Apoptosis,* DOI 10.1007/s10495-009-0418-7.

Articles in Press

Repouskou, A, Sourlingas, T.G., Sekeri-Pataryas, K.E., Prombona, A. The circadian expression of c-Myc is modulated by the histone deacetylase inhibitor trichostatin a in synchronized murine neuroblastoma cells. *Chronobiology Int.,* in press. (IF: 3.987).

2009 Proceedings to Conferences

A. Repouskou, K.E. Sekeri-Pataryas, T.G. Sourlingas, A. Prombona (2009). c-MYC expression is regulated by the circadian clock and displays a phase-dependent response to trichostatin A. 60th Meeting of the Hellenic Society for Biochemistry and Molecular Biology, Athens, 20 -22 November 2009 (Oral Presentation), Abstract book p. 72.

Educational Activities

Lecture within the framework of the Masters' Degree Program: "Applications of Biology in Medicine" of the course "Cell Cultures – Tissue Cultures" (Dept. of Biology, University of Athens). Title of lecture: "The cell cycle: Checkpoints and their function in the normal physiology of the cell**" (Dr. T. Sourlingas).**

Supervisor of the PhD thesis work of Marios Xidous, graduate student with a scholarship from NCSR "D". Title of thesis work: "Effect of the acetylation levels of histones in the regulation of the biological clock: consequences in cellular function".

Supervisor of the PhD thesis work of Paraskevi Salpea, graduate student with a scholarship from NCSR "D". Title of thesis work: "Study of the acetylation of histones and of the DNA linker histones with respect to the conformational changes of chromatin that take place during ageing".

Member Internal Advisory Committee for the doctoral theses of M. Xidous, A. Repouskou, P. Salpea and Y. Ninios.

Yiannis Ninios completed his PhD thesis and received a grade of excellent from the Dept. of Medicine, University of Athens in November, 2009. Title of thesis: "Study of apoptosis induction by histone deacetylase inhibitors in cancer cell lines".

Other Activities for the Institute of Biology

Member of the Education Committee of the IB/NCSR "D".

Member of the Examination Committee for the selection of doctoral candidates to be awarded IB/NCSR "D" scholarships (March, 2009).

Member of the Committees for the (1) selection, (2) receipt and (3) destruction of unusable material.

Other Scientific/ Research Activities

Research Collaborations:

- With Prof. Bruce Howard, Head of the Laboratory of Molecular Growth Regulation of the National Institute of Child Health and Human Development; National Institutes of Health (NIH). Within the framework of this collaboration, P. Salpea, who is a doctoral candidate in our lab, received a second annual scholarship (pro-Forgarty, 2009-2010) to carry out experiments which will be a part of her thesis work in Prof. Howard's lab.
- With Dr. A. Prombona (lab: "Chronobiology"), Institute of Biology, NCSR "D". The general aims of this collaboration involve the study of the effects of histone acetylation on the expression levels of genes that regulate the mammalian biological clock and their potential consequences in cellular function and carcinogenesis.
- With the National Hellenic Research Foundation, Institute of Biological Research and Biotechnology (Drs. M. Patrinou-Georgoula, V. Pletsa, A. Guialis). This collaboration was within the framework of a project financed by "Reinforcement Programme of Human Research Manpower" (PENED) (Greek Ministry of Development-General Secretariat of Research and Technology). Title of project: "Study of the mechanisms of action of anti-cancer compounds during the apoptotic process of cells and their effectiveness as chemotherapeutic agents".
- With the Dept of Biological Chemistry, Medical School, University of Athens (Assist. Prof. C. Troungos). This collaboration was within the framework of a project financed by "Reinforcement Programme of Human Research Manpower" (PENED) (Greek Ministry of Development-General Secretariat of Research and Technology). Title of project: " Study of the mechanisms of action of anti-cancer compounds during the apoptotic process of cells and their effectiveness as chemotherapeutic agents".
- With the Dept of Biological Chemistry, Medical School, University of Athens (Assoc. Prof. P. Moutsatsou). The collaboration involves the study of the "Induction of apoptosis by ursolic acid in the breast cancer cell line, MCF-7".
- With the Neurobiology Research Institute of the Th. Th. Cozzika Foundation. The project involves the study of the changes in the H1 DNA linker histone subtype composition of chromatin and changes in their expression levels in leucocytes of patients with schizophrenia.
- With the University of Goettingen, Prof. D. Doenecke. The collaboration involves the analysis of changes in the H1 DNA linker histone subtype profile during ageing using Capillary Zone Electrophoresis (CZE).

Impact Factors (for 3 publications): 8,696

Citations 2009 (without self-citations): 20

Citations 2006-2009: 72

h-factor: 6

Current External Funding

"Reinforcement Programme of Human Research Manpower" (PENED) (Greek Ministry of Development-General Secretariat of Research and Technology). Title of project: "Study of the mechanisms of action of anti-cancer compounds during the apoptotic process of cells and their effectiveness as chemotherapeutic agents". Scientific Supervisor: Dr. K. Sekeri,.
Duration: 1/1/2006 -30/6/2009
Total programme funding for 2009: 12.000 €
Funding for our lab for 2009: 5.500 €

Research programme amongst our lab and the Neurobiology Research Institute of the Th. Th. Cozzika Foundation. Title of programme: "Study of the changes in the H1 subtype composition of chromatin and changes in their expression levels in leucocytes of patients with schizophrenia".
Duration: 1/1/2007 –
Funding for our lab for 2009: 9.000 €

Research Group: Cell & Matrix Biochemistry/Pathobiology

Research Staff
Fotini-Effie Tsilibary, Research Director
Athina Tzinia, Senior Researcher
Angelika Chroni, Senior Researcher
Paraskevi Kitsiou, Researcher
Garyfallia Drossopoulou, Lecturer
Apostolia Fragouli, Postdoctoral Fellow
Nikos Tsotakos, Graduate Student
Myrto Kostomiri, Graduate Student
Katerina Kapodistria, Graduate Student
Theodore Koutmos, Graduate Student
John Daphnis, Graduate Student
Ioanna Tsagaraki, Graduate Student
Georgios Daniel, Graduate Student (MSc) - *MSc obtained in 2009*
Nefeli Lagopati, Collaborating Graduate Student
Sofia Verouti, Collaborating Graduate Student
Alexia Faidonos, Undergraduate Student – *undergraduate dissertation completed in 2009*
Maria Arvanitie, Undergraduate Student
Kiriakos Hassapis, Training Student
Eleni Kotsopoulou, Research Technician
Letta Argyri, Research Technician

Research Interests

- Transcriptional regulation of podocytic phenotype: Functional and expressional studies involving the anti-adhesive protein podocalyxin and slit diaphragm proteins implicated in human podocytopathies (Drossopoulou/Tsilibary)
- Investigation of the role of podocalyxin and nephrin during nephrogenesis and podocyte differentiation of the monoisomorphic RET51/51 animals (Drossopoulou/Tsilibary)
- In vitro studies of the function of vitamin D on podocyte function (Drossopoulou/Tsilibary)
- In vivo studies of the neurotrofic effect of MMP-9 and its protective role against amyloid plaque formation in an animal model of Alzheimer's disease (Tzinia/Tsilibary)
- In vitro studies of the neurotrofic effect of MMP-9 on PC12 cells after NGF-induced differentiation (Tsilibary/Tzinia)
- Inhibitors of Abeta peptide accumulation and amyloid plaque formation in Alzheimer's disease (Tzinia/Tsilibary, collaboration with Dr M. Pelecanou)
- The effect of cytokines and growth factors on apoptotic and anti-apoptotic mechanisms of osteoblasts (Tzinia/Tsilibary)
- The effect of neuropeptide Calcitonin on human osteoblasts (Tzinia/Tsilibary)
- Nephrin signalling in pancreatic β-cells: Cross talk between nephrin signalling and insulin survival signaling (Kitsiou/Tsilibary)
- Regulation of megalin expression by glucose in cultured pancreatic β-cells: Does insulin signalling interfere with megalin expression? (Kitsiou/Tsilibary)
- Molecular mechanisms of atherosclerosis. Structure function relationship of proteins involved in lipoprotein metabolism pathways (Chroni)
- Role of lipids and lipoproteins in Alzheimer's disease. Insights on the relationship between apolipoprotein E4 and A_β metabolism in brain (Chroni/ Tzinia/ Tsilibary)

2009 Findings

1. Like all epithelial cells, podocytes are polarized with luminal and basolateral cell membrane domains. The most abundant of cell membrane proteins is podocalyxin, a sialomucin expressed on the apical domain of foot processes above the level of the slit diaphragm. In the mature kidney, the slit diaphragm is the only site of cell-cell contact between foot processes of adjacent podocytes. The slit diaphragm is thought to be a modified adherens junction that is composed of a growing number of proteins, including nephrin, P-cadherin, FAT, podocin, and neph1. The actin cytoskeleton is also linked to the slit diaphragm complex through interaction with adaptor proteins such as ZO-1, catenins, and CD2AP. Several studies revealed direct interactions among nephrin, podocin, and CD2AP. Based on insights into the molecular pathology of podocyte injury, two major identified causes that lead to the uniform reaction of foot processes effacement and proteinuria are: interference with the slit diaphragm complex and its lipid rafts; and interference with the negative surface charge of podocytes. In view of these facts we aim to investigate the connection between altered podocyte morphology and development of podocytopathies. For this purpose we use T-SV40 immortalized Human Glomerular Epithelial Cells: HGEC, cultured either in the presence of normal glucose levels (HGEC:5mM), or non-physiological (high) glucose levels (HGEC:25mM).

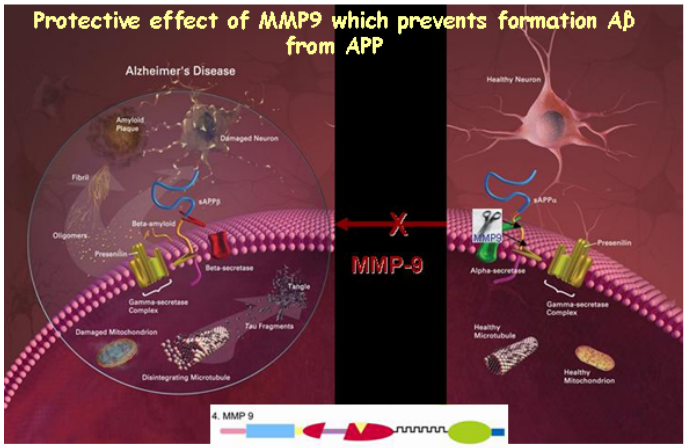
2. In vitro culturing HGEC, in the presence of high glucose levels, results in irreversible downregulation of podocalyxin and nephrin expression. On the other hand, downregulation of ZO-1, CD2AP and podocin expression are completely reversible. Nuclear WT1 and Sp1 transcription factor protein levels exhibited no significant differences in HGEC exposed to high glucose levels, compared to HGEC exposed to normal (5mM) glucose levels. In the presence of 5mM glucose levels WT1 and can bind to the podocalyxin and nephrin promoter regions as shown by Chromatin Immunoprecipitation Assays (ChIP analysis). Immortalised podocytes continuously cultured in the presence of physiological glucose levels (HGEC:5mM) begin exhibiting reduced WT1 binding to both the podocalyxin and nephrin promoter regions, after being cultured in the presence of 25mM glucose. Additionally, in order to determine the differentiation status of high glucose-stimulated HGEC, we determined the surface expression levels of a standard glomerular epithelial marker, CD10 (CALLA), and observed that CD10 also became suppressed by high glucose in a non-reversible manner. Our results indicate that downregulation of both nephrin and podocalyxin is gradual and simultaneous in HGEC that are exposed to high-glucose-containing culture medium. The suppression of the expression of these components may be associated with loss of the characteristic podocytic phenotype as shown by the concurrent downregulation of CD10 and morphological observations. Based on these findings, we propose that glucose induces a gradual de-differentiation of podocytes which is complete when podocalyxin, nephrin, and CD10 expression become almost totally suppressed, resulting among other, in loss of the specialized, podocyte-specific processes.

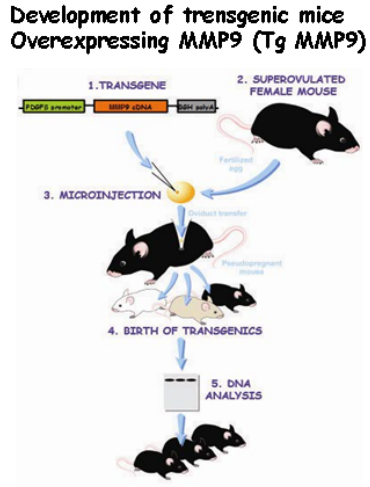
3. Additionally, we have established collaboration with Dr. Vassilis Pachnis (MRC, London, UK) in an effort to investigate the role of podocalyxin and nephrin during nephrogenesis and podocyte differentiation. The *c-Ret* proto-oncogene encodes a receptor tyrosine kinase (RTK) that is expressed widely in mammalian embryos and has diverse roles in development and disease. In mammals, *c-Ret* encodes two major isoforms, RET9 and RET51, which are generated by alternative splicing and differ only at their C-terminal tails. we used mice that express only a single RET isoform, RET51 (RET51/51),generatedby Dr. Pachnis's team. It has been shown that mice expressing only RET51 have severe defects in renal development as well as in innervation of the gut. This suggested that although signalling by RET51 is capable of supporting the early stages of ureteric bud outgrowth and branching, it is unable to sustain normal nephrogenesis at later stages of embryogenesis. Our aim was to investigate whether this induced impairment of nephrogenesis is also associated with an impaired podocalyxin and WT1 expression. WT1 and podocalyxin expression were analyzed by immunohistochemistry in developing kidneys at developmental stages E15.5. WT1 expression was significantly reduced in RET51/51 mice compared to wild type ones. More specifically, the developing nephrons of RET51/51 mice appeared to have less WT1 positive cells, while the intensity of WT1 expression in these cells was significantly reduced. Podocalyxin expression was also impaired in

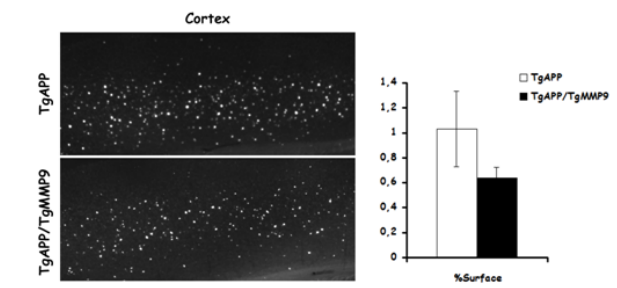
RET51/51 mice. We observed a small but significant reduction of podocalyxin expression in RET51/51 compared to controls.

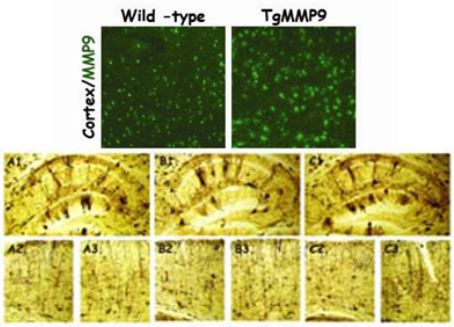
4. Vitamin D functions have been reported to be highly pleiotropic, involving amongst others protective role against kidney pathology. In view of the fact that the role of vitamin D on kidney glomerulus is not fully analyzed we initiated an investigation of the effects of vitamin D on podocyte function. HGEC exposure to either vitamin D or its analogue, paricalcitol, does not influence WT1 and CD2AP expression levels. On the other hand, podocalyxin expression levels are upregulated (both at the protein an mRNA), following exposure to vitamin D or paricalcitol for 4 days. Additionally, significantly high expression levels of the vitamin D receptor (VDR) have been detected. VDR protein and mRNA levels are upregulated in the presence of vitamin D or paricalcitol. Our initial data could therefore suggest that vitamin D may have a protective role on podocyte morphology.

5. In order to study the in vivo role of MMP9, we have generated transgenic mice over-expressing human MMP9 (TgMMP9) under a neuron-specific promoter that restricts its expression in the brain. TgMMP9 were crossed with mice overexpressing APP (AD mouse models). Behavioural studies revealed that TgMMP9 animals have improved cognitive abilities **(**3rd Meeting of the Hellenic Society for Neuroscience 2009)**.**









The number of amyloid plaques in the CNS of double transgenic animals (TgAPP/TgMMP9) was substantially decreased compared to transgenic animal models of Alzheimer disease (TgAPP)

6. Concerning the effect of MMP-9 in differentiated neuronal cells, it was shown that neurotrofic factor NGF shifts APP processing towards the α-secretase pathway and increases MMP9 expression. Concomitantly, inhibition of the β-secretase BACE1 and induction of the α-secretases ADAM17 was observed. Taken together our results suggest that NGF-induced expression of MMP9 shifts towards α-secretase cleavage of APP precluding the formation of neurotoxic Aβ peptides (2nd FEBS-MPST and 60th Scientific conference of Hellenic Society of Biochemistry & Molecular Biology, 20-22 November, 2009, Athens)

7. As an inhibitor of Alzheimer's amyloid beta accumulation we have examined Oleuropein, a constituent of oil with antioxidant properties. Our results have shown that Oleuropein promotes a-secretase pathway for processing the amyloid precursor protein APP (2nd FEBS-MPST 2009 and 3rd Panhellenic Meeting on: "Current trends in lipids", 2009)

8. A selective inhibitor of MMPs, (TIMP-1), interacts with $\alpha v\beta 3$ integrin receptor to confer protection against TNF-α-induced apoptosis of MG63 osteosarcoma cells. This involves activation of cell survival pathways through NF-κB (pending revisions).

9. The neuropeptide Calcitonin has been shown to inhibit bone resorption in vitro. In our system, human osteosarcoma cells, Calcitonin might be involved in cell metastasis, since it greatly enhanced the production of Fibronectin, an extracellular matrix component with a major role in cancer cell invasion

10. The role of nephrin in insulin-producing pancreatic β-cells: The slit diaphragm is a specialised cell junction between adjacent podocytes in the kidney glomeruli, required for functional glomerular filtration. It consists of several proteins including nephrin and nephrin-associated protein complex [CD2-associated protein (CD2AP), podocin, and zonula occludens-1 (ZO-1)]. The structural and functional integrity of the glomerular filtration barrier is dependent on interactions amongst these proteins. The presence of some proteins of the glomerular slit diaphragm complex in the islets β-cells, suggest that these proteins could be shared by the pancreas and kidney. It should be noted that the slit diaphragm proteins nephrin, CD2AP, and podocin, in addition to their structural functions, are able to initiate PI3K/AKT-dependent signal transduction in glomerular podocytes thus protecting them against apoptosis. Today, little is known about the role of the slit diaphragm protein-complex in pancreatic β-cells. The challenge for future studies is to confirm and identify key signaling molecules that interact with survival pathways thus promoting pancreatic beta-cell survival. This approach could yield targets for novel therapy to prevent pancreatic beta-cell loss in type 2 diabetes. Our results showed that cultured pancreatic β-cells (βTC-6) express nephrin, CD2AP, podocin and ZO-1 proteins. In addition, CD2AP interacted with $p85^{PI3K}$ and *vice versa.* CD2AP not only interacted with $p85^{PI3K}$, but also with nephrin. These data suggest that the nephrin/CD2AP complex may indeed participate in PI3K-mediated signalling in pancreatic beta cells.

11. Megalin (or LRP2, low-density lipoprotein receptor related protein-2) is a multi-ligand endocytic receptor at the intersection of endocytosis and signalling. Increased shedding of megalin could contribute to albuminuria in diabetes. To date, little is known about the role of megalin in pancreatic beta-cells. Cultured βTC-6 cells expressed megalin when cultured in low and high glucose. Exposure of βTC-6 cells to increased glucose concentrations resulted in significant increase of megalin mRNA and protein levels. Treatment of the cells with insulin also resulted in upregulation of megalin expression. In addition, PI3K inhibitors, which interfere with the IRS/PI3K system and work as antagonists of insulin-mediated signaling pathways, suppressed insulin-induced megalin expression. These results indicate that glucose could regulate megalin expression via insulin-IRS/PI3K signaling pathway. To evaluate the effect of glucose on megalin expression in pancreatic islets, pancreata from diabetic mouse (db/db lepr-/-) and control (lepr+/-) animals were examined by immunohistochemistry. The results obtained indicated that megalin expression was decreased (~8 fold) in islets of diabetic animals compared to control animals. Aberrant expression of megalin could contribute to β-cell dysfunction and apoptosis in type 2 diabetes. This hypothesis is currently under investigation

12. The cytotoxicity of photoactivated titanium dioxide nanoparticles (TiO_2) was investigated in terms of determining the conditions for photocatalytic induced-cancer cell death and also exploring the molecular mechanisms involved in this process. A selective action of the photocatalytically activated titania was observed on the highly malignant MDA-MB-468 breast cancer epithelial cells. Upon irradiation, these cells were induced to undergo apoptotic cell-death, compared to the MCF-7 cells which were still unimpaired. The molecular mechanism of apoptosis is associated at least in part with increase of caspase-3-mediated poly(ADP-ribose) polymerase (PARP) cleavage.

13. Lipids are transferred between cells and plasma by lipoproteins (chylomicrons, VLDL, LDL, HDL), that constitute complexes of amphipathic proteins with lipids. Lipoproteins are synthesized and

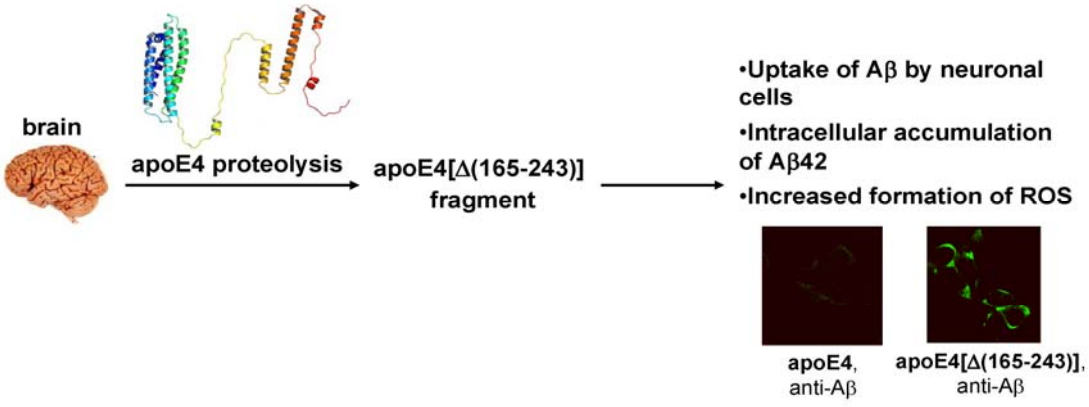
catabolized through complex and interrelated pathways, in which different proteins, such as apolipoproteins (apoA-I, apoE), enzymes (LCAT), lipid transfer proteins, lipoprotein receptors (SR-BI) and lipid transporters (ABCA1, ABCG1) participate. Genetic alterations in different steps of the lipoproteins metabolism pathways affect lipid homeostasis in cells and in the circulation and promote the development of atherosclerosis.

A) Characterization of composition, structure and function of HDL from family subjects with low HDL syndromes that carry mutations in apoA-I, ABCA1 or LCAT showed that specific mutations in various genes of the HDL biosynthesis pathway affect differentially the antioxidant/antiinflamatory properties of HDL and lead to distinct HDL subpopulations profile. These types of analyses could prove valuable in differential diagnosis of familial low HDL syndromes.

B) The study of interactions of HDL with the lipid transporter ABCG1 showed that ABCG1-mediated cholesterol efflux was completely reduced in the presence of reconstituted HDL (rHDL) containing by N-terminal, C-terminal and double N-and C-terminal deletion mutants of apoA-I. In contrast, rHDL containing deletion mutants of internal regions of apoA-I only partially reduced the ABCG1-mediated cholesterol efflux compared to rHDL containing WT apoA-I. These data suggest that apoA-I in HDL interacts with ABCG1 and that the C- and N-terminal regions of apoA-I are necessary for the apoA-I/ABCG1 interactions.

14. A hallmark of Alzheimer's disease (AD) is the deposition of plaques containing amyloid β peptide (A_β) in the brain. However, over the past decade, accumulating evidence points to an important role of intraneuronal Aβ as a trigger of the pathological cascade of early events leading to neurodegeneration and eventually to AD. Brain cells cholesterol levels and proteins involved in cholesterol metabolism can affect the formation, deposition and catabolism of A_β. ApoE (299 residues, 3 common isoforms apoE2, apoE3 and apoE4) is the only apolipoprotein in brain that participates in the formation of lipoproteins and lipid homeostasis. ApoE4 is a major risk factor for Alzheimer's disease. ApoE4 has been found to be much more susceptible to proteolysis than apoE3, creating bioactive carboxy-terminal truncated fragments in brains of AD patients. It has been suggested that these apoE4 fragments may play a key role in the pathogenesis AD.

A) A specific apoE4 fragment, apoE4[Δ(166-299)], can promote the uptake of extracellular Aβ40 and Aβ42 by human neuroblastoma cells SK-N-SH. A longer length fragment, apoE4[Δ(186-299)], or full-length apoE4 failed to elicit this effect. ApoE4[Δ(166-299)] effected reduction of cellular sphingomyelin levels, as well as changes in cellular membrane micro-fluidity. Following uptake, approximately 50% of Aβ42 remained within the cell for at least 24h, and led to increased formation of reactive oxygen species, and therefore increased oxidative stress. Overall our findings provide an association between two molecular events, the proteolysis of apoE4 and the intraneuronal presence of Aβ, both of which are considered to be early events in the pathogenesis of AD. We therefore propose that specific short apoE4 proteolytic fragments produced in the brain under pathologic conditions may promote intraneuronal accumulation of Aβ42 leading to neuronal dysfunction.



B) We produced in E.coli and purified the apoE4 mutants apoE4[Leu28Pro], that is a natural occurring mutant associated with increased risk of AD, and apoE4[Gln284Ala], that has been associated with

neurotoxicity. We will produce more apoE4 mutants associated with the pathogenesis of AD and subsequently we will study their structure and function.

2009 Publications

Drossopoulou GI, Tsotakos NE, Tsilibary EC. Impaired transcription factor interplay in addition to advanced glycation end products suppress podocalyxin expression in high glucose-treated human podocytes. Am J Physiol Renal Physiol. 2009; 297(3): F594-603

Aggeli, A., Kitsiou, P., Tzinia, A., Boutaud, A., Hudson, B., Tsilibary, E. (2009). Selective binding of integrins from different renal cell types to NC1 domain of α3 and α1 chains of collagen IV. *J. Nephrol.* 22,130-136

Articles in Press

Venieratos PD, Drossopoulou G, Tsilibary EC, Kitsiou PV: High glucose induces suppression of insulin signalin and apoptosis via upregulation of endogenous IL-1β and SOCS-1 in mouse pancreatic beta cells, *Cellular Signaling* 22 (2010) 791–800 (2010) (I. F.: 4,451)

Dafnis J, Stratikos E, Zanis, V, Tzinia A, Tsilibary EC, Chroni A: An apolipoprotein E4 fragment can promote intracellular accumulation of amyloid peptide beta 42, *J. Neurochem.* (accepted, 2010) (I.F.: 4.5)

Tsitrouli D, Rabias I, Karakosta E, Kehagias T, Diamantopoulos G, Fardis M, Maris P, Falaras P, Drossopoulou G, Tsilibary EC, Papavassiliou, G. Efficient Magnetic Heating by Highly Charged Coated Maghemite Nanoparticles in micro litre volume, *Biomicrofluidics,* **4**, pii 024111 (2010) (I.F. 2,311)

Lagopati N, Kitsiou PV, Kontos AI, Venieratos P, Kotsopoulou E, Kontos AG, Dionysiou DD, Pispas S , Tsilibary EC, Falaras P: Photo-induced cancer treatment using nanostructured titanium dioxide solution, *(Journal of Photochemistry and Photobiology A,* 214: 215-223, 2010) (I.F.: 2.362)

Tsagaraki I, Tsilibary, EC, Tzinia: TIMP-1 interaction with αvβ3 integrin confers resistance to human osteosarcoma cell line MG63 against TNF-α-induced apoptosis, *Cell & Tissue Research, Accepted (2010)* (I.F. 2.7)

Fragkouli A, Tzinia A, Charalampopoulos I, Gravanis A, Tsilibary EC: Matrix metalloproteinase-9 participates in NGF-induced α-secretase cleavage of amyloid precursor protein in PC12 cells, *J. Alzheimer Dis, pending revisions* (I.F.5.1)

Fragkouli A, Papatheodoropoulos C, Georgopoulos S, Stamatakis A, Stylianopoulou F ,. Tsilibary EC, Tzinia AK: Over-expression of matrix metalloproteinase 9 facilitates neuronal plasticity in vivo, *submitted Eur. J. Neuroscience* (I.F. 3.5)

Book Chapters and Conference Proceedings

Zannis V., Drosatos K., Kypreos K., Vezeridis A. and Chroni A. The role of apolipoprotein E in triglyceride homeostasis and the biogenesis of HDL. *Atherosclerosis Supplements*, Vol. 10, Issue 2, e190 (2009)

Zannis V., Sanoudou D., Koukos G., DuKa A., Chroni A. and Kardassis D. ApoA-I, HDL biogenesis and sites of regulation. *Atherosclerosis Supplements*, Vol. 10, Issue 2, e1577 (2009)

Zannis V. and Chroni A. Functions of apoE-containing HDL in the brain. *Atherosclerosis Supplements*, Vol. 10, Issue 2, e1577 (2009)

2009 Presentations at Scientific Conferences

K. Kapodistria, E. Tsilibary, V. Kaltezioti, P. Kavvadas, A. Charonis, D. Kerjaschki, P. Kitsiou. (2009). Megalin/gp330 occurs in pancreatic beta-cells and is modulated by glucose. 60th Scientific conference of Hellenic Society of Biochemistry & Molecular Biology, 20-22 November, 2009, Athens.

Fragkouli A, Tzinia A, Tsilibary E.NGF promotes a-secretase cleavage of APP and increases MMP9 expression in PC12 cells, 2nd FEBS-MPST Advanced Lecture Course, Matrix Pathobiology, Signaling and Molecular Targets (2009).

Kostomoiri M., Fragkouli A., Sagnou M., Skaltsounis L., Pelecanou M., Tsilibary E., Tzinia A. Oleuropein prevents the formation of extracellular amyloid β-peptide aggregates and leads to increased production of neuroprotective sAPPa, 2nd FEBS-MPST Advanced Lecture Course, Matrix Pathobiology, Signaling and Molecular Targets (2009).

Fragkouli Apostolia, Georgopoulos Spiros, Stamatakis Antonis, Stylianopoulou Fotini, Tsilibary Effie and Tzinia Athina Cognitive performance of mice over-expressing matrix metalloproteinase 9 (MMP9), 3rd Meeting of the Hellenic Society for Neuroscience (2009)

Apostolia Fragkouli, Athina K. Tzinia, Ioannis Charalampopoulos, Achille Gravanis, Effie C.Tsilibary. Matrix metalloproteinase 9 (MMP9) participates in NGF-induced α-secretase cleavage of amyloid precursor protein in PC12 cells, metalloproteinase 9 (MMP9), 3rd Meeting of the Hellenic Society for Neuroscience (2009)

Apostolia Fragkouli, Athina K. Tzinia, Ioannis Charalampopoulos, Achille Gravanis, Effie C.Tsilibary. Matrix metalloproteinase 9 (MMP9) participates in NGF-induced α-secretase cleavage of amyloid precursor protein in PC12 cells, 60th Scientific conference of Hellenic Society of Biochemistry & Molecular Biology, 20-22 November, 2009, Athens

Kostomiri M, Fragkouli A, Sagnius M, Skaltsounis L, Pelecanou M, Tsilibary EC, Tzinia A: Oleuropein, the anti-oxidant component of olive oil enhances the non-amyloidogenic pathway during metabolism of amyloid precursor protein (APP). 3th Panhellenic Meeting on "current trends in lipids", EIE, Athens (2009)

Zannis V., Drosatos K., Kypreos K., Vezeridis A. and Chroni A. The Role of apolipoprotein E in triglyceride homeostasis and the biogenesis of HDL. *XV International symposium on Atherosclerosis*, June 14-18, 2009, Boston, MA , USA

Zannis V. and Chroni A. Functions of apoE-containing HDL in the brain. *XV International symposium on Atherosclerosis*, June 14-18, 2009, Boston, MA , USA

Zannis V., Sanoudou D., Koukos G., DuKa A., Chroni A. and Kardassis D. ApoA-I, HDL biogenesis and sites of regulation. *XV International symposium on Atherosclerosis*, *Post-Satellite Symposio: High Density Lipoproteins and Atherosclerosis*, June 19-20, 2009, Newport, RI , USA

Daniil G., Zannis V. I. and Chroni A. Effect of amino-terminal, carboxy-terminal and internal deletion mutants of apoA-I on ABCA1- and ABCG1-mediated lipid efflux. *"Protein Modules and Networks in Health and Disease" FEBS Workshop*, September 5 - 10, 2009, Tirol, Austria

Dafnis J., Stratikos E., Tzinia A., Tsilibary E. C., Zannis V. I. and Chroni A. Carboxy-terminal truncated apolipoprotein E4 promotes the accumulation of amyloid peptide beta 42 in neuronal cells. 32nd European Lipoprotein Club meeting, 7-10 September 2009, Tutzing, Germany

Dafnis J., Stratikos E., Tzinia A., Tsilibary E. C., Zannis V. I. and Chroni A. Uptake and accumulation of amyloid peptide beta 42 in neuronal cells treated with Carboxy-terminal truncated apolipoprotein E4. 23rd Hellenic Neuroscience Society meeting, 13-14 September 2009, Rhodes, Greece

Daniil G., Phedonos A. A. P., Argyri L., Kuivenhoven J. A. and Chroni A. Antioxidant/Anti-inflammatory Properties of HDL and Distribution of ApoA-I–Containing HDL Subpopulations from Family Subjects with Low HDL Syndromes. 12th Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease meeting, 9-10 October 2009, Athens, Greece

Daniil G., Argyri L., Phedonos A. A. P., Kuivenhoven J. A. and Chroni A. HDL Antioxidant/Anti-inflammatory Properties and Subpopulation Profile in Familial Low HDL Syndromes. 3rd Meeting of working groups of Hellenic Atherosclerosis Society, 4-6 December 2009, Athens, Greece

G. Drossopoulou, N. Tsotakos, E. Kotsopoulou and E.C. Tsilibary. "Transcriptional regulation of matrix anti-adhesin podocalyxin-like protein, PCLP, and the slit diaphragm protein, NEPHRIN, in cultured immortalized human glomerular epithelial cells". 10th Annual Meeting of the Hellenic Research Club for Connective Tissue and Matrix Biology (Athens, Greece), 2009.

Drossopoulou G., Zaimidou E., Tsotakos N., Dimitraki P., Zoidakis I., Vlahou A. and Tsilibary E. "High glucose induces multiple proteomic changes in human glomerular epithelial cells (HGEC)". 60th Meeting of Hellenic Society of Biochemistry and Molecular Biology (HSBMB Athens), 2009.

Fragkouli A, Tzinia A, Tsilibary EC: NGF effects on the expression of MMPs and TIMPs in serum-deprived undifferentiataed PC12 cells, 22nd Conference of the Hellenic Society for Neuroscience, Athens, October 16-19, 2008

Tsotakos N, Drossopoulou G, Kotsopoulou E, Tsilibary EC: Glucose-induced changes of slit diaphragm may be associated with altered WT1-Sp1 binding to the relevant promoter regions and may affect podocalyxin expression, 21st Annual Meeting of the European Renal Cell Study Group (ERCSG), Delphi, March 26-28, 2009

Fragkouli A, Tzinia A, Tsilibary EC: NGF promotes a-secretase cleavage of APP and increases MMP9 expression in PC 12 cells, 10th Annual Meeting of the Hellenic Research Club for Connective Tissue & Matrix Biology Meeting, HSBMB, Patras, July 10 & 11, 2009

Other Scientific/Research Activities

EC Tsilibary" i) Reviewer for scientific journals: Cells-Tissues- Organs, Journal of Anatomy, Diabetes, Journal of Cellular and Molecular Medicine ii) Reviewer of proposals for the "Sotiris Papastamatis" Award of the Hellenic Medical Assocaition, iii) Invited speaker for the 15th seminar Bioengineering for Spine and Biotechnology; "*Perspective of stem cell use in regenerative medicine in spinal cord injuries*", P. Konialides Auditorium of EEXOT, 11 - 13 December, 2009. Title: "Perspectives of stem cell use in combination with matrix-derived peptides for regenerative medicine of the CNS" iv) Member of the committee for the selection of abstracts of theWorld Congress of Nephrology in collaboration with ISN (internationela Society for Nephrology), May 22-26, Milan, Italy v) Member: "COST ACTION ON KIDNEY URINE PROTEOMICS" (KUP)

A. Chroni: i) Reviewer for scientific journals Atherosclerosis and Journal of Biomedicine and Biotechnology **ii)** Lectures or scientific presentations (other than oral presentations in scientific meetings):

1) A. Chroni, «Cardiovascular disease and Alzheimer's disease: common molecular mechanisms», Meting about "The contribution of Research Centers in Research, Technological Advance and Innovation", 1 April 2009, Athens
2) A. Chroni, «Carboxy-terminal truncated apolipoprotein E4 promotes intracellular accumulation of amyloid peptide beta 42 in neuronal cells», Symposium to honor Professor Emeritus Vassilis I. Zannis, University of Crete Medical School, 16 October 2009, Herakleio, Crete

iii) Participation in consortium meeting of EU funded STREP grant (title: «Functional genomics of inborn errors and therapeutic interventions in high density lipoprotein (HDL) metabolism», 7-8 May 2009, Amsterdam, the Netherlands.

Awards

A. Chroni: i) L'ORÉAL-UNESCO Greek Award for Women in Science, **ii)** Best poster presentation award : Daniil G., Phedonos A. A. P., Argyri L., Kuivenhoven J. A. and Chroni A. Antioxidant/Anti-inflammatory Properties of HDL and Distribution of ApoA-I–Containing HDL Subpopulations from Family Subjects with Low HDL Syndromes. 12th Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease meeting, 9-10 October 2009, Athens, Greece

Educational Activities

EC Tsilibary: i) Three-hour presentation for the post-graduate course "*Pathobiochemistry*", Univerity of Athens, Department of Biology, 28/4/2009. Title: "Diabetes Mellitus: Molecular mechanisms and therapeutic approaches" ii) Thress-hour presentation for the post-graduate course "*Molecular and applied physiology*", Univerity of Athens, 27/10/2009. Title: "Cell apoptotic processes in human disease: beneficial or adverse?"

A. Chroni: i) «Is "good" cholesterol, HDL, always good?», NCSR Demokritos Summer School, 6-17 July 2009 (1 hour). **ii)** "Lipoprotein metabolism pathways and atherosclerosis. The association between atherosclerosis and Alzheimer's disease." Lecture for graduate class "Human Biochemistry", Biochemistry Graduate Program, Department of Chemistry, University of Athens, 18 June 2009 (3 hours- 15 students). **iii)** Instructor for the graduate course "Cell signaling", Institute of Biology, NCSR «Demokritos» (10 hours- 8 students)

P. Kitsiou: Exploring the mechanisms of cell-apoptosis for the treatment of diseases: The paradigm of Diabetes mellitus. Health Science and Biotechnology SUMMER SCHOOL, NCSR "Demokritos", 6-17 of July 2009 (1 hour)

P. Venieratos completed his Ph.D. thesis in 2009. "Effect of increased glucose concentration on cellular signaling and physiology of pancreatic β-cells". Medical School, University of Athens (Advirors: EC Tsilibary, P.Kitsiou)

G. Daniel completed his MSc thesis in 2009 "Analysis of Composition and Functions of HDL from Patients with Familial Low and High HDL Syndromes. Study of Interactions of Apolipoprotein A-I with the ABCG1 Cholesterol Transporter", Department of Chemistry, University of Athens (Advisor; A. Chroni)

A. Phedonos completed her undergraduate dissertation in 2009. "Role of lipids and lipid metabolism proteins in atherosclerosis and Alzheimer's disease. Analyses of composition and properties of HDL obtained from family subjects carrying ABCA1 mutations." Department of Biology and Biochemistry, University of Bath, UK

Other Activities for the Institute of Biology

- Head, Institute of Biology, NCSR Demokritos (Tsilibary, EC)
- Co-ordinator of the Committee for Bioethics, NCSR Demokritos (Tsilibary, EC)
- Member of the Executive Committee NCSR Demokritos (Tsilibary, EC)
- Appointed by GSRT (Greek Decretariat for research and Technology) as member of the National committee for "Referral Strategic Frame" (ESPA) for Health,((Tsilibary, EC)
- Representative from NCSR Demokritos &Member of the committee for EATRIS (European Advanced Translational Research Infrastructures), ESFRI (Tsilibary, EC)
- Coordinator of work package 4 (WP4) for "EATRIS" (European Advanced Translational Research Infrastructures), ESFRI (Tsilibary, EC)
- Member of the sub-committee for Research & Education, of the executive committee of NCSR Demokritos (Tsilibary, EC)
- Member of the committee for the evaluation of support personnel for the Instituite of Information & TelecommunicationsTechnology (IIT) (Tsilibary, EC)
- Member of the Advisory Committee of Ph.D. students N. Tsotakos and P. Salpea (P. Kitsiou)
- Scientist in charge for the organization of seminars with invited speakers (2009-2010) (P. Kitsiou)
- Member, Institute of IB Education Committee (A. Chroni)
- Member of the Institute of Biology Advisory Committee of PhD students N. Tsotakos and I. Vaggelatos (A. Chroni)
- Chair of Institute of Biology committee for Receipt of Materials and Destruction of Useless Material (A. Chroni)
- Scientist in charge for the operation of FPLC (A. Chroni)

Impact Factors (for 2 publications): 5,627

Citations 2009 (without self- citations): 179

EC Tsilibary: 73, A. Tzinia: 7, P. Kitsiou: 5, A. Chroni: 62, G. Dreossopoulou: 39.

Total Citations 2006-2009 (without self- citations): 760
EC Tsilibary: 297, A. Tzinia: 51, P. Kitsiou: 30, A. Chroni: 206, G. Drossopoulou: 176.

h-factor: EC Tsilibary: 28**,** (ISI, for the name Tsilibary, EC; Including other initials: PCT, PECT, FCT, κλπ: h-factor: 29), A. Tzinia: 8, P. Kitsiou: 5, A. Chroni: 11, G. Drossopoulou: 8

Current External Funding

Program etitled *Combinatorial use of biomedical and nanotechnological methods for interfering with pathological conditions* funded by the GSRT, EPAN Activity 4.5, Action 4.4.1) with Co-ordinator Dr. EC Tsilibary
Duration: 1/6/2005-31/5/2008
Total funding:1.300.000 €, for the IB 500.000 €
Funding of the IB for 2009 (interest): 3.631,57 €

Program etitled *Promoting Excellence for Research and Technology-Supporting Research Activities of the Institute of Biology of NCSR Demokritos* funded by the GSRT (EPAN, Activity 3.3, Action 3.3.1) with Co-ordinator Dr. EC Tsilibary
Duration: 3/2005 - 6/2008
Total funding for the IB: 246.913 €
Funding of the IB for 2009: 12.689,03 €

Program etitled *Uprading and Development of the Laboratories for Muman Tissue Grafts and Animal Colonies* funded by the GSRT (AKMON, Activity 4.2, Action 4.2.2) with Co-ordinator Dr. EC Tsilibary
Program duration: 1/6/2005-31/5/2008
Maximal possible funding (depending on sales): 360.000 €
Funding for the IB from GSRT during 2009: 11.159,95 €
Services from the Laboratory of "Cell & Matrix Pathobiology" for spin-off company: Biophylaxis AE (Co-ordinator:EC Tsilibary).
Duration: 1/6/2008 – 30/6/2011
Funding for 2009: 20.524,41 €

Program etitled *Functional genomics of inborn errors and therapeutic interventions in high density lipoprotein (HDL) metabolism* funded by the European Union with the Principal Investigator for NCSR "Demokritos": A. Chroni,
Duration: 2007-2009
Total funding (lab): 294.000€
Funding of the lab for 2009: 98.000 €.

Program entitled The role of hormone nuclear receptors and transcription factor FOXA2 (HNF-3β) in regulation of genes involved in biosynthesis and metabolism of HDL: New perspectives for the treatment of atherosclerosis. Funded by the Ministry of Health and Social Solidarity with the Principal Investigator for NCSR "Demokritos": A. Chroni (Coordinator: D. Kardassis, University of Crete),
Duration: 1/7/2009-31/6/2011

Note: The following proposals have been submitted and are under evaluation:

1. EEC, FP7-PEOPLE-ITN-2009 (Marie-Curie Training grant): "Cross- European PhD training programme to combat diabetic Kidney complications"(submitted on: 22.12.2009), Duration: 7.2010-7.2013. Co-ordinator: H. Holthofer (for: Ec Tsislibary:salary of one PhD student and consumables: ~65,000€)
2. Program"HRAKLEITOS": Photo-indiuced anti-cancer properties of TiO_2: Molecular nechanisms of action and applications (Submitted, N. Lagopati,, PhD candicate) Program duration: 1.5.2010-30.4.2013 Coordinator: PR. P. Papazafiri (EC Tsilibary, collaborating investigator) (45.000 €)
3. ESPA 2007-2013, "Collaboration"/long-range: "Development of Innovative Diagnostic and Therapeutic Magnetic Biocarriers for Breast Cancer Treatment" (DIAGNOHEAT ". Program

duration: 4 years (2011-2011) (Co-ordinator:EC Tsilibary) (1.500.000 €) (for EC Tsilibary: 350.000 €)

4. ESPA 2007-2013, "Collaboration" /mid-range: "Studies on viability and regeneration of pancreatic beta cells for interfering with Diabetes" Programduration: 3 years (2011-2013) (Co-ordinator: G. Chrousos) (1.150.000 €) (EC Tsilibary: 155.000 €)
5. Program "THALIS" "Exploring the protective role of MMP9 against amyloid plaque formation in transgenic animal models of Alzheimer disease": Duration: 2011-2013 (Co-ordinator: EC Tsilibary) (600.000 €) (For EC Tsilibary: 300.000 €)
6. Program "THALIS": "Mechanisms of development of chronic kidney disease in order to intervene preventively" Duration: 2011-2013 (Co-ordinator:AS Charonis) (600.000 €) (For EC Tsilibary: 150.000 €)
7. Human Science Frontier, Expression of Interest (30/03/2010) "Neuroprotective role of MMP9 in Alzheimer using animal models and in vivo molecular imaging". (Co-ordinator: EC Tsilibary) (participants: P.Francis, Wolfson Centre for Age-Related Diseases, King's College London, J. Herms, Zentrum für Neuropathologie, Ludwig Maximilians Universität, München, και V. Ntziachristos, Institute for Biological and Medical Imaging (IBMI, Helmholtz Zentrum München & Technische Universität München)

Research Group: Environmental Mutagenesis -Carcinogenesis

Research Staff
Gerassimos Voutsinas, Senior Researcher
Panagiotis Karkoulis, Graduate Student
Dimitra Anastasiou, Collaborating Graduate Student
Stefanos Kachrilas, Collaborating Graduate Student
Evmorphia Konstantatou, Collaborating Graduate Student
Katerina Vestaki, Collaborating Graduate Student (*MSc) – MSc obtained in 2009*
Sokratis Avgeris, Research Technician

Research Interests

1. Identification and validation of drug targets for cancer therapy
2. Development and evaluation of biomarkers for diagnosis, prognosis and response to treatment in human diseases
3. Development of genetic testing protocols for molecular diagnosis of human genetic diseases

2009 Findings

1. Grade-dependent effects on cell cycle progression and apoptosis in response to doxorubicin in human bladder cancer cell lines
Doxorubicin is an important component of combination therapy for muscle-invasive urinary bladder cancer. Treatment with this topoisomerase II poison is able to interfere with cell cycle progression and lead to cancer cell death. Using FACS analysis, Western immunoblotting and semi-quantitative RT-PCR, we studied the effects of doxorubicin on cell cycle progression and apoptosis, and also explored the possibility of using groups of genes as biomarkers of prognosis and/or response to doxorubicin treatment in human urinary bladder cancer cells. Doxorubicin induced dose-dependent G2/M and/or G1/S cell cycle arrest, followed by grade- and dose-dependent reduction in the amount of the cytosolic trimeric form of FasL, activation of Caspase-8, Caspase-9, Caspase-3, cleavage of PARP, Lamin A/C, Bcl-XL/S and interestingly Hsp90, and finally cell death. Data presented here also suggest the use of the expression patterns of *Cyclin-E2, Cyclin-F, p63, p73, FasL, TRAIL, Tweak, Tweak-R, XAF-1, OPG* and *Bok* genes for identification of the differentiation grade, and *Cyclin-B2, GADD45A, p73, FasL, Bik, Bim, TRAIL, Fas, Tweak-R, XAF-1, Bcl-2, Survivin, OPG, DcR2* and *Bcl-XL* genes for the detection of response to doxorubicin in human bladder cancer cells.

2. Mutational analysis of the *BRAF, RAS* and *EGFR* genes in human adrenocortical carcinomas
The serine/threonine kinase B-Raf plays a key role in the Ras/Raf/MEK/ERK pathway that relays extracellular signals for cell proliferation and survival. Several types of human malignancies harbor activating BRAF mutations, most frequently a V600E substitution. The epidermal growth factor receptor (EGFR), a transmembrane tyrosine kinase (TK) receptor that mediates proliferation and survival signaling, is expressed in a wide variety of normal and neoplastic tissues. EGFR inhibitors have produced objective responses in patients with non-small cell lung carcinomas harboring activating EGFR TK domain somatic mutations. We evaluated the presence of mutations in *BRAF* (exons 11 and 15), *KRAS* (exons 1 and 2), *NRAS* (exons 1 and 2) and *EGFR* (exons 18-21) in adrenal carcinomas (35 tumor specimens and 2 cell lines) by DNA sequencing. *BRAF* mutations were found in 2 carcinomas (5.7%). Four carcinomas (11.4%) carried EGFR TK domain mutations. One specimen carried a *KRAS* mutation, and another carried two *NRAS* mutations. No mutations were found in the 2 adrenocortical cell lines. *BRAF*- and *EGFR*-mutant tumor specimens exhibited stronger immunostaining for the phosphorylated forms of the MEK and ERK kinases that their wild-type counterparts. *EGFR*-mutant carcinomas exhibited increased phosphorylation of EGFR (Tyr 992) compared to wild-type carcinomas. We conclude that *BRAF*, *RAS* and *EGFR* mutations occur in a subset of human adrenocortical carcinomas. Inhibitors of the Ras/Raf/MEK/ERK and EGFR pathways represent candidate targeted therapies for future clinical trials in carefully selected patients with adrenocortical carcinomas harboring respective activating mutations.

3. Human bladder cancer cells undergo cisplatin-induced apoptosis that is associated with p53-dependent and p53-independent responses

Cisplatin is a first-line chemotherapeutic agent and a powerful component of standard treatment regimens for several human malignancies including bladder cancer. DNA-Pt adducts produced by cisplatin are mainly responsible for cellular toxicity and induction of apoptosis. Identification of the mechanisms that control sensitivity to cisplatin is central to improving its therapeutic index and to successfully encountering the acquired resistance frequently emerging during therapy. In the present study, using MTT-based assays, Western blotting and semi-quantitative RT-PCR, we examined the apoptosis-related cellular responses to cisplatin exposure in two human urinary bladder cancer cell lines characterized by different malignancy grade and p53 genetic status. Both RT4 (grade I; wild-type p53) and T24 (grade III; mutant p53) cell types proved to be vulnerable to cisplatin apoptotic activity, albeit in a grade-dependent and drug dose-specific manner, as demonstrated by the proteolytic processing profiles of Caspase-8, Caspase-9, Caspase-3, and the Caspase repertoire characteristic substrates PARP and Lamin A/C, as well. The differential resistance of RT4 and T24 cells to cisplatin-induced apoptosis was associated with an RT4-specific phosphorylation (Ser15; Ser392) pattern of p53, together with structural amputations of the Akt and XIAP anti-apoptotic regulators. Furthermore, cisplatin administration resulted in a Granzyme B-mediated proteolytic cleavage of Hsp90 molecular chaperone, exclusively occurring in RT4 cells. To generate functional networks, expression analysis of a number of genes, including *Bik, Bim, Bcl-2, FAP-1, Fas, FasL, TRAIL, Puma, Caspase-10, ATP7A, ATP7B* and *MRP1*, was performed, strongly supporting the role of p53-dependent and p53-independent transcriptional responses in cisplatin-induced apoptosis of bladder cancer cells.

4. Molecular targeting and gene delivery in bladder cancer therapy

Urothelial carcinoma of the bladder is the second most common genitourinary malignancy and the second most common cause of genitourinary cancer-related deaths with a worldwide estimate of about 300,000 new cases diagnosed every year. A significant problem in this type of cancer is the high recurrence rate of noninvasive primary tumors, leading to a high percentage of tumor progression and to a very poor 5-year survival rate. Targeted and gene therapy are currently the two major efforts in cancer treatment. Targeted therapy refers to strategies against specific cellular molecules deregulated in tumors, whereas gene therapy focuses on the genetic modification of tumor cells, mainly for correcting gene defects, inducing selective tumor cell death or modulating host's immune response. Recent advances in our understanding of the pathogenesis of bladder cancer at the molecular level have provided a significant number of cellular targets for therapy and have shown the importance of individualized therapy according to the molecular profile exhibited by the tumor cells. While the major problems of both targeted and gene therapy are far from being solved yet, both lines of cancer therapy hold promising results. This article aims at providing a brief general overview of this broad subject.

2009 Publications

Stravopodis, D.J., P.K. Karkoulis, E.G. Konstantakou, S. Melachroinou, D. Anastasiou, S. Kachrilas, A.D. Lampidonis, N. Messini-Nikolaki, I.S. Papassideri, G. Aravantinos, L.H. Margaritis and G.E. Voutsinas (2009) Grade-dependent regulation of cell cycle progression and apoptosis in response to doxorubicin in human bladder cancer cell lines, Int J Oncol 34, 137-160.

Kotoula, V., E. Sozopoulos, H. Litsiou, G. Fanourakis, T. Koletsa, G. Voutsinas, S. Tseleni-Balafouta, C.S. Mitsiades, A. Wellmann and N. Mitsiades (2009) Mutational analysis of the *BRAF, RAS* and *EGFR* genes in human adrenocortical carcinomas, Endocrine-Related Cancer 16, 565-572.

Konstantakou, E.G., G.E. Voutsinas, P.K. Karkoulis, G. Aravantinos, L.H. Margaritis, and D.J. Stravopodis (2009) Human bladder cancer cells undergo cisplatin-induced apoptosis that is associated with p53-dependent and p53-independent responses, Int J Oncol 35, 401-416.

Voutsinas, G.E. and D.J. Stravopodis (2009) Molecular targeting and gene delivery in bladder cancer therapy, J BUON 14 (suppl 1), S69-S78.

2009 Articles in Books and Conference Proceedings

Stavropoulou, C., S. Zachaki, K. Manola, G. Voutsinas, H. Orphanidou, V. Georgakakos and C. Sambani (2009) Association of NQO1 C609T polymorphism with chromosomes 5 and/or 7 abnormalities in patients with MDS/AML, 10th International Symposium on Myelodysplastic Syndromes, May 6-9, 2009, Patras, Greece, Leukemia Research 33, suppl. 1, S45-S46.

Voutsinas G.E. and Stravopodis D.J. (2009) Molecular targeting and gene delivery in bladder cancer therapy, 3rd Seminar of the Balkan Society of Oncology, 10-12 September 2009, Volos, Greece.

Karkoulis P.K., Stravopodis D.J., Konstantakou E.G., Melachroinou S., Anastasiou D., Margaritis L.H. and Voutsinas G.E. (2009) Hsp90 drug targeting in bladder cancer cells, 14th World Congress on Advances in Oncology and 12th International Symposium on Molecular Medicine, 15-17 October 2009, Loutraki, Greece, International Journal of Molecular Medicine 24 (suppl), S31.

Karkoulis P.K., Stravopodis D.J., Margaritis L.H. and Voutsinas G.E. (2009) Pharmacological targeting of Hsp90 molecular chaperone in human urinary bladder cancer cells, 1st International Conference on Molecular Cancer Research, 27-29 November 2009, Athens, Greece, p. 32.

2009 Presentations at Scientific Conferences

Gioni, V., T. Karabinas, G. Voutsinas, A.E. Roussidis, S. Papadopoulos, N.K. Karamanos and D. Kletsas (2009) Imatinib mesylate inhibits proliferation and collagen synthesis in human breast stromal fibroblasts, 15th Panhellenic Congress of Clinical Oncology, March 26-28, 2009, Athens, Greece.

Gioni V., Karabinas Th., Voutsinas G.E., Roussidis A.E., Papadopoulos S., Karamanos N.K. and Kletsas D. (2009) Imatinib mesylate inhibits the proliferation and collagen synthesis in human breast stromal fibroblasts, Proceedings of the 31st Scientific Conference of Hellenic Association for Biological Sciences, 14-16 May 2009, Patras, Greece, p. 60-61.

Anastasiou D.K., Stravopodis D.J. and Voutsinas G.E. (2009) DNA methyltransferase and histone deacetylase inhibitors synergize to induce apoptotic death in human urinary bladder cancer cells, Proceedings of the 31st Scientific Conference of Hellenic Association for Biological Sciences, 14-16 May 2009, Patras, Greece, p. 14-15.

Karkoulis P.K., Stravopodis D.J., Konstantakou E.G., Margaritis L.H. and Voutsinas G.E. (2009) 17-AAG induces cell cycle arrest and apoptosis in human urinary bladder cancer cell lines due to down-regulation of multiple Hsp90 clients in the Akt signaling pathway, Proceedings of the 31st Scientific Conference of Hellenic Association for Biological Sciences, 14-16 May 2009, Patras, Greece, p. 124-125.

Konstantakou E.G., Voutsinas G.E., Karkoulis P.K., Aravantinos G., Margaritis L.H. and Stravopodis D.J. (2009) Cisplatin-induced apoptosis of human bladder cancer cells is associated with p53-dependent and p53-independent regulatory responses, Proceedings of the 31st Scientific Conference of Hellenic Association for Biological Sciences, 14-16 May 2009, Patras, Greece, p. 172-173.

Setta-Kaffetzi N., Thanasopoulou A., Roumpelaki M., Samara A., Konstantakou E.G., Voutsinas G.E., Anagnou N., Dimas K., Stravopodis D.J. and E. Anastasiadou (2009) Xenograft animal models for the *in vivo* live imaging of human tumors, Proceedings of the 31st Scientific Conference of Hellenic Association for Biological Sciences, 14-16 May 2009, Patras, Greece, p. 172-173.

Karkoulis P.K., Stravopodis D.J., Margaritis L.H. and Voutsinas G.E. (2009) Inhibition of multiple signaling pathways by 17-allylamino-17-demethoxygeldanamycin in human urinary bladder cancer cells, 60th Meeting of the Hellenic Society of Biochemistry and Molecular Biology, 20-22 November 2009, Athens, Greece, p. 137.

Educational Activities

Course lectures on "Targeted cancer therapy", post-graduate courses of the Institute of Biology, NCSR "Demokritos".

Course lectures on "Cytotoxicity study on conventional and targeted chemotherapeutic drugs" (seminar and practical laboratory exercise) in the framework of the Post-Graduate Specialization

Diploma "Biological Applications in Medicine" of the Departments of Biology and Medicine of the National Kapodistrian University of Athens (NKUA), Athens.

Course lectures on the "Introduction to Molecular Biology" in the American College of Greece (Deree College), Aghia Paraskevi Attikis.

Ms K. Vestaki has completed in this lab her thesis for the **post-graduate specialization diploma** entitled "Comparative study of gene expression profiles as a genetic platform for the diagnosis and prognosis of human cancers" and successfully carried out the relevant presentation in the Department of Biology, NKUA.

Other Scientific/Research Activities

Participation in Greek and International scientific bodies and organizations:

1. Reviewer in International Journal of Cancer
2. Reviewer in the International Union Against Cancer
3. Greek Alliance for Rare Diseases (Treasurer)
4. Greek Alliance for Rare Diseases (Member of the scientific committee)
5. Tuberous Sclerosis Association of Greece (Member of the scientific committee)
6. Invited to the Dinner Debate on Rare Diseases "Patient Care: A Public Affair", 3 March 2009, European Parliament, Brussels.
7. Eurordis Membership Meeting 2009, 7-9 May 2009, Eugenides Foundation, Athens, Greece.
8. Participation to the Start-Up Central Workshop "Patients Partnering in Clinical Research", 11 June 2009, Brussels, Belgium.
9. Consultant of the Greek Ministry of Health and Social Solidarity in the meeting for the planning of the program E-Rare 2 for Rare Diseases, Brussels, June 2009.
10. Participation in the Organizing Committee of the 4th Conference of the Greek Alliance for Rare Diseases, "Rare Diseases with Hematological Manifestations – New Therapeutic Approaches", 11-12 December 2009.

Other scientific lectures or presentations:

1. Two lectures for the pupils of the 1st Primary School of Peania entitled "Rare Diseases: Patient Care is a Public Affair", 31 March and 7 April 2009, Peania Attikis.
2. Invited speaker for presentation of the subject "Biology: The Science for Life", 26 May 2009, Department of Philosophy, University of Ioannina, Ioannina.
3. Presentation entitled "Molecular Diagnosis of Rare Hematological Disorders", 4th Conference of the Greek Alliance for Rare Diseases, "Rare Diseases with Hematological Manifestations – New Therapeutic Approaches", 11 December 2009, Eugenides Foundation, Athens.
4. Presentation entitled "Therapeutic Approaches in Rare Diseases" in the framework of an event on "Rare Diseases from a Scientific and a Social Point of View" in the "2008-2009 Festival for Volunteers Without Borders », 20 December 2009, Hilton Hotel, Athens.

Other Activities for the Institute of Biology

Participated as an examiner in the relative committee for the selection of new scholars of the Institute of Biology (September 2009). Participated in 3 internal advisory committees of scholars working on their theses (A. Repouskou, M. Xydous, P. Karkoulis)

In charge for the operation of ABI Prism 310 Genetic Analyzer (Applied Biosystems), Mx3000P QPCR system (Stratagene), Image Analysis System (Vilber Lourmat), LAS-4000 Luminescent Image Analyzer (Fuji-Film) and FLA-7000 Fluorescent Image Analyzing System (Fuji-Film) of the Institute of Biology, NCSR "Demokritos".

Impact factors (for 4 publications): 11,305

Number of citations for 2009 (without self-citations): 54

Number of citations 2006-2009 (without self-citations): 172

h-factor: 10

Current Extramural Funding

Research project entitled *Structural and functional analysis of genes involved in the PI3K signal transduction pathway in urinary bladder cancer: effects on prognosis and therapy*, financed by the Ministry of Health and Social Solidarity, with Dr. G.E. Voutsinas as the Project Leader
Duration: 1/9/2008-31/8/2010
Total funding (lab): 12.000 €
Funding of the lab for 2009: 6.000 €.

Research project entitled *Decoding of the apoptotic potential of the specific inhibitor of proteasome activity Bortezomib (Velcade) in targeted chemotherapy of human urinary bladder cancer,* financed by the Association of Greek Oncologists Pathologists (ΕΟΠΕ), with Dr. D.J. Stravopodis (Dept of Biology, University of Athens) as the Project Leader
Duration: 1/12/2009 – 31/11/2010
Total funding (lab: 5.000 €
Funding of the lab for 2009: 1.250 €.

Note: Five (5) research proposals which have been submitted for funding are under evaluation**:**

1. «Targeted therapy of human urinary bladder cancer», Research proposal for funding submitted to the Miltiades Empeirikos Foundation.
2. «Molecular diagnosis of neurofibromatosis», Research proposal for funding submitted to the American College of Greece.
3. «Non-ionizing electromagnetic radiations: biologic effects», Research proposal for funding submitted to the Ministry of Education, Life-long Learning and Religion.
4. «Contribution of intracellular communication of ERα/β with EGR-R and IGF-R in the development and progression of breast cancer: functional properties of cells, expression of bio-active molecules and induction of EMT», Research proposal for funding submitted to the Ministry of Education, Life-long Learning and Religion.
5. «Sequencing and genome characterization of lactic acid bacteria Streptococcus macedonicus, Streptococcus thermophilus, Lactobacillus delbrueckii subsp. lactis and Lactobacillus acidipiscis. Physiological, evolutionary and technological extensions», Research proposal for funding submitted to the Ministry of Education, Life-long Learning and Religion.

PROGRAMME B:

MODEL SYSTEMS FOR THE STUDY OF CELL FUNCTION

Research Group: Molecular Genetics of Insects and Biotechnology

Research Staff
Kostas Iatrou, Research Director
Luc Swevers, Senior Researcher
Vassiliki Lampropoulou, Senior Researcher
Lydia Ignatiadou, Emeritus Scientist
Rodica Efrose, Postodoctoral Fellow
Konstantinos Koussis, Postdoctoral Fellow
Panagiota Tsitoura, Postdoctoral Fellow
Konstantinos Ioannides, Graduate Student
Christiana Magrioti, Graduate Student
Dimitris Raptopoulos, Administrative Assistant
Dimitra Stefanou, Technical Specialist
Dimitris Kopanelis, Research Technician

Research Interests

1. Regulatory mechanisms controlling insect physiological functions:

(a) Oogenesis in lepidopteran insects: a model for long-term differentiation programs induced by ecdysteroid hormones;
(b) Mechanisms of immunosuppression in lepidopteran insects following parasitization by hymenopteran endoparasitoids: the role of the interactions between proteins produced by hymenopteran endosymbiotic polydna viruses and hemocyte proteins of the lepidopteran hosts;
(c) Mechanisms controlling olfactory function in the malaria mosquito vector *Anopheles gambiae*.

2. Molecular biology and genetic manipulation of insect nuclear polyhedrosis viruses:

(a) Viruses expressing proteins harmful to the insect hosts;
(b) Recombinant viruses as vectors for insect genetic transformation;
(c) Modified viruses as vectors for human gene therapy and cellular immunization applications.

3. Functional genomics:

(a) Systems for production of proteins of economic importance in lepidopteran insect and mammalian cell lines;
(b) High throughput screening systems for detection of bioactive substances (activators and inhibitors of pharmacological targets) in chemical libraries and collections of natural products (plants and microorganisms).

2009 Findings

Silkmoth Oogenesis

RNA interference is a very promising technique to investigate the function of genes in organisms that are not readily amenable to classical genetic analysis. However, the silkmoth *Bombyx mori* is refractory to this technique, even when high amounts of dsRNA are administered. To investigate the cause of the absence of efficient RNAi in *Bombyx*, a gene profiling study was initiated that investigated the expression of key factors of the RNAi core and dsRNA uptake machinery in different larval and pupal tissues. Results indicate that the factor R2D2, an essential co-factor of Dicer-2, is present at very low levels in all tissues. Whether R2D2 is a limiting factor for efficient RNAi processing in the silkmoth, is currently investigated through rescue experiments (collaboration with Dr. Guy Smagghe, University of Ghent, Belgium).

Molecular mechanisms of endoparasitoidism in lepidopteran insects – the role of CcBV Ank proteins on the transcriptional activity of host immune genes

CcBV is the symbiotic bracovirus (or polydnavirus, PDV) of the wasp *Cotesia congregata* (Hymenoptera), which is injected in the wasp's lepidopteran host *Manduca sexta* along with wasp eggs during parasitism. Infection does not lead to replication of the viral genome and generation of new

viruses but it rather affects the caterpillar's overall physiology including its innate immunity system. Our group studies the relationship between CcBV and the hemocytes of the host *Manduca sexta* in order to understand the role(s) of CcBV proteins in the suppression of immune responses against the parasitoid wasp embryos. Among the CcBV gene families, there is the family of *ank* genes, which comprises members encoding proteins with similarities to the eukaryotic IκBα protein, the inhibitor of NF-κB transcription factor, and the inhibitory domain of the Cactus protein (IκBα homologue) of *Drosophila melanogaster*. Six out of the nine members of the *ank* gene family were cloned, initially into mammalian expression vectors, and expressed in mammalian HEK293 cells. The expressed proteins were examined for their ability to suppress TNFα–mediated induction of a luciferase reporter gene placed under the control of an NFκB-responsive promoter. Our results showed that at least two proteins, Ank4 and Ank7 are capable of inhibiting NFκB transcription by 40 and 30% respectively.

We also examined the ability of the CcBV Ank proteins to reduce the expression of antimicrobial peptides like Lebocin 4, Attacin and Cecropin B1, in insect cells. Expression constructs of the constitutively active transcription factor *Bm*Relish1-d2 were introduced into the insect cells by transfection together with reporter genes driven by the promoter element of the gene for the antimicrobial protein Attacin. Co-expression of six CcBV *ank* genes resulted in a dose-dependent inhibition of *Bm*Relish1-d2-driven transcriptional activity of the reporter genes. Immunofluorescence and confocal microscopy was employed to deduce the subcellular localization of the reconbinant Ank proteins. Most of the expressed proteins were found in both the cytoplasm and the nuclei of the cells. Interestingly, in the presence of *Bm*Relish1-d2, Ank 8 was found almost exclusively in the nucleus. This finding suggests that Ank proteins inhibit transcription of *Bm*Relish1-d2 in a manner different than that of the IκBα/NF-κB.

Proteins of the mosquito olfactory system

Our studies of the structural and functional characterization of the receptors of the olfactory neurons of the mosquito Anopheles gambiae, which specifically recognize and bind small volatile compounds that act as guiding cues during searches for food or mating partners continued, in collaboration with the the IB's Laboratory of Molecular Pharmacology (Z. Georgoussi) with the completion of studies of the membrane topology of the receptors. Through the use of (i) chimeric receptors fused with transactivators at the N- or C-termini which activate a reporter gene only when located intracellularly, and (ii) gene constructs that allow immunofluorescent detection of the receptors only when the fluorecent epitopes are exposed to the extracellular medium, we have shown that the transmembrane regions of the olfactory receptors OR1 and OR2, in the presence of absence of OR7, have an orientation opposite than that of classical GPCRs.

Furthermore, we cloned 6 receptors that are specifically expressed in the antennae of female mosquitoes (OR42, OR53, OR55, OR68 and OR72) into expression vectors that allow their expression either in authentic form or as tagged versions with different epitopes located at the N- or C-termini. The expression vectors for the receptors will be used for the production of stably transformed insect cell lines that will express the recombinant olfactory receptors.

The expression and intracellular location of the recombinant receptors OR1, OR2 and OR7 was determined in stably transformed insect cell lines established trough previous studies of our group, with the use of specific antibodies. More specifically, the detection of recombinant receptors tagged with Myc or Flag epitopes was carried out in whole cell extracts or membrane preparations with the use of specific antibodies against the epitopes. With this method, the expression of the receptor pairs mycOR1/flagOR7, OR1myc/flagOR7, mycOR2/flagOR7 and OR2myc/flagOR7 was verified in the transformed cell lines. It was also determined that co-expression of the OR7 receptor did not cause quantitative changes in the expression levels of OR1 and flagOR2. Moreover, the specific polyclonal antibodies raised in rabbits against small peptides of the OR1 and OR2 receptors were also tested and it was established that the antibodies specifically recognize the receptors.

During the same period, research efforts also concentrated on the development of a reliable reporter system for the activation of recombinant receptors in the cells after addition of specific ligands. The functional assays were based on (a) the fluorophore Fluo4-AM whose fluorescence increases when the

concentration of free intracellular calcium increase and (b) the increase in luminescence that is produced in the cell by the recombinant indicator protein aequorin when the levels of intracellular calcium increase. Results from control experiments have shown that the reporter systems produce significant and reproducible signals in the cells after addition of factors such as thapsigargin, ionomycin and Triton X-100, which induce the increase in intracellular free calcium levels. The experiments for the detection of the responses of activated olfactory receptors are still in progress.

Finally, for our studies for the identification of natural ligands for the odorant-binding proteins (OBPs) that could act as attractants or repellents for mosquitoes, we cloned in appropriate expression vectors and expressed in insect cells five OBPs (OBP1, OBP3, OBP5, OBP47 and OBP48), which are predominantly expressed in antennae of female mosquitoes. After their purification, the recombinant proteins were used, as described below, in *in vitro* assays that allow the fast analysis of complex mixtures of plant extracts for the detection of the presence of specific ligands in them.

Engineered Baculoviruses for Mammalian Cell Transduction and Gene Therapy

Baculoviruses that have incorporated the PiggyBac transposition system (PiggyBac transposase gene and recognition sequences at both ends of cloned genes whose over-expression is desired) were used to infect human cell lines. After infection of cells with virus that contained the YFP marker gene, efficient incorporation of the transgene in the genome of the host cells was observed and multiple clones that expressed YFP at high levels were selected. PCR experiments confirmed the correct excision of the expression cassette from the viral vector while the incorporation sites of the expression cassette in the cellular genome was determined by inverse PCR. The creation of a universal expression cassette that allow cloning of selected transgenes destined for stable chromosomal incorporation in mammalian cells with minimal effort is under construction.

Functional Genomics

Screening systems for ecdysteroid analogs that are specific for the beneficial lepidopteran insect, the domesticated silkworm *Bombyx mori*, and a lepidopteran pest, *Spodoptera littoralis*, were used to screen for compounds with high molting hormone activity. While the results of the *in vitro* screens showed minimal activity differences of the tested compounds between the two species, we have observed that compounds with high ecdysone activity showed great differences in *in vivo* toxicity when examined in larval toxicity assays. Thus, species-specific insecticides based on the activation of the ecdysone receptor can be developed but their specificity is not based on a differential capacity to activate the receptor but rather on other unidentified *in vivo* parameters, such as differential uptake, excretion or metabolism (collaboration with Dr. Guy Smagghe, University of Ghent, Belgium and with Dr. Yoshiaki Nakagawa, University of Kyoto, Japan).

To discover natural ligands for the cloned odorant-binding proteins (OBPs) of the malaria vector *A. gambiae*, we screened a collection of aromatic plant extracts that were prepared by the collaborating group of Chemical Ecology and Natural Products (M. Konstantopoulou) of the IB. The screening system for fast detection of ligands for the recombinant OBPs was based on the displacement of the non-specific fluorescent ligand N-phenyl-1-naphthylamine (1-NPN) by the specific ligands that are present in the plant extracts. The extracts that were found to contain ligands for OBPs underwent differential fractionation based on polarity and the obtained fractions were re-screened for the presence of ligands. In collaboration with the research team of P. Guerin (University of Neuchatel, Switzerland) and F. Dani (Interdepartmental Center for Mass Spectrometry, University of Firenze, Italy), extracts and fractions that contained OBP ligands were used in (i) *in vivo* behavioral assays (repellence or attraction of mosquitoes), (ii) separation by gas chromatography coupled to physiological analyses of compounds (electro-antennograms, EAG), and (iii) mass spectrometry for structural characterization. More in-depth analysis of individual compounds from the extracts that were found to produce strong physiological responses of the mosquitoes in EAG assays, including their binding properties to recombinant OBPs and their effect in the behavioral assays (as repellents or attractants), is in progress.

2009 Publications

Soin, T., Masatoshi, I., Swevers, L., Rougé, P., Janssen, C.R., and Smagghe, G. (2009). Towards Coleoptera-specific high-throughput screening systems for compounds with ecdysone activity: development of EcR reporter assays using weevil (*Anthonomus grandis*)-derived cell lines and *in silico* analysis of ligand binding to *A. grandis* ligand-binding pocket. *Insect Biochem. Mol. Biol.* 39, 523-534.

Georgomanolis, T., Iatrou, K., and Swevers, L. (2009). *BmCAP*, a silkmoth gene encoding multiple protein isoforms characterized by SoHo and SH3 domains: expression analysis during ovarian follicular development**.** *Insect Biochem. Mol. Biol.* 39, 892-902.

Ignatiades, L., Gotsis-Skretas, O., Pagou, K., Krasakopoulou, E. 2009. Diversification of phytoplankton community structure and related parameters along a large-scale longitudinal east-west transect of the Mediterranean Sea. *J. Plankton Res.* 31, 411-428.

Articles in Press

Soin, T., De Geyter, E., Mosallanejad, H., Iga, M., Martín, D., Ozaki, S., Kitsuda, S., Harada, T., Miyagawa, H., Stefanou, D., Kotzia, G., Efrose, R., Labropoulou, V., Geelen, D., Iatrou, K., Nakagawa, Y., Janssen, C.R., Smagghe, G., and Swevers, L. (2010). Assessment of species specificity of molting accelerating compounds in Lepidoptera: comparison of activity between *Bombyx mori* and *Spodoptera littoralis* by *in vitro* reporter and *in vivo* toxicity assays. *Pest Management Science*.

Biessmann, H., Andronopoulou, E., Biessmann, M.R., Douris, V., Dimitratos, S.D., Eliopoulos, E., Guerin, P.M., Iatrou, K., Justice, R.W., Kröber, T., Marinotti, O., Tsitoura, P., Woods, D.F., and Walter, M.F. (2010). The *Anopheles gambiae* Odorant Binding Protein 1 (AgamOBP1) mediates indole recognition in the antennae of female mosquitoes. *PLoS One*.

Efrose, R., Swevers, L. and Iatrou, K. (2010). Baculoviruses deficient in *ie1* gene function abrogate viral gene expression in transduced mammalian cells. *Virology*.

2009 Articles in Books

Swevers, L., and Iatrou, K. (2009). Ecdysteroids and ecdysteroid signaling pathways during insect oogenesis. In: Ecdysone, structures and functions (Smagghe, G., ed.). Part II – In the Post-Genomic Era, Ecdysteroid Genetic Hierarchies in Insect Growth and Reproduction, pp. 127-164. Springer-Verlag, Dordrecht, Nederland.

Hannan, G.N., Hill, R.J., Dedos, S.G., Swevers, L., Iatrou, K., Tan, A., Parthasarathy, R., Bai, H., Zhang, Z., and Palli, S.R. (2009). Applications of RNA interference in ecdysone research. In: Ecdysone, structures and functions (Smagghe, G., ed.). Part II – In the Post-Genomic Era, Ecdysteroid Genetic Hierarchies, pp. 205-227. Springer-Verlag, Dordrecht, Nederland.

2009 Presentations at Scientific Conferences

Efrose, R. L. Swevers, V. Douris and K. Iatrou (2009). Baculovirus knockout and transposition mutants as gene transfer vectors for mammalian cells: toward safer gene therapy and more efficient protein expression vectors. 12th Annual Baculovirus & Insect Cell Culture meeting, San Antonio, Texas, USA, February 1-4, 2009.

Iatrou, K. (2009). Toward the functional characterization of mosquito smell components in cultured lepidopteran cells. EMBO Conference Series on the Biology of Disease Vectors: Population and molecular biology of vectors, July 16-19, 2009, Kolympari, Crete, Greece.

Soin, T., De Geyter, E., Mosallanejad, H., Iga, M., Martín, D., Kotzia, G., Efrose, R.,Labropoulou, V., Iatrou, K., Ozaki, S., Kitsuda, S., Harada, T., Nakagawa, Y., Geelen, D., Smagghe, G., and Swevers, L. (2009). Evaluation of the specificity and efficiency of ecdysone agonists in *Bombyx mori* and *Spodoptera littoralis* by *in vitro* reporter assays and *in vivo* toxicity assays. 8th International Workshop on the Molecular Biology and Genetics of the Lepidoptera, August 23-29, Orthodox Academy of Crete, Kolympari, Crete, Greece.

Ioannidis, K., Swevers, L., and Iatrou, K. (2009). Baculovirus engineering: a viral RNA polymerase-deficient virus. 8th International Workshop on the Molecular Biology and Genetics of the Lepidoptera, August 23-29, Orthodox Academy of Crete, Kolympari, Crete, Greece.

Magkrioti C., Iatrou K. and Labropoulou V. The role of Ankyrin-repeat proteins of the polydnavirus CcBV on lepidopteran immunity signaling pathways. 8th International Workshop on the Molecular Biology and Genetics of the Lepidoptera, August 23-29, Orthodox Academy of Crete, Kolympari, Crete, Greece.

Swevers, L., Efrose, R., and Iatrou, K. (2009). Baculovirus engineering for development of safer gene therapy vectors and improved protein expression in mammalian cells. 8th International Workshop on the Molecular Biology and Genetics of the Lepidoptera, August 23-29, Orthodox Academy of Crete, Kolympari, Crete, Greece.

Efrose, R., Swevers, L., and Iatrou, K. (2009). Generation and characterization of BmNPV-based *piggyBac* baculoviruses for efficient and stable gene transduction in mammalian HEK293 cell line. 8th International Workshop on the Molecular Biology and Genetics of the Lepidoptera, August 23-29, Orthodox Academy of Crete, Kolympari, Crete, Greece.

Tsitoura, P., Koussis, K., Konstantopoulou, M., Swevers, L., and Iatrou, K. (2009). Toward the functional characterization of mosquito smell components in lepidopteran tissue culture cells. 8th International Workshop on the Molecular Biology and Genetics of the Lepidoptera, August 23-29, Orthodox Academy of Crete, Kolympari, Crete, Greece.

Iatrou, K. (2009). ENAROMaTIC, a coordinated European effort for the control of the malaria mosquito vector populations by way of olfactory disorientation. Joint workshop of the EU Commission (RTD F3 & B3) and the Imperial College of London (INFRAVEC) on "EU-funded Research for Malaria Vector Control", September 16, 2009 London, UK.

Iatrou, K. (2009). Functional genomics tools and applications: silkworm-derived expression platforms for functional expression of genes of choice in insect and mammalian cells. International Symposium on "Bombyx mori Functional Genomics and Modern Silk Road", October 23-25, 2009, Chongqing, China.

Iatrou, K., Agalou, M., Andronopoulou, E., Douris, V., Eliopoulos, E., Georgoussi, Z., Koussis, K., Labropoulou, V., Swevers, L., Tsikou, D., Tsitoura, P. (2009). Mosquito Olfaction as Target for malaria control. 60th Annual meeting of the Hellenic Society of Biochemistry and Molecular Biology, November 20-22, Athens, Greece.

Biessmann, H., A.C. Maranhao, E. Andronopoulou, M.R. Biessmann, V. Douris, S.D. Dimitratos, E. Eliopoulos, P.M. Guerin, K. Iatrou, R.W. Justice, T. Kröber, O. Marinotti, P. Tsitoura, D.F. Woods, and M.F. Walter (2010). The *Anopheles gambiae* Odorant Binding Protein 1 (AgamOBP1) mediates indole recognition in the antennae of female mosquitoes. Keystone Symposia Global Health Series: Molecular Targets for Control of Vector-Borne Diseases - Bridging Lab and Field Research. April 11-16, 2010, Copper Mountain, Colorado, USA.

Koussis, K, H Biessmann, F Dani, S Dimitratos, P Guerin, M Konstantopoulou, T Kroeber, P Pelosi, L Swevers, P Tsitoura, M Walter, DF Woods and K Iatrou (2010). Volatile constituents of plant origin bind selectively various odorant binding proteins of the malaria vector *Anopheles gambiae* and act as strong repellents capable of interfering with the vector's host seeking and blood feeding activities. Keystone Symposia Global Health Series: Molecular Targets for Control of Vector-Borne Diseases - Bridging Lab and Field Research. April 11-16, 2010, Copper Mountain, Colorado, USA.

Gotsis-Skretas, O., Ignatiades, L. 2009. The role of 'sporadic' phytoplanktonic species in eutrophic and oligotrophic waters. *9th Panhellenic Symposium in Oceanography and Fisheries, 13-16 May 2009, Patra, Greece*, Book of Abstracts, 1285-1289.

Other Scientific/ Research Activities

Deputy Editor of "The Journal of Insect Science". (K. Iatrou)

Member of the editorial board of "Sericologia", "Insect Biochemistry and Molecular Biology", "Archives of Insect Biochemistry and Physiology", "Open Biotechnology Journal" and "Journal of Biomedicine and Biotechnology" (K. Iatrou)
Member of the editorial board of "Archives of Insect Biochemistry and Physiology" (L. Swevers)
Referee for "Insect Biochemistry and Molecular Biology", "Insect Molecular Biology", "Journal of Insect Science", "Sericologia", "PLoS ONE", "BMC Physiology", "FEBS Journal","Journal of Molecular Biology", "Journal of Biotechnology" (K. Iatrou)

Referee for "Insect Biochemistry and Molecular Biology"(3x), "Insect Molecular Biology", "BMC Genomics", "Comparative Biochemistry and Physiology Part B", "Archives of Insect Biochemistry and Molecular Biology", "FEBS Journal" (L. Swevers)

Referee for "Marine Ecology", "Mediterranean Marine Science Journal" and "Limnology and Oceanography" (L. Ignatiadou)

Deputy Member of the Convention of the National Council of Research and Technology (ESET) (K. Iatrou)

Deputy Member of the Sector on Biology and Biotechnology (TES) of the National Council of Research and Technology (K. Iatrou)

Participation in the work group for the coordination of the European research effort for the "Control of malaria through the control of mosquito vector populations in Africa", Imperial College, London, UK, September 2, 2009 (K. Iatrou)

Invited speaker at the 12th Annual Baculovirus & Insect Cell Culture meeting on «Baculovirus knockout and transposition mutants as gene transfer vectors for mammalian cells: toward safer gene therapy and more efficient protein expression vectors», San Antonio, Texas, USA, February 1-4, 2009 (K. Iatrou)

Invited speaker at the Joint workshop of the EU Commission (RTD F3 & B3) and the Imperial College of London (INFRAVEC) on "EU-funded Research for Malaria Vector Control", London, UK, 15-16 September, 2009 (K. Iatrou)

Organizer of the 8th International Workshop on the "Molecular Biology and Genetics of Lepidoptera", Kolymbari, Crete, Greece, August 23-29, 2009 (K. Iatrou)

Invited speaker at the International Symposium on "Bombyx mori Functional Genomics and Modern Silk Road", October 23-25, 2009, Chongqing, China (K. Iatrou)

Invited visiting professor and speaker at the Institute of Sericulture and Apiculture, College of Animal Sciences, Zhejiang University, Hangzhou, China, October 26-November 1, 2009 (K. Iatrou)

Participation in the 12-member committee of the European Union for the drawing of the Guide for the maintenance of the quality of the European Sea Environment with title "Marine Strategy Framework Directive Guidance" Eutrophication Quality Descriptor 2009-2010 (L. Ignatiadou)

Participation at the First Meeting of the EU-Eutrophication Task Group for preparation of Directive concerning the protection the European Marine Environment, Ispra, Italy, 28-29 May 2009. (L. Ignatiadou)

Participation at the Second Meeting of the EU- Eutrophication Task Group for preparation of Directive concerning the protection the European Marine Environment, Lisbon, Spain, 19-20 October, Barcelona, Spain. (L. Ignatiadou)

Visiting Scientist, Faculty of Bioscience Engineering, Department Crop Protection, Ghent University, Ghent, Belgium. September-December 2009 (L. Swevers)

Scholarship, Foreign Researcher, Special Research Fund, University of Ghent (L. Swevers)

Participation in common research program with duration of 4 years (1/2009-12/2012) with title "Key mechanisms of systemic RNA interference (RNAi) in insects" that is funded in Belgium by FWO

Vlaanderen (project F 6/12) by 1.170.200 € (collaboration with Dr. G. Smagghe, University of Ghent and Dr. J. Vanden Broeck, Catholic University of Leuven) (L. Swevers)

Referee for postdoctoral scholarships of the Funds for Scientific Research – Flanders (FWO Vlaanderen), Belgium (L. Swevers)

Referee for postdoctoral scholarship of the Keeley Visiting Fellowships, Oxford University, UK (L. Swevers)

Member of the Scientific Organizing Committee, 8th International Workshop on the Molecular Biology and Genetics of Lepidoptera, Kolympari, Crete, Greece, August 23-29 2009 (L. Swevers)

External Referee of the Evaluation Committee for the Ph.D. thesis of Hadi Mosallanejad: "Resistance mechanisms for methoxyfenozide: *in vitro* and *in vivo* approaches" (Applied Biological Sciences, April 2009, Faculty of Bioscience Engineering, Department Crop Protection, Ghent University, Ghent, Belgium) (L. Swevers)

External Referee of the Evaluation Committee for the Ph.D. thesis of Thomas Soin: "The ecdysone receptor in insects and crustaceans: from fundamentals to endocrine disruption" (Applied Biological Sciences, June 2009, Faculty of Bioscience Engineering, Department Crop Protection, Ghent University, Ghent, Belgium) (L. Swevers)

Member of the Advisory Committee for the Ph.D. thesis of Sotiris Tsatsarounis, National and Kapodistrian University of Athens (L. Swevers)

Educational Activities

Two-hour presentation with title "Engineering of Baculovirus Vectors for protein production and cellular transformation" at the summer school of NCSR "Demokritos" (L. Swevers)

Other Activities for the Institute of Biology

Deputy member of the committee for recruitment of researchers (K. Iatrou)

Member of the Education Committee (L. Swevers)

Member of the Examination Committee for the recruitment of new graduate students at the IB (Biology) (K. Iatrou)

Supervision of the Ph.D. thesis of the graduate student Theodoros Georgomanolis at the IB (University of Athens) (K. Iatrou)

Supervision of the Ph.D. thesis of the graduate student Konstantinos Ioannidis at the IB (University of Athens) (K. Iatrou)

Supervision of the Ph.D. thesis of the graduate student Christiana Magkrioti at the IB (University of Athens) (K. Iatrou)

Member of the Examination Committee for the recruitment of new graduate students at the IB (Biology) (L. Swevers)

Co-supervision of the Ph.D. thesis of the graduate student Theodoros Georgomanolis at the IB (University of Athens) (L. Swevers)

Co-supervision of the Ph.D. thesis of the graduate student Konstantinos Ioannidis at the IB (University of Athens) (L. Swevers)

Member of the Internal Committee for supervision of graduate students with scholarship from NCSR "Demokritos" at the IB: Sophia Aliberti, Christiana Magkrioti, Konstantinos Ioannidis, Maria Papakonstandinou (L. Swevers)

Responsible for the functioning of the following instruments: Fluostar Microplate Fluorometer, HPLC Hewlett Packard, microplate luminometer TECAN InfiniTE M-200 (L. Swevers)

Member of the Examination Committee for the recruitment of new graduate students at the IB (Biochemistry) (V. Labropoulou)

Co-supervision of the Ph.D. thesis of the graduate student Christiana Magkrioti at the IB (University of Athens) (V. Labropoulou)

Member of the committee for organization of seminars at the IB (V. Labropoulou)

Impact Factors (for 3 publications): 6,959

Citations 2009 (without self- citations): 163
Iatrou K. (including citations with L. Swevers and V. Labropoulou): 74
Swevers L. (including 41 citations with K. Iatrou): 46
Labropoulou V. (including 12 citations with K. Iatrou and L. Swevers): 45
Ignatiadou L.: 52

Total Citations 2006-2009 (without self- citations): 681
Iatrou K. (including citations with L. Swevers and V. Labropoulou): 251
Swevers L. (including 119 citations with K. Iatrou): 133
Labropoulou V. (including 24 citations with K. Iatrou and L. Swevers): 181
Ignatiadou L.: 181

h-factor:
22 (K. Iatrou)
13 (L. Swevers)
8 (V. Lampropoulou)
15 (L. Ignatiadou)

Current External Funding

EPAN project entitled *Baculovirus Artificial Chromosomes (BVACs) and technologies for gene therapy and continuous high-level expression of therapeutic proteins in insect production systems*, funded by GSRT (Coordinator K. Iatrou).
Duration: 1/2004-1/2008
Total funding (lab): 391.000 €
Funding of the lab for 2009: 40.920 €.

Bilateral Collaboration Greece-USA entitled *The Olfactory Odorant Binding Proteins of the Malaria Mosquito Anopheles gambiae as Targets for Vector Control*, funded by GSRT (Coordinator K. Iatrou).
Duration: 9/2004-5/2007
Total funding (lab): 162.000 €
Funding of the lab for 2009: 9.250 €.

PENED 2005 project entitled *Mechanisms of immunosuppression in lepidopteran insects following parasitization by hymenopteran endoparasitoids: the role of the interactions between proteins produced by hymenopteran endosymbiotic polydna viruses and hemocyte proteins of the lepidopteran hosts*, funded by GSRT (Coordinator K. Iatrou).
Duration: 3/2006-3/2009
Total funding (lab): 57.600 €
Funding of the lab for 2009: 0 €.

PENED 2005 project entitled *Regulatory mechanisms that govern oogenesis in Lepidopteran insects: identification and functional characterization of factors that play a key role during successive stages of oogenesis in the silkworm Bombyx mori*, funded by GSRT (Coordinator L. Swevers).
Duration: 3/2006-3/2009
Total funding (lab): 23.080€
Funding of the lab for 2009: 3.641 €.

Bilateral Collaboration Greece-Japan entitled *Insect cell-based high-throughput screening systems for the identification of compounds with ecdysteroid mimetic and order-specific insecticide activities in synthetic libraries*, funded by GSRT GSRT (Coordinator L. Swevers).
Duration: 3/2006-3/2008
Total funding (lab): 60.000 €
Funding of the lab for 2009: 8.600 €.

Project entitled *ENAROMaTIC - European Network for Advanced Research on Olfaction for Malaria Transmitting Insect Control*, funded by the European Union GSRT (Coordinator K. Iatrou).
Duration: 12/2008-12/2012
Total funding (consortium): 2.500.000 €
Total funding (lab): 406.706 €
Funding of the lab for 2009: 4.710 €

Note: The following proposals have been submitted and are under evaluation for the program "Thalis" as "Central Research Group":

1. Title: Prostaglandin receptors during insect development – study of signal transduction and isolation of agonists and antagonists in natural products. Coordinator: S. Dedos, University of Athens (Coordinating IB scientist: K. Iatrou).
2. Title: Role of the insulin signal transduction cascade and the FOXO transcription factor in the regulation of lifespan and metabolism during diapause of lepidopteran insects. Coordinator: A. Kourti, Agricultural University of Athens (Coordinating IB scientist: K. Iatrou).
3. Title: Genomic and functional approach to understand the resistance of insects and mites against insecticides and development of applications for its management. Coordinator: J. Vontas, University of Crete (Coordinating IB scientist: K. Iatrou).

The laboratory also participates in the "Thalis" program as member of a "Central Research Group":

Title: Regulation of cellular signalling by autophagy and heat-shock proteins after exposure to ionizing radiation. Coordinator: E. Sivridis, University of Thrace (Coordinating IB scientist: N. Grammatikakis) (V. Labropoulou).

Finally, the laboratory participates in the proposal for funding of a research consortium w entitled *NIMVeCTA (New and Improved Malaria Vector Control in Africa)* that was submitted to the European Union under the call: HEALTH.2010.2.3.2-4: Controlling Malaria by Hitting the Vector: New or Improved Vector Control Tools. FP7–CALL–FOR-AFRICA-2010. Coordinator: Fulvio Esposito, University of Camerino, Italy (Coordinator for Greece: K. Iatrou).

Research Group: *Pending (Collaboration with the laboratory of Molecular Genetics of Insects and Biotechnology –In charge: Dr. K. Iatrou)*

Research Staff
Maria Konstantopoulou, Researcher
Elias Siskos, Postodoctoral Fellow
Kelly Martinou, Postdoctoral Fellow
Anatsassia Pantazi – Mazomenou, Research Technician

Research Interests

- Chemical ecology: isolation and identification of biologically active substances, relating to the chemical communication of insects and to plant – insect interaction (pheromones, volatile compounds of plant origin etc.) that may be used in integrated pest management programs.
- Isolation and identification of secondary metabolites (mainly of plant origin) acting on insect physiology and/or behavior (behavior modifying agents - infochemicals). Laboratory and field evaluation of bioactivity of the isolated metabolites; study of their mode of action.
- Development of specialized chemical attractants for insects and technologies for their application in pest population control.
- Biochemistry of insect olfactory receptors with emphasis on the localization and isolation of protein receptors of semiochemicals.
- Endosymbiotic bacteria of insects: Isolation and studies on their mutualistic relations with the host insects.
- Microorganisms and Biotechnology: Isolation of naturally occurring microorganisms and their biologically active secondary metabolites (toxins) aiming to incorporate them in insect population management.

2009 Findings

In the context of the EC funded project ENAROMaTIC (a collaboration with the Laboratory of "Insect Molecular Genetics and Biotechnology", Prof. K. Iatrou), 200 endemic botanical species were sampled from botanical gardens and various regions in Greece. Steam distillation technique in a modified Clevenger apparatus was employed to extract their essential oils. The crude extracts were tested with the high throughput screening assay for OBPs ligand identification. Extracts exhibiting positive response were fractionated by means of solid phase extraction using increasing polarity solvents. Fractions were evaluated for biological activity. Gas chromatography was employed to identify differences in eluates between crude and their derived fractions. A tentative identification of biologically active compounds was done using Mass Spectroscopy. The experimental procedure is currently under way aiming to isolate and identify substances with repellant or attractive action.

The effect of the secondary metabolites produced by the entomotoxic strain (SMU-21) of *Mucor hiemalis* isolated from natural insect populations was studied on Hemiptera (*Aphis*) and Diptera (*Culex*) insects. Toxicity levels of the bioactive secondary metabolites, mode of action and the most efficient isolation techniques were evaluated. The chemical characterization of the toxins is under way using up-to-date organology (HPLC, NMR, FAB-MS, FTIR).

Aiming to identify semiochemicals with kairomonal activity on parasitoids that may increase egg-parasitism in IPM programmes, the effect of *Prays oleae* sex pheromone was tested on *Trichogramma* behavior. Using a Y type olfactometer, three different *Trichogramma* species were tested for their ability to detect the pheromone of their natural host.

Mating disruption was deployed in olive orchards in Egypt against the *Zeuzera pyrina*, using pheromone dispensers emitting the insect's pheromone at the optimum concentration. Monitoring and mass trapping methods against *Prays oleae* and *Palpita unionalis* using pheromone traps were developed and applied in Egypt.

2009 Publications

Hegazi E.M., M.A. Konstantopoulou, A. Herz, B.E. Mazomenos, W.E. Khafagi, E. Agamy, A. Zaitun, G.M. Abd El-Aziz, S. Showiel, S.M. Abdel-Rahman, (2009). Is mating disruption effective in controlling the olive moth, *Prays oleae*? Crop Prot. 28, 181-189. DOI: 10.1016/j.cropro.2008.10.005.

Siskos E.P., M.A. Konstantopoulou and B.E. Mazomenos, (2009). Insecticidal activity of *Citrus aurantium* peel extract against of *Bactrocera oleae* and *Ceratitis capitata* adults (Diptera: Tephritidae). J. Appl. Entomol. 133, 108-116, doi: 10.1111/j.1439-0418.2008.01312.x.

Milonas P., B.E. Mazomenos, and M. Konstantopoulou, (2009). Kairomonal effect of sex pheromone components of two Lepidopteran olive pests on *Trichogramma* wasps. Insect Sci. 16, 131-136, DOI 10.1111/j.1744-7917.2009.00264.x.

Hegazi, E., Khafagi, W. E., Konstantopoulou, M., Raptopoulos, D., Tawfik, H., Abd El-Aziz, G. M., Abd El-Rahman, S. M., Atwa, A., Aggamy, E., Showeil, S. Efficient Mass-Trapping Method as an Alternative Tactic for Suppressing Populations of the Leopard Moth, (Lepidoptera: Cossidae), (2009). Ann. Entomol. Soc. America 102(5), 809-818.

Milonas P. G., A.F. Martinou, D. Ch. Kontodimas, F. Karamaouna, and M. A. Konstantopoulou, (2009). Attraction of Different *Trichogramma* Species to Olive Moth Sex Pheromone. Ann. Entomol. Soc. America 102(6), 1145-1150.

2009 Presentations at Scientific Conferences

Hegazi E.M., W.E. Khafagi, M.A. Konstantopoulou, H. Tawfik, G.M. Abd El-Aziz, E. Agamy, S.M. Abdel-Rahman, S. Showiel, A.A.Atwa , S. Showeil (2009). Seasonality in the occurrence of two Lepidopterous olive pests in two different olive growing zones, in Egypt. 4th European Meeting of the IOBC/WPRS Working Group "Integrated Protection of Olive Crops", June 1-4. Cordoba, Spain, 2009. Abstract: 75

E. Hegazi. W. E. Khafagi. M. Konstantopoulou, D. Raptopoulos, H. Tawfik. G. M. Abd El-Aziz. S.M. Abd El-Rahman, A.A.Atwa, E. Agamy, and S. Showeil, (2009). Efficient Mass-Trapping Method as an Alternative Tactic for Suppressing the Population of the Leopard Moth, *Zeuzera pyrina*. 4th European Meeting of the IOBC/WPRS Working Group "Integrated Protection of Olive Crops", June 1-4. Cordoba, Spain, (2009). Abstract: 76

Tsitoura, P., Koussis, K., Konstantopoulou, M., Swevers, L., and Iatrou, K. (2009). Toward the functional characterization of mosquito smell components in lepidopteran tissue culture cells. 8th International Workshop on the Molecular Biology and Genetics of the Lepidoptera, August 23-29, Orthodox Academy of Crete, Kolympari, Crete, Greece.

Koussis, K, H Biessmann, F Dani, S Dimitratos, P Guerin, M Konstantopoulou, T Kroeber, P Pelosi, L Swevers, P Tsitoura, M Walter, DF Woods and K Iatrou (2010). Volatile constituents of plant origin bind selectively various odorant binding proteins of the malaria vector *Anopheles gambiae* and act as strong repellents capable of interfering with the vector's host seeking and blood feeding activities. Keystone Symposia Global Health Series: Molecular Targets for Control of Vector-Borne Diseases - Bridging Lab and Field Research. April 11-16, 2010, Copper Mountain, Colorado, USA

Siskos E. and Konstantopoulou M. (2009). Insecticidal activity of *Citrus aurantium* secondary metabolites. 110 Panhellenic Congress of Hellenic Botanical Society, Athens, Greece, 8-11 October. Abstract: 150.

Milonas P., A. Martinou, D. Kontodimas, F. Karamaouna and M. Konstantopoulou (2009). Attraction of different *Trichogramma* species to *Prays oleae* sex pheromone (Lepidoptera: Hyponomeutidae). 13o Panhellenic Congress of Hellenic Entomological Society, Alexadroupolis, 3-6 November, Proceedings: 268-269.

Other Research Activities

Member of the Examining Board to judge the doctoral thesis of Patricia Acin Viu, Faculty of Biology, University of Barcelona, Spain.

Reviewer of the following international scientific journals (I.F. 2008): Chemosphere (I.F.=2.297), Journal of Agricultural and Food chemistry (I.F.=2.562), Entomologia Experimentalis et Applicata (I.F.=1.248), Bulletin of Insectology, Journal of Applied Entomology (I.F.=0.703), Crop Protection (IF=1.201).

Member of the editorial board of the scientific journal "Tunisien Journal of Plant Protection" specialist for the Chemical Ecology issues.

Other Activities for the Institute of Biology

Responsible for radioprotection of the radioactive source Co-60, with activity 5470 Ci (March 2004-).

Impact Factors (for 5 publications): 5,477

Citations 2009 (without self- citations): 25

Total Citations 2006-2009 (without self- citations): 71

h-factor: 6

Current External Funding

Participation in the EU Research project entitled *ENAROMaTIC - European Network for Advanced Research on Olfaction for Malaria Transmitting Insect Control* (Coordinator: Prof. K. Iatrou).
Duration: 12/2008-12/2012
Total project funds: 2.500.000 €
Total funds for the Coordinator's laboratory (2009): 406.706 €
Funds (2009) for the laboratory derive through the total Coordinator's funds

Research submitted proposals

Three proposals were submitted in the action of "Thales" - Enhancing interdisciplinary and multidisciplinary research and innovation with the potential to attract high level researchers from abroad by conducting basic and applied research excellence. Ministry for Education Lifelong Learning and Religion:

- Title: Molecular mechanisms of florescence and bioactive secondary metabolites production of *Eruca sativa*. Coordinator: K. Papadopoulou, University of Thessaly (Research partner from Institute of Biology: M. Konstantopoulou)
- Title: Prostaglandin receptors for insect development. Coordinator: D. Skarlatos, National and Kapodistrian University of Athens (Research partner from Institute of Biology: Prof. K. Iatrou)
- Title: Genomic and functional analysis for insect and acari resistance to insecticides Coordinator: J. Vontas, University of Crete (Research partner from Institute of Biology: Prof. K. Iatrou)

Two proposals were submitted in the action "Innovation Vouchers for Small and Medium Enterprises", Secretariat for Research and Technology (GSRT) of the Ministry for Education Lifelong Learning and Religion:

- Research and development of innovative tools for palm pest management, *Rhynhophorous ferrugineus* (Coleoptera: Curculionidae).
- Development of ecological and innovative technologies for the population management of pine processionary moth, *Thaumetopoea pityocampa,* in urban and suburban environments

Participation in the research proposal entitled NIMVeCTA (New and Improved Malaria Vector Control in Africa) which was submitted in the HEALTH.2010.2.3.2-4: Controlling Malaria by Hitting

the Vector: New or Improved Vector Control Tools. FP7–CALL–FOR-AFRICA-2010. Coordinator: Fulvio Esposito, University of Camerino, Italy (Project Greek partner: Prof. K. Iatrou).

Research Group: Chronobiology

Research Staff
Anastassia Prombona, Senior Researcher
Anastasia Repouskou, Graduate Student
Aggeliki Galeou, Graduate Student
Dafni Antoniou, Undergraduate Student

Research Interests

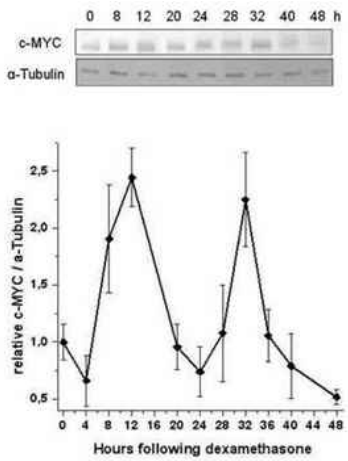
Investigation of the biological clock function in plants
Study of rhythmically expressed genes in *Phaseolus vulgaris*. Regulation of genes involved in the synchronization of the biological clock by input light signals and photoperiodism. Role of rhythmically expressed genes in the central oscillator function. Interaction of clock proteins.

Involvement of the biological clock function in carcinogenesis
Regulation of cell cycle and cell proliferation by the biological clock in mouse fibroblasts and cancer cell lines. Study of the effects of modulated acetylation levels on the biological clock function and the cell cycle. Elucidation of the role of the circadian time in proliferation of cancer cells during application of drugs (chronotherapy).

2009 Findings

Investigation of the biological clock function in plants
A. Galeou has isolated during her master's research a fragment of 900 bp from the 5' upstream region of the *PvTOC1* ORF. In order to identify any promoter elements contained in this region, the cloning of the complete 900 bp region and of truncated fragments of it upstream of GFP (green fluorescent protein) is in progress. In transient expression assays in *Phaseolus* protoplasts the putative regulatory elements will be tested. In addition, synchronization experiments by light application at the dark phase of the photoperiod indicate a role of *PvTOC1* in light-regulation of the biological clock in *Phaseolus.*

Involvement of the biological clock function in carcinogenesis
Our study (PhD thesis of A. Repouskou) focused on the study of gene expression of *Per1,* the central oscillator component of the mammalian biological clock and of *c-Myc*, because of its importance in the progression of the cell cycle (G_0/G_1 transition) and in carcinogenesis. N2A mouse neuroblastoma cell cultures exhibited oscillating (24-hour rhythm) *c-Myc* transcript and protein levels under serum starvation, whereas serum addition to the culture medium abolished the circadian rhythmicity of *c-Myc*, but not of *Per1*. Moreover, the role of trichostatin A (TSA, a specific inhibitor of histone deacetylases) in *c-Myc* transcript and protein levels was investigated after its application at different circadian times. Our results show that a low TSA dose (2-hour treatment at 0.33 μM) is adequate to decrease *c-myc* transcript and protein levels, if it is applied at specific circadian time(s) (Repouskou et al., 2010).



Τα επίπεδα της c-MYC ογκοπρωτεΐνης συσσωρεύονται ρυθμικά στη διάρκεια του 24-ώρου
Repouskou et al., 2010

This project is carried out in collaboration with Dr. T. Sourlingas ('Nuclear Proteins and Chromatin Function' group).

Articles in Press

Repouskou, A, Sourlingas, T.G., Sekeri-Pataryas, K.E., Prombona, A. The circadian expression of c-Myc is modulated by the histone deacetylase inhibitor trichostatin a in synchronized murine neuroblastoma cells. *Chronobiology Int.*, in press. (IF: 3.987).

2009 Presentations at Conferences

A. Repouskou, K.E. Sekeri-Pataryas, T.G. Sourlingas, A. Prombona (2009). c-MYC expression is regulated by the circadian clock and displays a phase-dependent response to trichostatin A. 60th Meeting of the Hellenic Society for Biochemistry and Molecular Biology, Athens, 20 -22 November 2009 (Oral Presentation), Abstract book p. 72.

Other Activities for the Institute of Biology

Member and Secretary of the Scientific Advisory Board of I.B.

Citations 2009 (without self- citations): 2

Total Citations 2006-2009 (without self- citations): 31

h-factor: 5

Participation in 2 "Thalis" program proposals:

1. Program Title: "Non-ionizing electromagnetic radiations: biological impact", University of Athens
2. "Investigation of the role of microRNAs in plant development and resistance by genomic approaches", Agricultural University of Athens

Research Group: Microbial Molecular Genetics

Research Staff
Vassiliki Sophianopoulou, Research Director
Eleftherios Sideris, Emeritus Scientist
Dimitris Lagos, Postdoctoral Fellow
Katerina Roumelioti, Graduate Student – *Phd obtained in 2009*
Dimitra Bouzarelou, Graduate Student
Ioannis Vaggelatos, Graduate Student
George Krezias, Undergraduate Student
Vassiliki, Pantazopoulou, Undergraduate Student
Andreas Panopoulos, Training Graduate Student

Research Interests

The last 13 years we have developed the genetic and molecular tools that introduce the fungus *Aspergillus nidulans (Figure 1)* as a model organism for studying the molecular mechanisms of amino acid recognition, transport and metabolism.

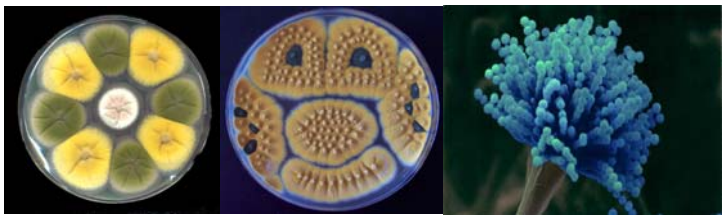


Figure 1: Ο μύκητας *Aspergillus nidulans*

The last 5 years we have used the fresh-water cyanobacterium *Synechococcus elongatus* as a biological system for studying the molecular mechanisms of Na^+/H^+ homeostasis.

A. ***Transporters of medical, pharmacological and agricultural importance***

Recognition and transport of amino acid-neurotransmitters (proline, glutamate), through cellular membranes via specific transmembrane transporters

Activities:
a) identification and regulation of the expression of genes encoding amino acid transporters b) isolation and characterization of factors that regulate directly or indirectly the activity of amino acid transporters ie *trans*-acting molecular determinants involved in topogenesis/recycling/endocytosis of amino acid transport systems (eisosomal proteins, CKI kinases, aldolases) and c) studies on structure-function relationships of amino acid transporters.

Intermediate and Long-term objectives: better understanding of the molecular basis of neurodegenerative diseases, possible identification of new pharmaceutical targets and future development of highly-targeted drugs.

B. ***Study of the molecular mechanisms involved in modification of fungal cell wall***

Activities: a) identification and regulation of the expression of genes encoding putative expansin-like protein(s) and cell wall enzymes in *Aspergillus nidulans* b) functional, developmental and physiological characterization of their products.

Intermediate and long-term objectives*:* novel biotechnological means with fungicidal activity, development of novel enzymatic applications, ial applications in food industry and environmental sciences (bio-furling agro-waste treatment industr).

C. ***Study of the molecular mechanisms involved in salt tolerance of fresh-water cyabobacteria***

Activities: Identification of a network of genes encoding putative Na^+/H^+ antiporters in the freshwater cyanobacterium *Synechococcus elongatus* and functional characterization of their products.

Intermediate and long-term objectives: understanding the molecular mechanisms involved in adaptation and acclimation of freshwater organisms to salt and pH tolerance in order to highlight features that might be applicable to plants of economical importance.

2009 Findings

a) We identified *agtA*, a gene encoding the specific dicarboxylic amino acid transporter of *Aspergillus nidulans*. The deletion of the gene resulted in loss of utilisation of aspartate as nitrogen source and of aspartate uptake, while not abolishing completely glutamate utilisation. Kinetic constants showed that AgtA is a high affinity dicarboxylic amino acid transporter. The gene is extremely sensitive to nitrogen metabolite repression, depends on AreA for its expression and is seemingly independent from specific induction. We showed that the localisation of AgtA in the plasma membrane necessitates the ShrA protein and that an active process elicited by ammonium results in internalisation and targeting of AgtA (and PrnB the main proline transporter) to the vacuole followed by degradation. Thus, nitrogen metabolite repression and ammonium-promoted vacuolar degradation act in concert to downregulate dicarboxylic amino acid transport activity.

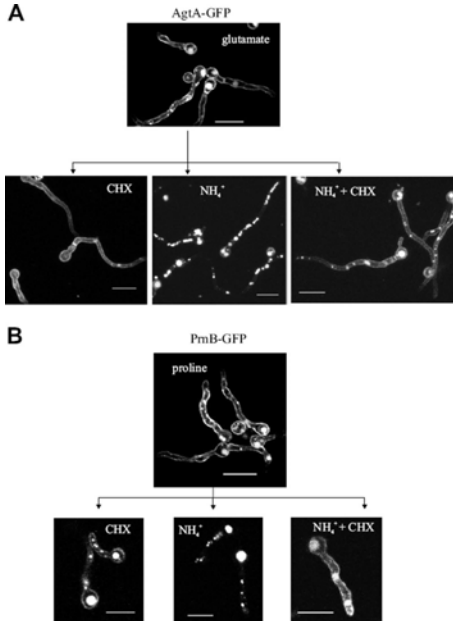


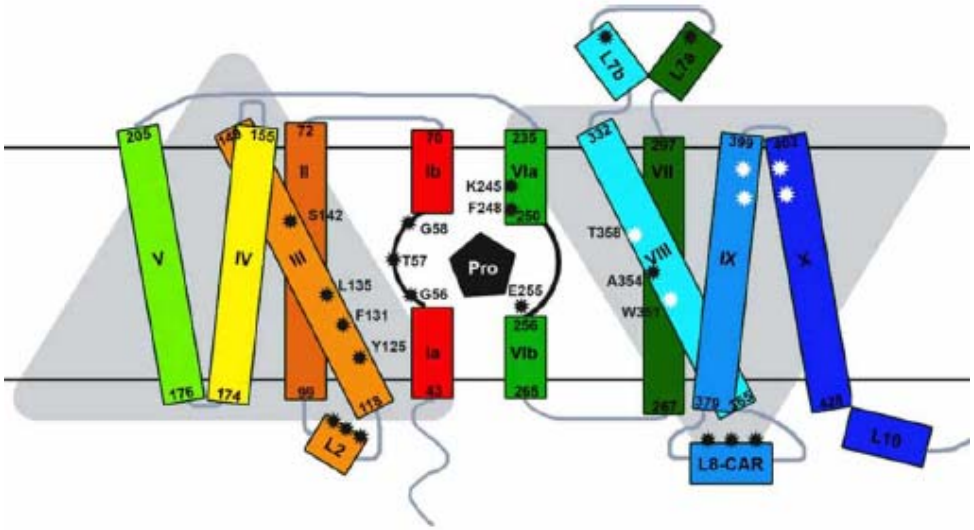
Figure 2
Protein synthesis is necessary for ammonium-dependent AgtA and PrnB internalization. Shown is the subcellular localization of AgtA and PrnB proteins in young hyphae of *A. nidulans* strains under conditions that inhibit protein synthesis. Representative pictures from LSCM (laser scanning confocal microscopy) show conidiospores of a wild-type strain that expresses functional AgtA-GFP (A) or PrnB-GFP (B) molecules grown in the presence of 10 mM glutamate as the sole nitrogen source, followed by the addition of 10 mM ammonium tartrate (NH4, 0.1 mg/ml cycloheximide (CHX), or both (NH4 - CHX) for the last 30 min of growth (A), or in the presence of 10 mM proline as the sole nitrogen source, followed by the addition of 10 mM ammonium tartrate (NH4, 0.1 mg/ml cycloheximide (CHX), or both (NH4 - CHX) for the last 30 min of growth (B). Bar 5 μM

b) We found that fructose 1,6-biphosphate aldolase (FBA), an enzyme involved in both glycolysis and gluconeogenesis, is involved in the regulation of amino acids transporters. In particular, in *Aspergillus nidulans* the *fbaA1013* mutation results in reduced or total loss of growth on glycolytic and gluconeogenic carbon sources, respectively. It also negatively affects growth on several amino acids (including L-proline, L-glutamate or L-aspartate) that the fungus can use as nitrogen source on glycolytic carbon sources. Complementation of the *fbaA1013* mutation using an *A. nidulans* genomic library resulted in cloning of the *fbaA* gene, which encodes a putative FBA protein. The *fbaA1013* mutation is a chromosome rearrangement in the 5′ regulatory region of the *fbaA* gene resulting in reduced or total loss of transcription in response to glycolytic and gluconeogenic carbon sources respectively. The *fbaA* gene is essential for growth. A functional FbaA protein is necessary for plasma membrane localization of the AgtA acidic amino acid (L-glutamate/L-aspartate) transporter, as the *fbaA1013* mutation results in targeting to and presumably subsequent degradation of AgtA in the vacuole. Thus, our results support a novel role of the FbaA protein that is, involvement in the regulation of amino acids transporters.

c) In an attempt to find possible substrates of a CKI (casein kinase I) protein of *Aspergillus nidulans*, point mutation alleles of which result in disruption of amino acid transporter topogenesis we have identified eisosomal proteins in filamentous fungi. Eisosomes are sub-cortical organelles hitherto described in *Saccharomyces cerevisiæ*. They comprise two homologue proteins, Pil1 and Lsp1, which colocalise with the transmembrane protein Sur7. These proteins are universally conserved in the

ascomycetes. We identify in *Aspergillus nidulans* (and in all the Pezizomycotina) two homologues of Pil1/Lsp1, PilA and PilB, originating from a duplication independent from that extant in the Saccharomycotina. In the Aspergilli there are three clades of Sur7-like proteins, with one strict orthologue of Sur7 in each species (SurG in *A. nidulans*). In *A. nidulans* conidiospores, as in *S. cerevisiæ*, the three proteins colocalise at the cell cortex. These structures are assembled late during the maturation of conidia. In mycelia, punctuated structures are present, but they are composed only of PilA, while PilB is diffused in the cytoplasm and SurG is located in vacuoles and endosomes. Deletion of each of the genes does not result in any obvious growth phenotype, except for a moderate resistance to itraconazole. Deletion of *pilA* results in larger, discrete peripheral PilB spots and considerable loss of SurG peripheral localization, while deletion of *surG* results in the collapse of PilB patches into large clusters.

d) The Amino acid-Polyamine-Organocation (APC) superfamily is the main family of amino acid transporters found in all domains of life and one of the largest families of secondary transporters. Using a sensitive homology threading approach and modelling we showed that the predicted structure of APC (Amino acid-Polyamine-Organocation) members is extremely similar to the crystal structures of several prokaryotic transporters belonging to evolutionary distinct protein families with different substrate specificities. All of these proteins, despite having no primary amino acid sequence similarity, share a similar structural core, consisting of two V-shaped domains of five transmembrane domains each, intertwined in an antiparallel topology. Based on this model, we reviewed available data on functional mutations in bacterial, fungal and mammalian APCs and obtained novel mutational data, which provide compelling evidence that the amino acid binding pocket is located in the vicinity of the unwound part of two broken helices, in a nearly identical position to the structures of similar transporters. Our analysis is fully supported by the evolutionary conservation and specific amino acid substitutions in the proposed substrate binding domains. Furthermore, it allows predictions concerning residues that might be crucial in determining the specificity profile of APC members. Finally, we show that two cytoplasmic loops constitute important functional elements in APCs. Our work along with different kinetic and specificity profiles of APC members in easily manipulated bacterial and fungal model systems could form a unique framework for combining genetic, *in-silico* and structural studies, for understanding the function of one of the most important transporter families.



2009 Publications

Apostolaki, A., Erpapazoglou, Z., Harispe, L., Billini, M., Kafasla, P., Kizis, D., Peñalva, M., Scazzocchio, C., and Sophianopoulou, V. (2009). AgtA, the dicarboxylic amino acid transporter of *Aspergillus nidulans*, is concertedly down-regulated by exquisite sensitivity to nitrogen metabolite repression and ammonium-elicited endocytosis. *Eukaryotic Cell* 8(3): 339-352.

Vangelatos, I., Vlachakis, D., Sophianopoulou, V*., Diallinas, G. (2009). Modelling and mutational evidence identify the substrate binding site and functional elements in APC amino acid transporters. *Mol. Membr. Biol*. 26(5): 356-70.

*corresponding author

Roumelioti, K., Vangelatos, I., and Sophianopoulou, V. (2010). A cryptic role of a glycolytic-gluconeogenic enzyme (aldolase) in amino acid transporter turnover in *Aspergillus nidulans Fungal Genet. Biol.* 47: 254-267.

Articles in Press

Vangelatos, I., Roumelioti, K., Gournas, C., Suarez, T., Scazzocchio, C., Sophianopoulou, V. (2010). Eisosome organisation in the filamentous ascomycete *Aspergillus nidulans Eukaryotic Cell* (6 August, Epub ahead of print).

2009 Articles in Books

M. Billini and V. Sophianopoulou (2009). Isolation and molecular characterization of genes encoding Na^+/H^+ antiporters in the freshwater cyanobacterium *Synechococcus elongatus*. In "Research Advances in Bacteriology" Invited Article (Global Research Network).

2009 Presentations at Scientific Conferences

I. Vangelatos, M. Billini, D. Vlachakis and V. Sophianopoulou, 2009. Molecular modelling study of PrnB, the major proline transporter of *Aspergillus nidulans*. Procceedings of the 34th Febs Congress on Life's Molecular Interaction, Prague, Czech Republic, July 4-8. Febs Letters, Volume 276, Issue S1, Functional Genomics, Proteomics and Bioinformatics, pages 123-124.

V. Sophianopoulou, 2009. Dicarboxylic amino acids transport in *Aspergillus nidulans.* 11th International Congress on amino acids, peptides and proteins. Vienna, August 3-7. Oral presentation.

K. Roumelioti*, I.Vangelatos and V. Sophianopoulou, 2009. A CRYPTIC ROLE OF A GLYCOLYTIC-GLUCONEOGENIC ENZYME IN AMINO ACID TRANSPORTER TURNOVER IN *Aspergillus nidulans.* 60th Panhellenic Congress of the Hellenic Society for Biochemistry and Molecular Biology, Athens, Greece, Abstract book page 44 (selected for oral presentation)

I. Vangelatos, D. Vlachakis,G. Diallinas and V. Sophianopoulou, 2009. MODELLING AND MUTATIONAL EVIDENCE IDENTIFY THE SUBSTRATE BINDING SITE AND FUNCTIONAL ELEMENTS IN APC AMINO ACID TRANSPORTERS. 60th Panhellenic Congress of the Hellenic Society for Biochemistry and Molecular Biology, Athens, Greece, Abstract book page 126.

I. Vangelatos, C. Scazzocchio and V. Sophianopoulou*, 2010. Eisosome organization in the filamentous fungus *Aspergillus nidulans*. Abstract of the IMC9 "The Biology of Fungi", Edinburgh, UK, 1-6 August (Selected for oral presentation).

I. Vangelatos, C. Scazzocchio and V. Sophianopoulou*, 2010. Eisosomes in the filamentous ascomycete *Aspergillus nidulans*. Leopoldina Workshop on Plasma Membrane Domains in Fungi and Plants. Patchy Prague 2010. Prague Czech Republic 10-13 October (Oral presentation).

Other Scientific / Research activities

Editorial Advisor Board Member of "The Open Mycology Journal" (Bentham Science Publishers) (V. Sophianopoulou).

Referee for Applied Microbiology, Molecular Genetics and Genomics (x2) (V. Sophianopoulou).

Invited speaker at the "Leopoldina Workshop on "Plasma Membrane Domains in Fungi and Plants". "Patchy Prague 2010". Prague Czech Republic, 10-13 October 2010 (V. Sophianopoulou).

Invited speaker at the 31th Annual Scientific Conference of the Hellenic Society for Biological Sciences. Presentation with title "Molecular mechanisms of endocytosis: eisosomal proteins in *Aspergillus nidulans"*. Patras Greece, 14-16 May 2009 (V. Sophianopoulou).

Member of the Scientific Organizing Committee of the 60th Panhellenic Congress of the Hellenic Society for Biochemistry and Molecular Biology, Athens Greece, 20-22 November 2009 (V. Sophianopoulou).

Invited speaker at the Department of Biological Chemistry, Medical School, University of Ioannina, Ioannina Greece, 14 February 2009. Presentation with title: "CKI kinases in *Aspergillus nidulans"* (I. Vangelatos).

Member of the Advisory Committee for the Ph.D. Thesis of D. Bouzarelou, National and Kapodistrian University of Athens (V. Sophianopoulou).

Member of the Advisory Committee for the Ph.D. thesis of I. Vangelatos, University of Ioannina (V. Sophianopoulou).

Member of the Advisory and the Evaluation Committee for the Ph.D. thesis of K. Roumelioti, National and Kapodistrian University of Athens (V. Sophianopoulou).

Educational Activities

Four-hour lectures on "Microbial Biotechnology-Model Systems of Molecular Microbiology" a Post-Graduate Course at University of Athens Department of Biology (V. Sophianopoulou).

Presentation with title: "Eisosomes: gates or blocks of endocytosis?" at the summer school of NCSR "Demokritos" (V. Sophianopoulou).

Coordinator of the Post-Graduate Course on "Chromatin structure and regulation of gene expression" at IB (V. Sophianopoulou).

Other Activities for the Institute of Biology (V. Sophianopoulou)

Member of the Scientific Consulting Board at IB

President of the Education Committee at IB

Member of the Education Committee at NCRS Demokritos

Supervision of the Ph.D. thesis of the graduate student K. Roumelioti at the IB (University of Athens). Defence December 2009. Grade "Excellent".

Supervision of the Ph.D. thesis of the graduate student I. Vangelatos at the IB (University of Ioannina)

Supervision of the Ph.D. thesis of the graduate student D. Bouzarelou at the IB (University of Athens)

Supervision of Diploma Thesis of the undergraduate student V. Pantazopoulou at the IB (University of Athens)

Supervision of Diploma Thesis of the undergraduate student G. Krezias at the IB (University of Athens)

Member of the Internal Committee for supervision of graduate students with scholarship from NCSR "Demokritos" at the IB: N. Sdralia.

Member of the Examination Committee for the recruitment of new graduate students at the IB (Biology)

Impact Factor (for 3 publications): 11, 13

Citations 2009 (without self-citations): 27

Total Citations 2006-2009 (without self-citations): 115

h-factor: 11

Current External Funding

PENED 2003 project entitled *Study of CNS transporters: glutamate transporters*, funded by GSRT (Coordinator V. Sophianopoulou).

Duration: 2006-2009
Total funding (lab): 57.600 €
Funding of the lab for 2009: 15.200 €.

PENED 2005 project entitled *Structure-function analysis of purine transporters for the systematic and targeted pharmacological treatment of pathogenic fungi* (Coordinator G. Diallinas, University of Athens).
Duration: 2006-2009
Total funding (lab): 15.000 €
Funding of the lab for 2009: 6.000 €.

FP7-PEOPLE-2009-RG Marie Curie Actions—European Re-integration Grants (ERG) project entitled *Eisosomal proteins in Aspergillus nidulans: regulators of endocytosis, cell wall synthesis, membrane sub-domain organization and cell cycle*.

Responsible scientist: V. Sophianopoulou

Fellow: Dr. Z. Erpapazoglou
Duration: 2011-2013
Total funding (lab): 45.000€
Funding of the lab for 2009: 0 €.

Note:

The following proposal has been submitted and is under evaluation for the program "Thalis" as "Central Research Group":

- Title: *Structure-function relations of bacteria transporters and their eukaryotic homologues* Coordinator: S.Frillingos, University of Ioannina (Coordinating IB scientist: V. Sophianopoulou).

The laboratory also participates in the "Thalis" program as member of a "Central Research Group":

- Title: *Membrane trafficking and homeostasis.* Coordinator: C. Delidakis, Institute of Molecular Biology and Biotechnology (IMBB)/University of Crete (Coordinating IB scientist: V. Sophianopoulou).
- Title: *Development of genetic and genomic tools with Minos transposon and their applications in model organisms*. Coordinator: Ch. Savakis, Biomedical Sciences Research Center Al. Fleming (Coordinating IB scientist: V. Sophianopoulou).

Research Group: Biophysics and Biotechnology of Membranes

Research Staff
Kostas Stamatakis, Senior Researcher
George Papageorgiou, Emeritus Scientist
Meropi Tsimilli – Michael, Collaboratig Scientist
Marianna Triantou, Summer Student

Research Interests

We investigate the time-dependent changes (induction) of chlorophyll a (Chl a) fluorescence in model cyanobacteria and higher plants, with emphasis on the role of carotenoids as photon collectors. In contrast to chlorophylls and phycobilins, the light harvesting mechanism of carotenoids and the supply of electronic excitation from them to the Chls a of the reaction centers of photosystems I and II (PSI, PSII) has not been described in satisfactory detail. Our research focusses on the role of carotenoids in the balanced excitation of the reaction centers of PSI and PSII, so that they turn over at the same rate and the quantum yield of photosynthesis becomes maximized.

Studies on the photosynthetic Hydrogen production.

2009 Findings

Study of Fluorescence induction curves in cyanobacteria
We investigated the dark-to-light transition in *Synechococcus* sp PCC 7942 cells by a detailed analysis of fluorescence transients induced by strong red light. The transients, recorded with high data-acquisition, revealed all the steps of the fast (OJIP; 10-5-1 s) and slow phase (PSM(T); 1-103 s), kinetically distinguished with precision. Focusing on the OJIP-rise, we show, for the first time, how the variable to initial fluorescence ratio and the relative height of J-level can serve as indexes of the plastoquinone redox poise and the established state in the dark; hence, differences among cyanobacteria can be recognized in a simple way. We establish a method by which we determine the fluorescence component not originating from photosystem (PS) II and we assess PSII dynamics during state 2-state 1 transition. The development of photochemical and non-photochemical quenching is also discussed, as well as evidences favouring the mobile antenna model.

2009 Publications

Tsimilli-Michael M, Stamatakis K, Papageorgiou GC (2009) Dark-to-light transition in Synechococcus sp PCC 7942 cells studied by fluorescence kinetics assesses plastoquinone redox poise in the dark and photosystem II fluorescence component and dynamics during state 2 to state 1 transition PHOTOSYNTHESIS RESEARCH 99, 243-255

Björn LO, Papageorgiou GC, Blankenship RE, Govindjee (2009) A viewpoint: Why chlorophyll *a*? Photosynth Res 99:85–98

Kaňa R, Prášil O, Komárek O, Papageorgiou GC, Govindjee (2009) Spectral characteristic of fluorescence induction in a model cyanobacterium, *Synechococcus* sp. (PCC 7942) Biochim Biophys Acta 1787: 1170–1178

Other Research Activities

Books:
Special issue of the journal Current Science on "Global Issues and Photosynthesis (invited editors: Papageorgiou GC, Govindjee, Tripathy BC).

Papageorgiou GC and Govindjee (eds) Chlorophyll Fluorescence: A Signature of Photosynthesis, 2010 (Springer, ISBN 978–90–481–3882–1, e-ISBN: 978–4020–3218–9)

Papageorgiou GC, Stamatakis K (2010, in press) Chlorophyll fluorescence: A sensitive reporter of osmotic properties and permeability properties of cyanobacterial cells. *In*: Bagchi SN, Kleiner D, Mohanty P (eds) Protocolls on Algal and Cyanobacterial Research, Narosha Publishing, New Delhi.

Papageorgiou GC (2010, in press) Fluorescence emission from the photosynthetic apparatus. In: Photosynthesis: A Comprehensive Treatise, Physiology, Biochemistry, Biophysics and Molecular Biology. Eaton-Rye JJ and Tripathy BC (eds), Springer

Referee in research articles:
G. C. Papageorgiou: BMC[1], Current Sci (3), J Photobiol[1], J Plant Physiol [1], PNAS [1], Photosynthetic*α* [6]

Scientific awards:
G. C. Papageorgiou*:*
(a) Honorary member of the Oxygen Club of California.
(b) Associate Editor of Photosynthetica (Springer; print version ISSN: 0300-3604; electronic version ISSN: 1573-9058; *α*πό 12 / 2009)

Invitation from Laboratory of Photosynthesis, Institute of Microbiology, Acad Sciences Czech Republic, for research collaboration with Prášil O, Kaňa R, andje Komárek O, & Govindjee. (Dr. G.C. Papageorgiou)

Member of scientific committee of emeritus researchers in NCSR Demokritos (Gr. G. C. Papageorgiou)

Other Activities for the Institute of Biology

Responsible for the program "Open doors" in Ins. of Biology (K. Stamatakis)

Impact factor (for 3 publications): 8,113

Citations 2009 (without self-citations): 18

Total citations 2006-2009 (without self-citations): 39

h-factor: 6

PROGRAMME C:

STRUCTURAL AND COMPUTATIONAL BIOLOGY

Research Group: Theoritical Biology and Computational Genomics

Research Staff
Yannis Almirantis, Research Director
Spyros Papageorgiou, Emeritus Scientist
Alexandros Klimopoulos, Collaborating Graduate Student *(MSc)*
Yannis Tsiagas, Collaborating Graduate Student *(MSc)*
Labrini Athanassopoulou, Collaborating Graduate Student *(MSc)*
Dimitris Polichronopoulos, Collaborating Graduate Student *(MSc)*

Research Interests

Probabilistic and statistical aspects in genome organization – Non-randomness at several length scales.

- Deviations from randomness at the level of nucleotide n-tuplets. Patterns related to the functionality of genomic regions and to the global genome structure.
- Deviations from randomness at the "middle" length scale, expressed as clustering of similar nucleotides. Use of such approaches for the distinction of coding and non-coding segments.
- Long range correlations and Zipf laws in the genome structure. Power laws in the distribution of exons and of other genomic functional localizations.
- DNA sequences seen as genomic text – Linguistic features in the genome: redundancy – multiple coding – asymmetry etc.
- "Conservation laws" at the genome structure. The case of "Chargaff's 2nd parity rule". The use of deviations from this law in the study of genomic dynamics and evolution.
- Evolution at the genomic level. Formulation of minimal evolutionary scenarios compatible with the observed probabilistic features of genomes. Interpretation of the above mentioned probabilistic features either by selectionist or mutationist causality.

Pattern formation in biological systems – Self-organization and evolution.

- Early development – Left-right asymmetries – Mechanisms of activation of Hox genes during limb development.
- Reaction-diffusion systems – Spontaneous symmetry breaking and pattern-formation in systems with feedbacks.
- Prebiotic and early evolution as a complex self-organization procedure.

2009 Findings

Repetitive DNA sequences derived from transposable elements (TEs) are often distributed in a highly non-random way, showing co-clustering with other classes of repeated elements, genes, segmental duplications and other genomic components. They may also exhibit GC-content preferences. We investigate the large-scale features of the spatial arrangement of all principal repeat classes in genomes from distant organisms, by studying the size distribution of inter-repeat distances. Power-law like size distributions (linearity in double-log scale) are observed in several cases. In a previous work we have reported such distributions by studying the spatial arrangement of Alu and LINE1 elements in the human genome, while here we find that this property is widespread in several genomes, with non-LTR retroelement populations often giving rise to the most extended power-laws. A simple evolutionary scenario, formulated initially for the explanation of power-laws observed in retroelement distributions in the human genome is extended here, in order to incorporate segmental duplications and cut-and-paste events (characteristic of the DNA-elements' life-cycle). Simulations using this model reproduce the main features of the genomic size distributions presented herein. On these grounds the dependence of power-law appearance and extent on several factors like: type of the considered repeat class (SINE vs LINE, retroelement vs. DNA-element), age, rate of subsequently inserted repeats in the genomic region under examination and some specific features of the genome, e.g. high deletion rates or propensity to recombination events, may be justified.

2009 Publications

Papageorgiou S. (2009). A biophysical mechanism may control the collinearity of Hoxd genes during the early phase of limb development Hum. Genomics 3, 275-280.

Sellis D., Almirantis Y. (2009). Power-laws in the genomic distribution of coding segments in several organisms: an evolutionary trace of segmental duplications, possible paleopolyploidy and gene loss. Gene 447, 18-28.

2009 Presentations at Conferences

S. Papageorgiou 'An explanation of the puzzling *Hox* gene collinearity' 4th Conference of the Hellenic Society for Computational Biology and Bioinformatics, 18-20 December 2009, NHRF, Athens.

Educational Activities

Teaching in the framework of the postgraduate program "Bioinformatics", the course "Introduction to Computational Biology" (I. Almyrantis)

Other Activities for the Institute of Biology

Vice director of the Institute of Biology (Yannis Almirantis)

President of the Scientific Advisory Board of the Institute (Yannis Almirantis)

Reviewer of scientific articles for the following research Journals: BMC Evolutionary Biology, BMC Genomics, GENE, Journal of Theoretical Biology (Yannis Almirantis)

S. Papageorgiou 'Expression collinearity of *Hox* genes: Experiments and Theory' Complex Systems and Applications (COSA) Seminar, 23 February 2009, NCSR Demokritos.

Impact Factors (for 1 publication): 2,578

Citations 2009 (without self- citations): 24

Total Citations 2006-2009 (without self- citations): 75

h-factor: 10

Research Group: NMR Studies of Biomolecules and Parmaceuticals

Research Staff
Maria Pelekanou, Research Director
Marina Sagnou, Lecturer
Demetra Benaki, Postdoctoral Fellow
Angeliki Panagiotopoulou, Technical Specialist

Research Interests

Studies of the **structure**, **interactions** and **structure-function relationships** of bioactive compounds of pharmacological interest for the diagnosis and/or therapy of various diseases. We focus on two major types of compounds:

- Peptides and small proteins
- Labelled compounds designed as potential radiopharmaceuticals

The areas of application of our work is mainly Alzheimer's disease and cancer, but also other diseases of the central nervous system, bacterial infections, etc. Our main tools are NMR spectroscopy and circular polarimetry (CD) for the structural studies in combination with methodologies of organic synthesis, radiolabelling, and in vitro and in vivo biological assessment.

2009 Findings

In 2009 the application of an NMR method for the detection of interactions of the β-amyloid peptide (β-AP) of Alzheimer's disease with small molecules-potential inhibitors of its aggregation was completed and published. It is the first time that interactions of β-AP are detected by NMR with the histological dye thioflavin T, the hormone melatonin, and the antioxidant oleuropein, basic constituent of olive oil. In conjunction with the NMR studies, extended evaluation of the behavior of solutions of β-AP in the presence of potential inhibitors of its aggregation was performed. Among the compounds studied, of special interest is curcumin a constituent of curry powder. Spectroscopic data based on NMR and CD studies indicate for the first time its interaction with the β-AP (publication in preparation). In the area of bioactive peptides, our team participated in the structural characterization of peptide derivatives labeled with suitable radioisotopes for biodistribution and mechanistic studies. Examples include the neuroprotective humanin labeled with ^{125}I, the neuropeptide bombesin and the immunopeptide thymosin labeled with ^{99m}Tc.

In the field of development of radiopharmaceuticals for labeling of specific targets in the body related to diseases, the ^{99m}Tc complex of the anticancer agent 2-(4'-aminophenylbenzothiazole was administered in SCID mice bearing MCF-7 xenografts. The complex demonstrated clear tumor uptake allowing the delineation of the tumor with the γ-camera, thus becoming a potential candidate for radiodiagnosis or radiotherapy of breast cancer. In addition, new stable complexes of the tricarbonyl $M(CO)_3^+$ core (M = Re, ^{99m}Tc) with a variety of tridentate ligands with SNO and NNO donor atoms, as well as bidentate ligands with NN donor atoms, were synthesized. With the attachment of the proper pharmacophore molecule like quinazoline, WAY 100635, or norfloxacine, these model compounds led to the synthesis of complexes for imaging of the EGF receptor, the serotonin receptor, and for the distinction of infection vs. aseptic inflammation. Special report is made to the design and synthesis of the tricarbonyl $Re(CO)_3^+$ complex of acetylacetone, that led to the synthesis of the corresponding complex of curcumin. The curcumin complex exhibits affinity and specificity for the amyloid plaques of Alzheimer's disease and further reinforces the activity of the team in the area of development of radiodiagnostics for Alzheimer's disease.

2009 Publications

Benaki, D., Stathopoulou, K., Leondiadis, L., Ferderigos, N., Pelecanou*, M., Mikros, E. (2009) Study of the interaction of the *ß*-amyloid peptide of with small molecules employing transferred NOEs. J. Pept. Sci. 15, 435-41

Gourni, E., Bouziotis, P., Benaki, D., Loudos, G., Xanthopoulos, S., Paravatou-Petsotas, M., Mavri-Vavagianni, M., Pelecanou, M., Arhimandritis, S.C., Varvarigou, A. (2009) Structural Assessment and Biological Evaluation of Two New N_3S Bombesin Derivatives. J. Med. Chem. 52, 4234-4246

Evangelou, A., Zikos, C., Benaki, D., Pelecanou, M., Bouziotis, P., Papadopoulos, M., Borovickova, L., Vesela, I., Elbert, T., Kunešová, G., Pirmettis, I., Paravatou-Petsotas, M., Slaninová, J., Livaniou, E. (2009) In vitro binding and in vivo biodistribution studies of the neuroprotective peptide humanin using [125I]humanin derivatives. Peptides 30, 2409-2417

Manta, S., Tzioumaki, N., Tsoukala, E., Panagiotopoulou, A., Pelecanou, M., Balzarini, J., Komiotis, D. (2009) Unsaturated dideoxy fluoro-ketopyranosyl nucleosides as new cytostatic agents: A convenient synthesis of 2,6-dideoxy-3-fluoro-4-keto-ß-d-glucopyranosyl analogues of uracil, 5-fluorouracil, thymine, N-4-benzoyl cytosine and N-6-benzoyl adenine. Eur. J. Med. Chem. 44, 4764-4771

Kyprianidou, P., Tsoukalas, C., Patsis, G., Papagiannopoulou, D., Nicolic, N., Jankovic, D., Djokic, D., Raptopoulou, C., Pelecanou, M., Papadopoulos, M., Pirmettis, I. (2009) Rhenium(I) and technetium-99m(I) *fac*-tricarbonyl complexes with 4-(imidazolin-2-yl)-3-thiabutanoic acid derivatives as tridentate ligands: Synthesis and structural characterization. Polyhedron 28, 3171-3176

Bourkoula, A., Paravatou-Petsotas, M., Papadopoulos, A., Santos, I., Pietzsch, H.J., Livaniou, E., Pelecanou, M., Papadopoulos, M., Pirmettis, I. (2009) Synthesis and characterization of rhenium and technetium-99m tricarbonyl complexes bearing the 4-[3-bromophenyl]quinazoline moiety as a biomarker for EGFR-TK imaging. Eur. J. Med. Chem. 44 4021-4027

Karagiorgou, O., Papagiannopoulou, D., Kyprianidou, P., Patsis, G., Panagiotopoulou, A., Tsoukalas, C., Raptopoulou, C., Pelecanou, M., Pirmettis, I., Papadopoulos, M. (2009) Synthesis and structural characterization of novel neutral *fac*-$M(CO)_3(NSO)$ complexes (M=Re, ^{99m}Tc) with N-acetyl-cysteine derivatives as tridentate NSO ligands. Polyhedron 28, 3317-3321

Articles in Press

Sagnou, M., Tsoukalas, C., Triantis, C., Raptopoulou, C. P., Terzis, A., Pirmettis, I., Pelecanou*, M., Papadopoulos, M. *(2010)* A new tricarbonyl *fac*-M(acac)(isc)$(CO)_3$ complex (M = Re, ^{99m}Tc) with acetylacetonate (acac) and isocyanide (isc) in a 2+1 combination. Inorg. Chim. Acta, *available online* (imp. fact: 1.94)

Papagiannopoulou, D., Makris, G., Tsoukalas, C., Raptopoulou, C. P., Terzis, A., Pelecanou, M., Pirmettis, I., Papadopoulos, M. S. (2010) Rhenium(I) and technetium(I) *fac*-M(NSO)$(CO)_3$ (M = Re, ^{99m}Tc) tricarbonyl complexes, with a tridentate NSO bifunctional agent: Synthesis, structural characterization, and radiochemistry. Polyhedron 29, 876-880 (imp. fact: 1.801)

Chiotellis, A., Tsoukalas, C., Pelecanou, M., Pirmettis, I., Papadopoulos, M. New $^{99m}Tc(CO)_3(NNO)$ complexes in the development of 5-HT_{1A} receptor imaging agents. Eur. J. Med. Chem., *in press* (imp. fact: 2.882)

Tzanopoulou, S., Sagnou M., Paravatou-Petsotas M., Gourni, E., Loudos, G. Xanthopoulos, S., Lafkas, D., Kiaris, H., Varvarigou, A., Pirmettis, I., Papadopoulos, M., Pelecanou M. Evaluation of Re and ^{99m}Tc complexes of 2-(4'-aminophenyl)benzothiazole as potential breast cancer radiopharmaceuticals. J. Med. Chem., *in press* (imp. fact: 4.898)

2009 Proceedings to Conferences

D. Benaki, C. Zikos, P.Klimentzou, I. Pirmettis, M. Papadopoulos, E. Livaniou, M. Pelecanou (2009). NMR Structural Analysis of the $^{185/187}$Re=O Complex of a Thymosin Alpha1 Derivative. In "Collection Symposium Series, Biological Active Peptides, XIth Conference, Prague, Czech Republic" (J. Slaninova, eds) vol. 11, p. 9-12, 2009

E. Gourni, P. Bouziotis, D. Benaki, G. Loudos, S. Xanthopoulos, M. Pelecanou, S.T. Archimandritis, A. D. Varvarigou (2009). Assessment and Biological Evaluation of two N3S Bombesin Derivatives. In "Collection Symposium Series, Biological Active Peptides, XIth Conference, Prague, Czech Republic" (J. Slaninova. eds) vol. 11, p. 40-42, 2009

A. Evangelou, C. Zikos, D. Benaki, M. Pelecanou, P Bouziotis, M. Papadopoulos, L. Borovičková, I. Veselá, T. Elbert, G. Kunešová, I. Pirmettis, M. Paravatou-Petsotas, J. Slaninová, E. Livaniou (2009). *In Vitro* and *In Vivo* Studies of the Neuroprotective Peptide Humanin Using ^{125}I-Radiolabeled Humanin Derivatives. In "Collection Symposium Series, Biological Active Peptides, XIth Conference, Prague, Czech Republic" (J. Slaninova, eds) vol. 11, p. 34-36, 2009

2009 Presentations at Scientific Conferences

D. Benaki, C. Zikos, P.Klimentzou, I. Pirmettis, M. Papadopoulos, E. Livaniou, M. Pelecanou (2009). NMR Structural Analysis of the $^{185/187}$Re=O Complex of a Thymosin Alpha1 Derivative. XIth Czech and Slovak National Conference on Biologically Active Peptides, April 22-24, 2009, Prague, Czech Republic.

E. Gourni, P. Bouziotis, D. Benaki, G. Loudos, S. Xanthopoulos, M. Pelecanou, S.T. Archimandritis, A. D. Varvarigou (2009). Assessment and Biological Evaluation of two N3S Bombesin Derivatives. XIth Czech and Slovak National Conference on Biologically Active Peptides, April 22-24, 2009, Prague, Czech Republic.

A. Evangelou, C. Zikos, D. Benaki, M. Pelecanou, P Bouziotis, M. Papadopoulos, L. Borovičková, I. Veselá, T. Elbert, G. Kunešová, I. Pirmettis, M. Paravatou-Petsotas, J. Slaninová, E. Livaniou (2009). *In Vitro* and *In Vivo* Studies of the Neuroprotective Peptide Humanin Using ^{125}I-Radiolabeled Humanin Derivatives. XIth Czech and Slovak National Conference on Biologically Active Peptides, April 22-24, 2009, Prague, Czech Republic, 2009.

I. Veselá, D. Benaki, M. Pelecanou, T. Elbert. Tyrosine containing Peptide Resistent to Radiiodination. What is behind? 16th *Workshop* of the International Isotope Society-Central European Division: The synthesis and applications of isotopes and isotopically labelled compounds, Bad Soden, Germany, October 1-2, 2009

Kostomoiri M., Fragkouli A., Sagnou M., Skaltsounis L., Pelecanou M., Tsilibary E., Tzinia A. Oleuropein prevents the formation of extracellular amyloid β-peptide aggregates and leads to increased production of neuroprotective sAPPa**,** 2nd FEBS-MPST Advanced Lecture Course, Matrix Pathobiology, Signaling and Molecular Targets (2009).

Educational Activities

"Introduction to NMR spectroscopy" teaching session within the framework of the graduate course of "Protein Biotechnology", Department of Biology, University of Crete (5 hours, 6 students), M. Pelecanou

"Applications of Nuclear Magnetic Resonance in Medicine" Summer School 2009, NCSR "Demokritos" (1 hour, 20 students), M. Pelecanou

Alzheimer's disease: Current status in diagnosis and therapy" Lecture of M. Pelecanou within the seminar cycle organized by senior/retired researchers of NCSR "Demokritos" May 2009

Other Scientific/Research Activities

Reviewer for the scientific journals: British Journal of Pharmacology, Inorganic Chemistry, Journal of Medicinal Chemistry (M. Pelecanou)

Member of the Greek network to optimize exploitation of the INSTRUCT (Integrated Structural Biology) infrastructure for Europe, the EATRIS-GR National Consortium of Biomedical and Medical Institutions to improve the way biomedical research is conducted across the country, and the Greek XFEL network to optimize use of the European XFEL (X-ray Free Electron Laser) by the greek research community (M. Pelecanou)

Other Activities for the Institute of Biology

Responsible for the operation of the Circular Spectropolarimeter (M. Pelecanou) with the support of the specialized technical scientist Dr. A. Panagiotopoulou (M. Pelecanou)

Co-responsible (along with Drs K. Yannakopoulou and L. Leondiadis, Institutes of Physical Chemistry and Radioisotopes/Radiodiagnostic Products, respectively) for the NMR Lab, comprising two spectrophotometers of 250 and 500 MHz. (M. Pelecanou)

Member of the Scientific Advisory Board of the Institute of Biology (M. Pelecanou)

Member of the Committee for Education of the Institute of Biology and member of the examination committee for the award of graduate scholarships at the Institute of Biology (M. Pelecanou)

Responsible for fire security in the Institute of Biology (M. Pelecanou)

Responsible for the operation of the Confocal microscope (M. Sagnou)

Organizer of the seminars (research and bibliographical) of graduate students of the Institute of Biology (M. Sagnou)

Member of the examination committee for the award of graduate scholarships at the Institute of Biology (M. Sagnou)

Responsible for the operation of the Blood Bank of the employees of NCSR "Demokritos" (D. Benaki)

Impact Factors (for 7 publications): 18,597

Citations 2009 (without self- citations):
M. Pelecanou: 33
M. Sagnou: 14

Total Citations 2006-2009(without self- citations):
M. Pelecanou: 153
M. Sagnou: 44

h-factor:
M. Pelecanou: 13
M. Sagnou: 6

Current External Funding

Programme entitled *Synthesis and chasracterization of specific cold and radiolabeled derivatives for the in vitro and in vivo study of immunoactive peptides*, funded by the Greek State's Scholarship Foundation (IKYDA) with Coordinator Dr. E. Livaniou (Institute of Radioisotopes/Radiodiagnostic Products, NCSR "Demokritos"
Duration: 2008-2009

Total programme funding: 4000 €
Total funding (lab): 1000 €
Funding of the lab for 2009: 800 €

Programme entitled *Development of a diagnostic drug for Alzheimer's disease*, funded by the ASPIS Bank with Coordinator Dr. M. pelecanou
Duration: 2007-2010
Total funding (lab): 30.000 €
Funding of the lab for 2009: 5000 €

Programme entitled *Development of ^{99m}Tc and ^{186}Re labelled small biomolecules for cancer diagnosis and/or radiotherapy* funded by the International Atomic Energy Agency (IAEA) with Coordinator: Dr. I. Pirmettis(Institute of Radioisotopes/Radiodiagnostic Products, NCSR "Demokritos")
Duration: 2007-2009
Total funding (lab): 1.000 €
Funding of the lab for 2009: 500 €

Programme entitled *Development of ^{99m}Tc radiopharmaceuticals for sentinel node detection and cancer diagnosis* funded by the International Atomic Energy Agency (IAEA) with Coordinator Dr. I. Pirmettis(Institute of Radioisotopes/Radiodiagnostic Products, NCSR "Demokritos")
Duration: 2007-2009
Total funding (lab): 3.000 €
Funding of the lab for 2009: 1.500 €

Programme entiled *EUropean research initiative to develop imaging probes for early in-vivo diagnosis and evaluation of response to therapeutic substances* funded by EU (Health 2008-2012) with Coordinator Dr. A. Varvarigou (Institute of Radioisotopes and Radiodiagnostic Products , NCSR "Demokritos")
Duration: 2008-2012
Total programme funding: 318.000 €
Total funding (lab): 16.000 €
Funding of the lab for 2009: 4.000 €

Note 1:
The following program etitled *Diagnostic imaging of amyloid plaques of Alzheimer's disease* was submitted and approved for funding in 2009 by the John S. Latsis Public Benefit Foundation with Coordinator Dr. M. pelecanou
Duration: 1/1/2010-31/12/2010
Total programme funding: 12.000 €
Funding of the lab for 2009: 0 €

Note 2: Submissions under evaluation:

1. "Biosynthesis and genetic identification of cyclic peptide with potential therapeutic properties against Alzheimer's disease: inhibitors of Aβ protein aggregation"
   General Secretariat for Research and Technology
   3rd Community Support Program for Greece 2007-2013
   within the framework of "Thalis"
   Coordinator: E.S. Gonos, Laboratory of Molecular and Cellular Aging, Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens
2. "Synthesis of pyrrolo-quinoline and pyrrolo-isoquinoline nuclesidic derivatives with potential antiviral and antitumour properties"
   General Secretariat for Research and Technology
   3rd Community Support Program for Greece 2007-2013
   within the framework of "Thalis"

Coordinator: D. Komiotis, Department of Biochemistry & Biotechnology, University of Thessaly, Greece

3. "Innovative imaging techniques using THz frequencies for biomedical applications"
   General Secretariat for Research and Technology
   3rd Community Support Program for Greece 2007-2013
   within the framework of "Thalis"
   Coordinator: H. Avramopoulos, School of Electrical and Computer Engineering, National Technical University of Athens, Greece
4. Novel osteophilic ^{99m}Tc and $^{186/188}$Re based radiopharmaceuticals for imaging/therapy of bone metastatic tumours and inflammation
   General Secretariat for Research and Technology
   3rd Community Support Program for Greece 2007-2013
   within the framework of "Thalis"
   Coordinator: N. Efstathopoulos, 2nd Department of Orthopaedics, Medical School, University of Athens, Greece
5. "Strengthening and sustaining research capacity in design and synthesis of nanocarriers and bioactive molecules with potential pharmaceutical applications in the NCSR "Demokritos"
   FP7-REGPOT-2010-1 (Regional Potential)
   Coordinator: I. Mavridis, Institute of Physical Chemistry, NCSR "Demokritos"
6. "Development and screening of novel beta amyloid peptide inhibitors for Alzheimer's disease"
   NSRF 2007-2013 National Action: COOPERATION Sub-Action II: Large Scale Cooperative Projects
   Coordinating Institution: The Goulandris Natural History Museum, GAIA Research Center, Bioanalytical Department
7. "Synthesis and structural characterization of Ru(II) complexes; investigation of biological activity in vitro"
   Ministry of Education
   within the framework of "Heraklitos"
   March 2009
   Coordinator: K. Methenitis, Department of Chemistry, University of Athens

Research Group: Protein Structure and Molecular Modeling

Research Staff
Metaxia Vlassi, Research Director
Dimitris Vlachakis, Postodoctoral Fellow
Athanassios Tartas, Collaborating Collaborating Graduate Student – *Phd obtained in 2009*
Diamadis Sellis, Graduate Associate

Research Interests

Our current research activities focus on

1) Structural studies of proteins with emphasis on sequence repeat-containing proteins aiming to elucidate sequence/structure relationships and the structural determinants of sequence repeat mediated protein interactions. The approach followed mainly includes structural bioinformatics techniques such as *in silico* 3D-modelling (homology/comparative modelling & threading), docking, molecular dynamics simulations etc.

2) Studying the dynamics of protein structure by means of molecular dynamics (MD) simulations and development of related bioinformatics tools.

3) 3D-modelling of enzymes of mainly medical interest and of potential inhibitors towards a structure-based drug design.

2009 Findings

1) In silico 3D-modelling of Arginine-Serine (RS) repeats

Arginine-Serine (RS) repeats have been found in a large number of proteins with various functions and have been implicated in protein interactions. In a previous work, we have studied the conformation of a similar protein interaction module, namely the TPR repeats of the Ssn6 protein, by a combination of experimental methods and molecular dynamics (MD) simulations and found that conformational changes take place upon the TPR-mediated interaction of Ssn6 with its partner, Tup1 (Palaiomylitou et al., 2008).

In this work and with the aim to elucidate the role of serine phosphorylation in the conformation of RS repeats, we performed MD simulations on the unphopshorylated and phosphorylated RS domain of lamin b receptor (LBR) consisting of four (human orthologue) and five RS consecutive repeats (vhicken orthologue). Apart from commercially available software (MOE), we also used the Gromita software we developed recently (Sellis et al., 2009, see below).

Our study showed that serine phosphorylation of both RS peptides induces order-to-disorder transition. Our data in conduction with data from the literature suggest that the phosporylation-induced lack of ordered structure is a common feature of RS repeats (Manuscript in preparation).

A grant application for similar studies on other aminoacid repeats and their modifications has been submitted to the GSRT in the framework of the "THALIS" program (see the grant applications section).

2) Development of a bioinformatics tool for molecular dynamics simulations of proteins in solution.

In the framework of the DEMOEREVNA program (financed by NCSR "Demokritos", coordinator: M. Vlassi) we have developed the computer program, Gromita, which is a Graphical User Interface (GUI) to the widely used MD computer program, Gromacs. In 2009, we developed a new version of Gromita (Fig. 1), which is the only GUI available compatible with the laest Gromacs version, v4. In addition, we constructed a website (http://bio.demokritos.gr/gromita) including a manual and installation instructions.

The new version of Gromita is published in *Bioinformatics & Biology Insights* (**Sellis et al., 2009**).

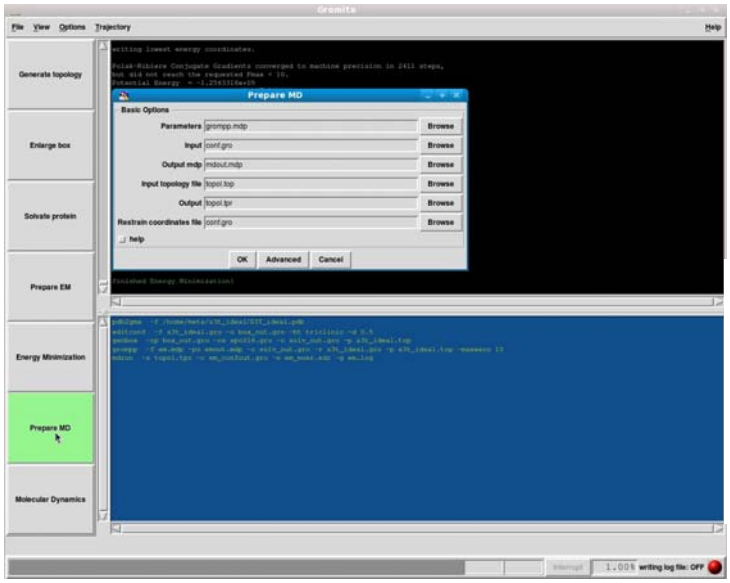


Fig 1. **The main window of the Gromita GUI**. All options have been included in clickable buttons, switches or drop-down menus. (**Sellis et al., *Bioinformatics & Biology Insights* 2009**)

3) Molecular Modelling of inhibitors of enzymes of mainly medical importance

3a) Using molecular docking experiments followed by MD simulations we showed that a series of synthetic fluoro-pyranosyl nucleosides can efficiently dock into the active site of the human poly(A)-specific ribonuclease (PARN) (Fig. 2).

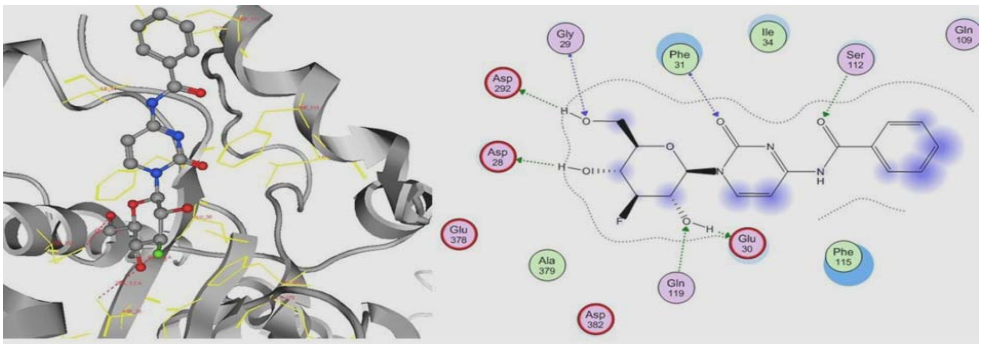


***Fig* 2.** (Left): Molecular Docking of a potential inhibitor (compound A6, in balls-and-sticks) into the active site of the human PARN (in ribbon representation). (Right): Details of the enzyme-compound interactions .(**Balatsos et al., *Biochemistry* 2009**)

Our in silico data in conjunction with *in vitro* experiments (from our collaborators) suggest that human PARN is indeed among the molecular targets of such compounds. Such compounds can therefore, serve as leading compounds for the development of novel inhibitors of PARN_with potential use for novel therapeutic approaches. This work was published in ***Biochemistry*** **(Balatsos et al., 2009**).

Docking experiments with a new series of synthetic nucleoside analogues targeting PARN are in progress.

3b) Aiming at the development of new antiviral agents, we designed *in silico* a novel series of potential inhibitors of the Dengue virus (type II) helicase by a *de novo* structure-based approach including the "grow" technique and the known crystal structure of a homologous helicase. Evaluation of the compound from the synthesis point of view is in progress.

4) The 3D-model of histone-like DNA-binding protein, HU from the thermophile bacterium *Thermoplasma volcanium* (HUTvo) that we have previously constructed by applying homology

modelling techniques in combination with thermal stability studies of the enzyme was published in ***Extremofiles*** (**Orfaniotou et al., 2009**).

2009 Publications

Sellis, D., Vlachakis, D., Vlassi, M. (2009) Gromita: A fully integrated graphical user interface to Gromacs 4. *Bioinformatics & Biology Insights* 3, 99-102

Balatsos, N., Vlachakis, D., Maragozidis, P., Anastasakis, D., Kyritsis, A., Vlassi, M., Koumiotis, D., Stathopoulos, C (2009) Competitive Inhibition of Human Poly(A)-Specific Ribonuclease (PARN) by Synthetic Fluoro-Pyranosyl Nucleosides. *Biochemistry* 48(26), 6044-6051

Orfaniotou F, Tzamalis P, Thanassoulas A, Stefanidi E, Zees A, Boutou E, Vlassi M, Nounesis G, Vorgias CE (2009) The stability of the archaeal HU histone-like DNA-binding protein from Thermoplasma volcanium. *Extremophiles* 13(1), 1-10.

2009 Presentations at Scientific Conferences

Vlachakis D, Vlassi M (2009) «Molecular modeling of a novel series of Dengue virus (typeII) helicase potential inhibitors, by a de novo structure-based drug design approach». 34th FEBS Congress, July 4-9, 2009, Prague, Czech Republic. Abstract in FEBS J. 276:171

Vlassi, M (2009) «Research Activities related to Bioinformatics & Computational Biology at NCSR "Demokritos"», Meeting of the Hellenic Society of Computational Biology & Bioinformatics (HSCBB) – GSRT-ELIXIR, 30-31 March, 2009, Athens, Greece.

Other Research/Scientific Activities

Member of the national network "INSTRUCT" ("INSTRUCT: An Integrated Structural Biology Infrastructure for Europe).

Member of the national network BE/OPT-XFEL (Network to Optimize use of the European X-FEL by the Greek Research Community)

Member of the network "Center for Crystallographic Studies of Macromolecules"

Reviewer of the journal *Theoretical chemistry Accounts*

Vlassi, M. (2009). "Development of Bioinformatics Tools for Molecular Dynamic Simulations of Proteins in solution". Presentation in the framework of the Gala: "Demoerevna Results",1-12-2009, NCSR "Demokritos".

Educational Activities

Lecture on "Principles of X-Ray Crystallography: Applications in Structural Biology" in the framework of the post-graduate program (towards a Masters degree) entitled "Clinical Biochemistry – Molecular Diagnosis" (Dr. M. Vlassi, Dept. of Biology, National & Kapodistrian University. of Athens)

Lectures on "Structural Biology: Crystallography – NMR" in the framework of the post-graduate program (towards a Masters degree) entitled "Introduction to Experimental Methodology" (Dr. M. Vlassi, Medical School, National & Kapodistrian University. of Athens)

A. Tartas **completed his PhD thesis and received a grade of excellent** from the Dept. of Chemistry, University of Patras in March 2009. Title of thesis: "Biochemical & Structural Characterization of the TPR domain of the transcriptional repressor, Ssn6 from S. cerevisiae".

Other Activities for the Institute of Biology

President of the Committee for computing and network support of IB

Supervisor of the PhD thesis of the graduate student, A. Tartas (completed: March 2009, University of Patras)

Member of the supervising committee of the PhD thesis of E. Mavrogonatou

Member of the committee for consumables delivering at IB

Member of the committee for instrumentation delivering at IB in the framework of EPAN

Responsible scientist for the use of common instrumentation (i.e. shaking incubator etc)

Member of the Committee for examining the level of knowledge of the Greek language of EU citizens

Impact Factors (for 2 publications): 5,973

Citations 2009 (without self- citations): 32

Total Citations 2006-2009 (without self- citations): 113

h-factor: 11

Current External Funding

Note 1:
A grant application entitled "BE/OPT-XFEL:Network to Optimize use of the European X-FEL by the Greek Research Community" submitted to GSRT has been approved (5-1-2010).
Duration: 20/1/2010-20/10/2010
Total programme funding: 125.000 €
Total funding (lab): 22.200 €
Funding of the lab for 2009: 0 €.
Part of the budget corresponds to the organization cost of a workshop, which will take place at NCSR "D", 18-19 June, 2010 (Main Organizer: M. Vlassi)

Note 2: Grant applications submitted

(i) A grant application entitled *Strengthening and sustaining research capacity in Design and synthesis of nanocarriers and bioactive molecules with potential pharmaceutical application in the NCSR «Demokritos»* has been submitted in the framework of FP7-REGPOT-2010-1 program (submitted December 2009)

(ii) A grant application entitled *Intrinsically disordered proteins: A complete in vitro, in vivo και in silico analysis by a combination of state-of-the art techniques* has been submitted to the GSRT in the framework of the THALIS program (Submitted February 2010)

(iii) A grant application entitled *Signaling in bacteria: from understanding the biochemical and molecular pathways of the AtoS/AtoC system to developing specific antimicrobials* has been submitted to the GSRT in the framework of the THALIS program (Submitted December 2009)

(iv) A grant application entitled *Dysfunction of mitochondria in neurodegenerative diseases* has been submitted to the GSRT in the framework of the THALIS program (Submitted February 2010)

S E R V I C E U N I T S

- ***HUMAN TISSUE BANK***
- ***EXPERIMENTAL ANIMAL COLONY***
- ***LASER CONFOCAL MICROSCORY***
- ***CHARACTERIZATION OF PROTEINS AND BIOACTIVE MOLECULES***

HUMAN TISSUE BANK

Research Staff
Helen Vavouraki, Reseracher
Stilianos Kakkos, Research Technician
Vassilki Stefou, Assistant Technician

Lab Description – Research Interests

Human Tissue Bank collects tissues of human origin, processes them, produces grafts and delivers them to Hospitals or Medical Laboratories. It follows the International stds as well as the European Directives 23/2004, 17/2006 and 86/2006. The established know-how in processing methods of various human tissue parts is unique in Greece, being always in progress. Since 1971 when established –among the first three Tissue Banks in Europe, it has delivered over 42000 grafts of proven quality. The whole activities are computerized and accredited according to ISO 9001/2008 .

Our permanent task is the continuous search of human tissues from suitable donnors, the effort for the optimization of the production processes, the study of activity mechanisms of our grafts, the introduction of new techniques and methods, the application of new quality controls according to the latest national and international standards and legislation for this type of products. -

In research level, our collaboration with the Faculty of Dentistry/ University of Athens is being continued on:

a) the effect of PRP (platelet rich pegma) in the osteoconductive capacities of DBM. (deminarilised bone matrix) (PhD thesis is being written)
b) Attachment and proliferation of human osteoblast-like cells on guided bone regeneration membranes in the absence or presence of nicotine (PhD Thesis, published papers).

2009 Graft production - Findings

There is a collaboration between the Human Tissue Bank and a third party (ORTHOMEDICAL Ltd) concerning the promotion and delivery of some of our bone grafts in hospitals. For this purpose we have produced 334 cancellous bone grafts, 277 of which were delivered by ORTHOMEDICAL.

Concerning other scientific collaborations we have prepared either grafts of other tissues, or new processed grafts**.**

2009 Publications

Markou N., Perelassi E., Vavouraki H., et al (2009). Treatment of Periodontal Endosseous Defects With Platelet-Rich Plasma Alone or in Combination with Demineralized Freeze-Dried Bone Allograft: A Comparative Clinical Trial. Journal of Periodontology, Vol 80 (12), 1911- 1919.

Articles in Press

Papaioannou, K., Markopoulou, C., Gioni V., Mamalis A. Vavouraki H.,et al. Attachment and Proliferation of Human Osteoblast-Like Cells on Guided Bone Regeneration (GBR) Membranes in the Absence or Presence of Nicotine: An In Vitro Study International Journal of Oral and Maxillofacial Implants (IntJOMI). (I.F 1,972)

Markou, N., Pepelassi, E., Kotsovilis, S., Vavouraki, H., et al. Platelet- Rich plasma combined with Demineralized Freeze-dried bone allograft in the therapy of periodontal endosseous defects: report of two clinical cases. The Journal of the American Dental Association (JADA).(IF 1,849)

2009 Presentations at Scientific Conferences

Vavouraki H.N, Human tissue processed grafts, Biomaterial 2009. 5th international technology transfer days, 19 - 20 February 2009, Erfurt/Germany

Other Activities for the Institute of Biology

Responsible of Quality Process according ISO 9001/2008.

Students training in the Bank activities

Other Scientific/Research Activities:

Member of the European Committee for the establishment of a unique European nomenclature and coding of human tissues and cells.

Collaboration with the National Transplant Organisation, and the Ministry of Health and Social Solidarity for the adaptation of the above Directives into the National Law, and launch of guidelines for the establishment of Tissue Banks

Member of European Net of Human Tissue and Cell Banking Inspectors EUSTITE.

Training Certification of Kapodistrian University if Athens in "Business Administration"

Impact Factors (for 1 publication): 1,961

Citations 2009 (without self- citations): 7

Total Citations 2006-2009 (without self- citations): 34

h-factor: 3

EXPERIMENTAL ANIMAL COLONY

Research Staff
Dimitris Kletsas, Research Director
Ioannis Zafiropoulos, Research Technician
George Doulgeridis, Research Technician

Description

The animal facility maintains and reproduces inbred strains of experimental animals. The following strains are currently available:

- Mice, strain SWR SWISS ALBINO
- Rats, strains WISTAR ALBINO and ETB
- Rabbits, strain NZW ALBINO
- Mice, strain SCID

During 2009, the Animal Facility provided the following animals:

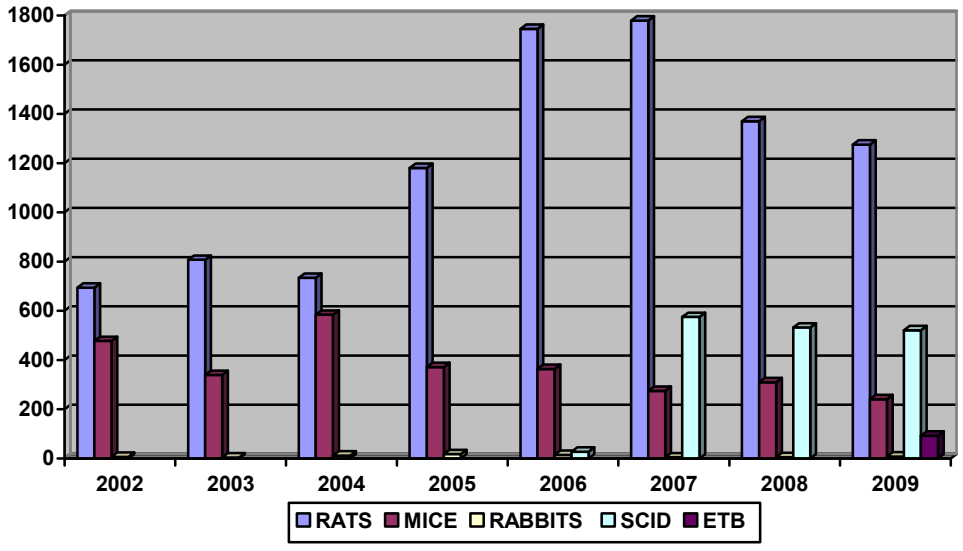
Users	*Rats*	*Rats ETB*	*Mice SWR*	*Rabbits NZW*	*SCID mice*
Institute of Biology	60	0	0	0	18
Institute of Radioisotopes & Radiodiagnostics	80	0	241	7	421
External Users	1135	92	0	0	82
Total	**1275**	**92**	**241**	**7**	**521**

The number and species of animals produced are dictated by the needs of research programs within the Institutes of NCSR "Demokritos", mainly the Institutes of Biology and Radioisotopes-Radiodiagnostic Products. In addition, animals are provided outside the Centre in research laboratories, hospitals, pharmaceutical companies, etc. Research projects in collaboration with Thriassio Hospital and Ioannina University Medical School are ongoing.

The colonies of SWR and SCID mice have been renewed. A new colony of ETB rats for ELPEN Pharma has been developed. Two new colonies of transgenic animals, one expressing MMP9 in neurons (TgMMP9) and one being a model for Alzheimer disease (5xFADD) have also been developed.

The Unit has been certified according to ISO 9001:2000. Accordingly, the building has (and is currently being) upgraded, new instruments have been purchased, the quality of animals has been tested locally by a veterinarian and by a certified laboratory abroad.

DISPOSAL OF LABORATORY ANIMALS 2002-2009



LASER CONFOCAL MICROSCOPY

Research Staff
Marina Sagnou, Lecturer

Description

The current Unit activities include:

a) The study of cellular, molecular and biochemical phenomena on cells and tissues using confocal microscopy imaging techniques

b) The use of confocal microscopy as a tool to explore the surface area and penetration potential of novel and known material

c) The application of immunohistochemistry, face-contrast, Nomarsky etc techniques on both fixed and living cells.

2009 Findings

During the year 2009, there seemed to be a rather increased demand for the the study of cellular, molecular and biochemical phenomena using confocal microscopy imaging techniques by both the Local Institute of Biology researchers, and those from the University of Athens, The Agricultural University, the Technical University as well as some Hospital Units.

Furthermore, it was this year's achievement, to initiate the exploration of the surface area and penetration potential of novel and known material, as a new ground of application for this technique, by both NCSR "D" researchers and external industry collaborators.

CHARACTERIZATION OF PROTEINS & BIOACTIVE MOLECULES

Research Staff
Metaxia Vlassi, Research Director
Maria Pelecanou, Research Director
Aggeliki Panagiotopoulou, Technical Specialist

Description

The service unit for Characterization of Molecules and Biomolecules (CMB) has been established in 2003 and comprises two pre-existing laboratories: 1) the Centre for Crystallographic Studies of Macromolecules (CCM) and 2) the Nuclear Magnetic Resonance laboratory (NMR).

- CCM consists of a) a state-of-the-art X-ray system for diffraction experiments on macromolecules and b) a Circular Dichroism (CD) spectropolarimeter. CCM has been financed by the General Secretariat for Research and Technology (GSRT, EPET II program) as a network of related to molecular structure research groups from "Demokritos" and from other Research/Academic Institutions of Greece. CCM operates at NCSR "Demokritos" since fall 1998.
- The NMR laboratory consists of a) a 250 MHz NMR spectrometer and b) a 500 MHz ADVANCE DRX NMR spectrometer. The latter has been funded in the framework of a GSRT program entitled: 'Up-grading the infrastructure of NCSR "Demokritos" and is shared by the Institutes of Physical Chemistry, Biology and Radioisotopes & Radiodiagnostic Products.

2009 Findings

Both laboratories of the CMB service unit mainly support related to molecular structure research activities of the Physical Chemistry, Biology and Radioisotopes & Radiodiagnostic Products Institutes of NCSR "Demokritos", thus contributing to the research and development program of the Centre. In addition, the unit also serves external users mainly from other Research and Academic Institutions.

EDUCATIONAL ACTIVITIES

EDUCATION

The Institute of Biology continues its Graduate Course Programmme, which has been successfully carried out for the past 30 years. This Programme includes:

a. Training of young scientists at the postdoctoral level
b. Pre-graduate and graduate thesis work
c. Courses at the graduate level
d. Lecture Contributions to the Summer School of the NCSR "Demokritos"

During the year 2009, 12 scientists were trained at the postdoctoral level at our Institute. Furthermore, 27 graduate students worked toward the completion of their doctoral thesis research work under the supervision of scientists of the Institute and on projects which were given to them by their respective supervisors.

During the year 2009, 4 of our graduate students finished their thesis work and became PhDs.

Moreover, 7 students from the University are carrying out their pre-graduate project thesis work at the Institute and 3 students did practical job training. Additionally, 1 student from University abroad did practical lab training in laboratories at the Biology Institute.

In the framework of Graduate Programme, during the year 2009 the Biology Institute organized one course in which had as participants graduate students of the IB and of other Institutes of N.C.S.R. "Demokritos". The following course was given by scientists of the Biology Institute:

- *Cellular Signaling* [course lecturers: D. Kletsas, G. Voutsinas, A. Chroni, course coordinator:Z. Georgoussi].

In addition to the above, scientists of the Biology Institute carried out the following series of courses and seminars within the framework of the Graduate School Programme of the Greek Universities:

- *Lectures entitled "Cytotoxicity study on conventional and targeted chemotherapeutic drugs" (seminar and practical laboratory exercise) in the framework of the Post-Graduate Specialization Diploma "Biological Applications in Medicine"* (**Dr. G. Voutsinas**, Department of Biology, University of Athens)
- *Lecture entitled "Mechanisms of G Protein-Coupled Receptor signaling in novel drug development» in the framework of the postgraduate course "Biochemistry"* (**Dr. Z. Georgoussi**, Department of Molecular Biology and Biochenistry, University of Athens)
- *Participation in the postgraduate course "Biochemistry"* of the department of Molecular Biology and Biochenistry University of Athens (**Dr. Z. Georgoussi**, teaching responsibilities)
- *Lecture entitled "Cellular senescence and tissue homeostasis" in the framework of the postgraduate course "Biochemistry***"** (**Dr. D. Kletsas,** Department of Biology, University of Athens)
- *Lecture entitled "Cellular senescence and tissue homeostasis" in the framework of the postgraduate course "Physiology***"** (**Dr. D. Kletsas,** Medical School, University of Athens)
- *Lecture entitled "Growth Factors" in the framework of the postgraduate course "Composition and Metabolism of Mineralized Tissues",* (**Dr. D. Kletsas,** Dental School, University of Athens)
- *Teaching in the framework of the postgraduate programme "Application of Biology in Medicin", the course "Cell cultures – Tissue cultures*" (**Dr. D. Kletsas and Dr. H. Pratsinis**, Department of Biology, University of Athens)
- *Teaching in the framework of the postgraduate course: "Applications of Biology to Medicine", the course "Cell Cycle: Checkpoints and Consequences for Physiological Cell Function"* (**Dr. Th. Sourlingas,** Department of Biology, University of Athens).
- *Lecture with title "Matrix-mediated signal transduction in physiological and pathological conditions; the roleof growth factor receptors, proteoglycans and integrins" in the framework of the postgraduate course "Physiology - Anasology"* (**Dr. E. Tsilibary**, Department of Biology, University of Athens)

- *Lecture entitled title "Diabetes Mellitus: Molecular mechanisms and therapeutic approaches" in the framework of the course"Pathobiochemistry" (***Dr. E. Tsilibary**, Department of Biology, University of Athens)
- *Lecture entitled title "Cell apoptotic processes in human disease: beneficial or adverse?" in the framework of the course "Molecular and applied physiology", (***Dr. E. Tsilibary**, Medical School, University of Athens)
- *Lecture entitled "Lipoprotein metabolism pathways and atherosclerosis. The association between atherosclerosis and Alzheimer's disease" in the framework of the postgraduate course "Human Biochemistry"* (**Dr. A. Chroni**, Department of Chemistry, University of Athens)
- *Lecture entitled "Eukaryotic microorganism as model systems for functional expression and characterization of transmembrane transporters of higher organisms" in the framework of the postgraduate course "Model Systems of Molecular Microbiology, Microbial Biotechnology"* **(Dr. V. Sophianopoulou**, Department of Biology, University of Athens)
- *Teaching in the framework of the postgraduate program "Bioinformatics", the course "Introduction to Computational Biology"* **(Dr. I. Almyrantis,** Department of Biology, University of Athens)
- *Lecture on "Principles of X-Ray Crystallography: Applications in Structural Biology" in the framework of the post-graduate program (towards a Masters degree) entitled "Clinical Biochemistry – Molecular Diagnosis"* (**Dr. M. Vlassi**, Dept. of Biology, National & Kapodistrian University. of Athens)
- *Lectures on "Structural Biology: Crystallography – NMR" in the framework of the post-graduate program (towards a Masters degree) entitled "Introduction to Experimental Methodology"* (**Dr. M. Vlassi**, Medical School, University. of Athens)
- *Lecture entitled "Introduction to NMR spectroscopy" in the framework of the graduate course "Protein Biotechnology"* (**Dr. M. Pelecanou**, Department of Biology, University of Crete)

During July 2009, the Summer School of NCSR "Demokritos" was held and had included talks from the researchers of the Institute of Biology and of invited speakers coming from other Greek Institutions and abroad. The seminars of Biology related to the Summer School are presented analytically in the following pages.

Within the framework of the Graduate School Programme, are also organized, on a regular basis, bibliographical seminars and seminars presenting progress in current research work. These seminars are presented by all the graduate students of the Institute and supplemented by scientific seminars presented by other researchers of the Institute as well as invited guest speakers from other Greek or foreign Educational and/or Scientific Research Institutes. The seminars accomplished the past year (2009) are presented analytically in the following pages.

Finally, the educational endeavours of the Biology Institute also include those accomplished by **Dr. K. Stamatakis**, who gives informative seminars to High School, University and Military School students.

COMPLETION/AWARD OF DOCTORAL THESES IN 2009

GRADUATE STUDENT	TITLE OF DOCTORAL THESIS	ADVISOR (in Institute of Biology)	UNIVERSITY
Eleni Mavrogonatou	Study of ageing and regulation of cell proliferation in intervertebral disc cells	D. Kletsas	Department of Biology University of Athens
Ioannis Ninios	Study of apoptosis induction by histone deacetylase inhibitors in cancer cell lines	Th. Sourlingas	Medical School University of Athens
Katerina Roumelioti	Genes coding protein factors that participate in intracellular protein trafficking and topogenesis of acidic amino acids transporters in *Aspergillus nidulans*	V. Sophianopoulou	Department of Biology University of Athens
Athanassios Tartas	Biochemical & Structural Characterization of the TPR domain of the transcriptional repressor, Ssn6 from *S. cerevisiae*.	M. Vlassi	Department of Chemistry University of Patras

LECTURE CONTRIBUTIONS TO THE 2009 SUMMER SCHOOL OF THE NCSR "DEMOKRITOS"

(July 2009)

DATE	SPEAKER	TITLE
7/7/09	**Dr. V. Sophianopoulou & I. Vaggelatos** Institute of Biology, NCSR "Demokritos"	Eisosomal proteins: gates or barriers of endocytosis?
7/7/09	**Dr. A. Chroni** Institute of Biology, NCSR "Demokritos"	Is "good" cholesterol, HDL, always good?
7/7/09	**Dr. I. Georgoussi** Institute of Biology, NCSR "Demokritos"	Heptahelical receptors and drug development
7/7/09	**Dr. M. Pelecanou** Institute of Biology, NCSR "Demokritos"	Applications of Nuclear Magnetic Resonance in Medicine
13/7/09	**Dr. P. Kitsiou** Institute of Biology, NCSR "Demokritos"	Investigating the mechanisms of cell apoptosis for the treatment of diseases: The paradigm of Diabetes mellitus
15/7/09	**Dr. L. Swevers** Institute of Biology, NCSR "Demokritos"	Engineering of baculovirus vectors for protein production and cellular transformation

SEMINAR PROGRAMME 2009
INSTITUTE OF BIOLOGY

DATE	SPEAKER	TITLE
10/2/09	**P. Karkoulis** Institute of Biology, NCSR "Demokritos"	SATB1 reprogrammes gene expression to promote breast tumor growth ans metastasis
10/2/09	**Ch. Magrioti** Institute of Biology, NCSR "Demokritos"	Generation of pluripotent stem cells from adult mouse liver and stomach cells
17/2/09	**K. Roumelioti** Institute of Biology, NCSR "Demokritos"	CTA4 transcription factor mediates induction of nitrosative stress response in Candida albicans
23/2/09	**Dr. G. Papageorgiou** Institute of Biology, NCSR "Demokritos" (Seminar organised by the COSA group of NCSR "Demokritos")	Temporal and spatial colinearity in the expression patterns of homeostic genes (HOX): Experiments and Theories
24/2/09	**E. Salpea** Institute of Biology, NCSR "Demokritos"	Telomere length regulates the epigenetic status of mammalian telomeres and subtelomeres
5/3/09	**Dr. M. Kotsifakis** Institute of Allergy and Infectious Diseases National Institute of Health (NIH), USA	Molecular and biochemical approaches in blood-fed arthropods: carriers of human disease, source of pharmaceuticals
24/3/09	**M. Papakonstantinou** Institute of Biology, NCSR "Demokritos"	Rgs5 tergeting leads to chronic low blood pressure and a lean body habitus
31/3/09	**Dr. J. Urban** Department of Physiology, Anatomy and Genetics Oxford University	The strange environment of cells of the intervertebral disc
7/4/09	**I. Dafnis** Institute of Biology, NCSR "Demokritos"	Role of ABCG1 and ABCA1 in regulation of neuronal cholesterol efflux to apolipoprotein E discs and suppression of amyloid-beta peptide generation
14/4/09	**N. Tsotakos** Institute of Biology, NCSR "Demokritos"	Densin and beta-catenin form a complex and co-localize in cultured podocyte cell junctions
28/4/09	**A. Dimozi** Institute of Biology, NCSR "Demokritos"	Caveolin-1 Regulates the Antagonistic Pleiotropic Properties of Cellular Senescence through a Novel Mdm2/p53-Mediated Pathway
12/5/09	**G. Daniil** Institute of Biology, NCSR "Demokritos"	Adenovirus-Mediated Expression of Human Paraoxonase 3 Protects Against the Progression of Atherosclerosis in Apolipoprotein E_Deficient Mice
19/5/09	**A. Galeou** Institute of Biology, NCSR "Demokritos"	Establishing RNA Interference as a Reverse-Genetic Approach for Gene Functional Analysis in Protoplasts
26/5/09	**K. Kapodistria** Institute of Biology, NCSR "Demokritos"	A novel mechanism is involved in cationic lipid-mediated functional siRNA delivery
29/5/09	**Prof. K. Drainas**	Molecular Biology for the Environment and Industry:

	Dpt. of Chemistry, University of Ioannina	mechanisms of horizontal gene transfer
2/6/09	**M. Kostomiri** Institute of Biology, NCSR "Demokritos"	Small molecule inhibitors of aggregation indicate that amyloid β oligomerization and fibrillization pathways are independent and distinct
9/6/09	**K. Ioannides** Institute of Biology, NCSR "Demokritos"	Repair of infracted myocardium using mesenchymal stem cell seeded small intestinal submucosa in rabbits
16/6/09	**A. Repouskou** Institute of Biology, NCSR "Demokritos"	Circadian Control of the NAD+ Salvage Pathway by CLOCK-SIRT1
23/6/09	**S. Aliberti** Institute of Biology, NCSR "Demokritos"	Transcription analysis in the MeLiM swine model identifies RACK1 as a potential marker of malignancy for human melanocytic proliferation
17/7/09	**Dr. A. Raikhel** Department of Entomology, University of California , USA	Mosquito blood meals and development: ecdysteroid and nutritional regulation of mosquito vitellogenesis
5/11/09	**Dr. E. Douni** BSRC "Al. Fleming"	Forward genetics as a tool for the identification of novel disease targets
11/11/09	**Dr. E. Mavrogonatou** Institute of Biology, NCSR "Demokritos"	Effect of osmotic stress on the proliferation and senescence of intervertebral disc cells
18/11/09	**M. Kostomiri** Institute of Biology, NCSR "Demokritos"	Studies of the effects of oleuropein on the metabolism of proteins involved in the pathigenesis of Alzheimer disease
25/11/09	**K. Ioannides** Institute of Biology, NCSR "Demokritos"	Nuclear polyhedrosis viruses from lepidopteran insects: molecular characterization and use in biotechnological applications
4/12/09	**Dr. F. Kolissis** National Hellenic Research Foundation	Biotechnology for Sustainable Development friendly for the Environment and Industry: Biotranformations of Physical compounds-Structural and Functional variability of new products
9/12/09	**K. Roumelioti** Institute of Biology, NCSR "Demokritos"	Genes encoding factors involved in trafficking and topogenesis of acidic amino acid transporters
15/12/09	**Dr. I. Bossis** Dept. of Veterinary Medicine University of Maryland, USA	Polyionic Papillomavirus Virus-Like Particles: A Novel vaccine platform for induction of robust CTL response
16/12/09	**G. Daniil** Institute of Biology, NCSR "Demokritos"	Analysis of Antioxidant-Anti-inflammatory properties of HDL from subjects with familial low or high HDL syndromes
18/12/09	**Dr. A. Gavalas** National Foundation of Biomedical Research, Academy of Athens	Endocrine pancreas specification: from the embryo to embryonic stem cells and back

COLLECTIVE DATA

FINANCIAL REPORT 2009

1. INTERNAL FUNDING FROM THE SPECIAL ACCOUNT DEPARTMENT AND FUNDING FROM GSRT
(COORDINATOR: E. TSILIBARY, HEAD OF IB)

		PROGRAMMES				PROGRAMMES FROM SERVICES
INCOME	**464**	**1240** (IB, IPC, IMS)	**1269**	**1333**	**1397**	**[1334 - 1475 – 1164 - 1507]**
CARRIED OVER FROM 2008	32.545,74	121.825,27	15.767,84	6.013,45	5.092,45	-50.175,91
FUNDING FROM NCSR "D"	8.450,00	3.631,57	0,00	0,00	0,00	0,00
MATCHING FUNDS	20.957,55	0,00	12.596,61	11.159,95	0,00	0,00
INCOME FROM SERVICES	0,00	0,00	0,00	0,00	0,00	120.137,39
DONATIONS FROM COMPANIES	0,00	0,00	0,00	0,00	0,00	0,00
TRANSFER FROM OTHER SOURCES	33.908,45	0,00	92,42	0,00	0,00	23.570,62
TOTAL INCOME	**95.861,74**	**125.456,84**	**28.456,87**	**17.173,40**	**5.092,45**	**93.532,10**
EXPENSES		**I.B.**				
EQUIPMENT	12.234,39	124.541,40	0,00	0,00	0,00	3.919,26
SUPPLIES	22.395,96	0,00	0,00	0,00	0,00	26.773,09
SALARIES	0,00	0,00	0,00	0,00	0,00	48.866,46
TRAVELS	50,00	0,00	0,00	0,00	0,00	1.649,73
OTHER EXPENSES	21.708,45	0,00	-83,17	0,00	0,00	32.611,99
COMMITTED	6.107,92	0,00	0,00	0,00	0,00	6.570,56
TRANSFER FROM OTHER SOURCES	19.106,62	26.341,23	27.570,22	17.173,40	5.000,00	5.037,44
TOTAL EXPENSES	**81.603,34**	**150.882,63**	**27.487,05**	**17.173,40**	**5.000,00**	**125.428,53**

2. GOVERNMENTAL FUNDING

LIQUID NITROGEN	0,00
ANIMAL CHOW	4.991,76
TOTAL GOVERNMENTAL FUNDING	**4.991,76**

3. EXTERNAL FUNDING FROM THE PROGRAMMES OF THE INSTITUTE (Programmes that are coordinated by the Head of IB are included)

SOURCE OF FUNDING (number of programmes)	FUNDING (in EUROS)			
	Programme A	**Programme B**	**Programme C**	**INSTITUTE**
European Union (5)	278.000	4.700	4.000	286.700
General Secretariat for Research & Technology (10)	32.800	83.400	-	116.200
Ministry of health & Social Solidarity (2)	12.000	-	-	12.000
International Atomic Energy Ageny (IAEA) (2)	-	-	2.000	2.000
Aspis Bank (1)	-	-	5.000	5.000
National Scholarship Foundation (1)	-	-	800	800
Greek Society of Oncologists (1)	1.250	-	-	1.250
Kotsikas Foundation (1)	9.000	-	-	9.000
AO Foundation (1)	12.000	-	-	12.000
TOTAL	**345.050**	**88.100**	**11.800**	**444.950**

COLLECTIVE DATA ON PRODUCTIVITY OF SCIENTIFIC PROGRAMMES

	PROGRAMME			
	A	B	C	INSTITUTE
Researchers	11	7	4	23*
Technical Specialist	-	1	1	2
Emeritus & Collaborating Scientists	2	4	1	7
Postdoctoral Fellows	4	6	2	12
Graduate Students	20	7	-	27
Collaborating Graduate Students	10	-	5	15
Graduate Research Associates	-	1	1	2
Undergraduate Students	5	5	-	10
Research Technicians	3	2	-	9 @
Administrative Staff	-	-	-	2
Total Personnel	**55**	**33**	**14**	**109**
Publications in Peer-Reviewed Journals	**17**	**14**	**12**	**44#**
Publications (Average) in Peer-Reviewed Journals per Scientist	1.54	2	3	1.913
Cumulative Impact Factor in Peer-Reviewed Journals (number of publications)	**58.148 (17)**	**23.566 (14)**	**27.15 (12)**	**110.825# (44)**
Average Impact Factor in Peer-Reviewed Journals	3.420	1.683	2.262	2.518
Cumulative Impact factor per Scientist	5.286	3.366	6.787	4.818
Proceedings to Conferences	**8**	**3**	**3**	**14**
Proceedings (Average) per Scientist	0.727	0.428	0.75	0.608
Total Publications	**25**	**17**	**15**	**58#**
Publications (Average) per Scientist	2.272	2.428	3.75	2.521
Citations	**852**	**235**	**103**	**1197***
International Patents	-	-	-	-
Greek Patents	-	-	-	-
Presentations to International Conferences	**19**	**18**	**6**	**44£**
Presentations (Average) per Scientist to International Conferences	1.727	2.571	1.5	1.913
Presentations to Greek Conferences	**35**	**8**	**2**	**44&**
Presentations (Average) per Scientist to Greek Conferences	3.181	1.142	0.5	1.913
Total Presentations to Conferences	**54**	**26**	**8**	**88**
Presentations (Average) per Scientist to Conferences	4.909	3.714	2	3.826

** 1 Scientist of Human Tissue Bank is included*

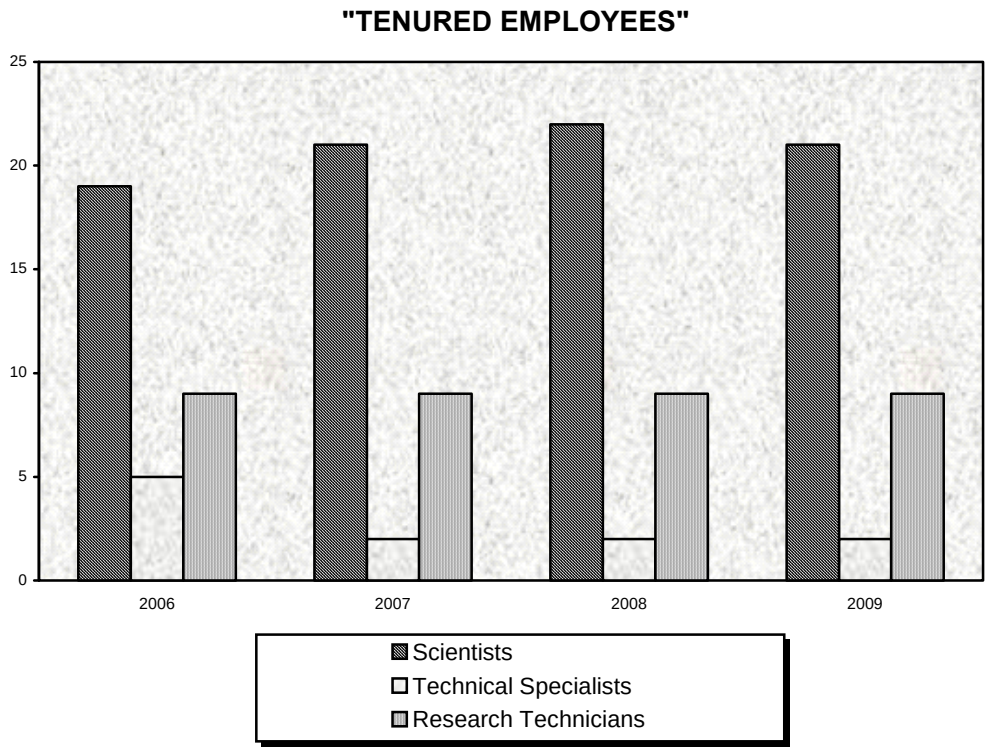
@ 2 Research Technicians who are occupied in Experimental Animal Colony and 1 Research Technician who is occupied in Human Tissue Bank are included

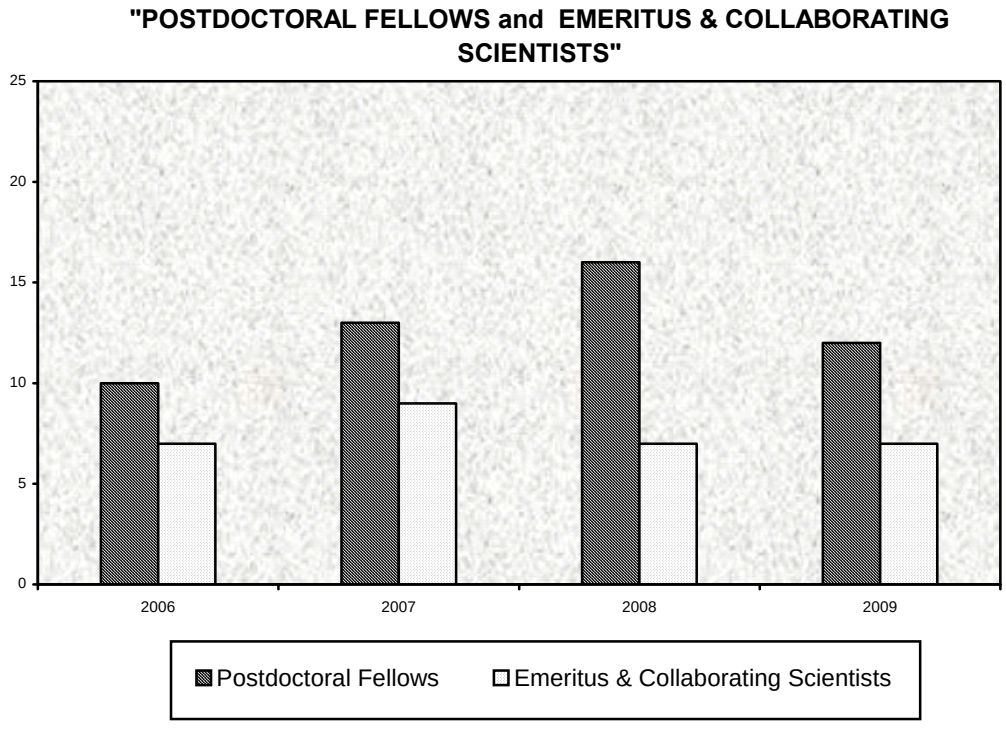
 # 1 publication of Human Tissue Bank is included

£ 1 presentation to International Conference of Human Tissue Bank is included

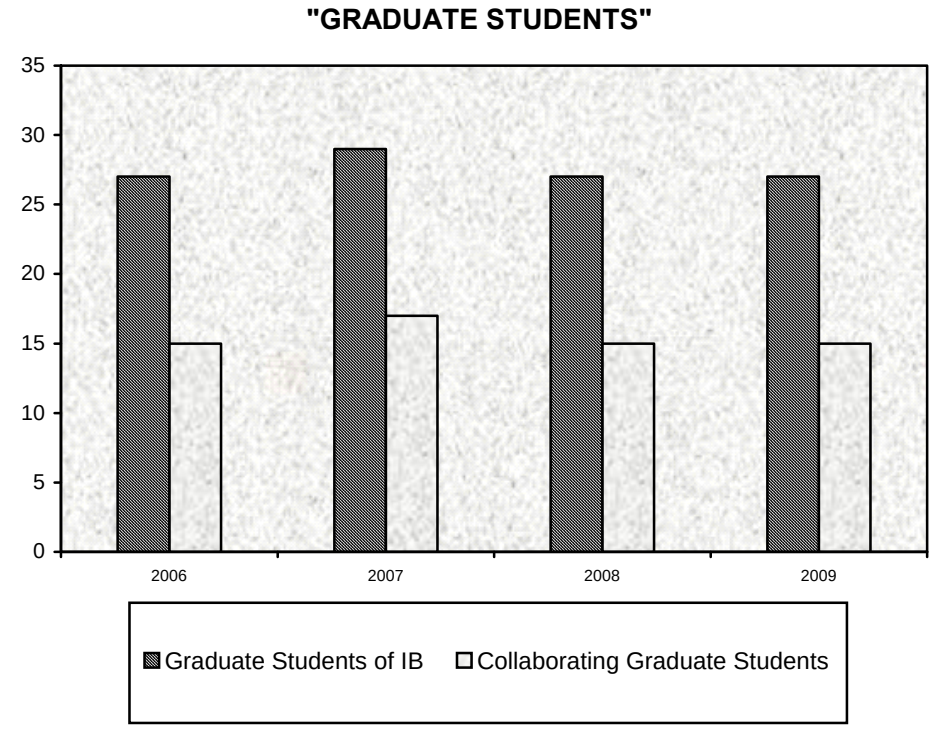
&There is 1 common presentation to Greek Conference of A and B programme

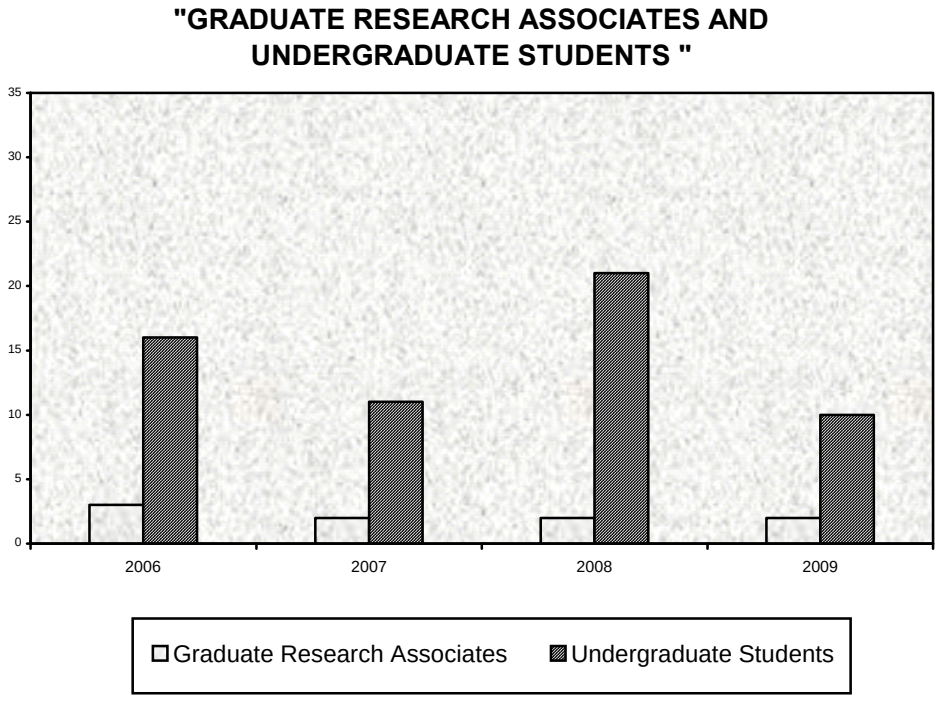
CHANGES OF IB STAFF DURING 2006-2009





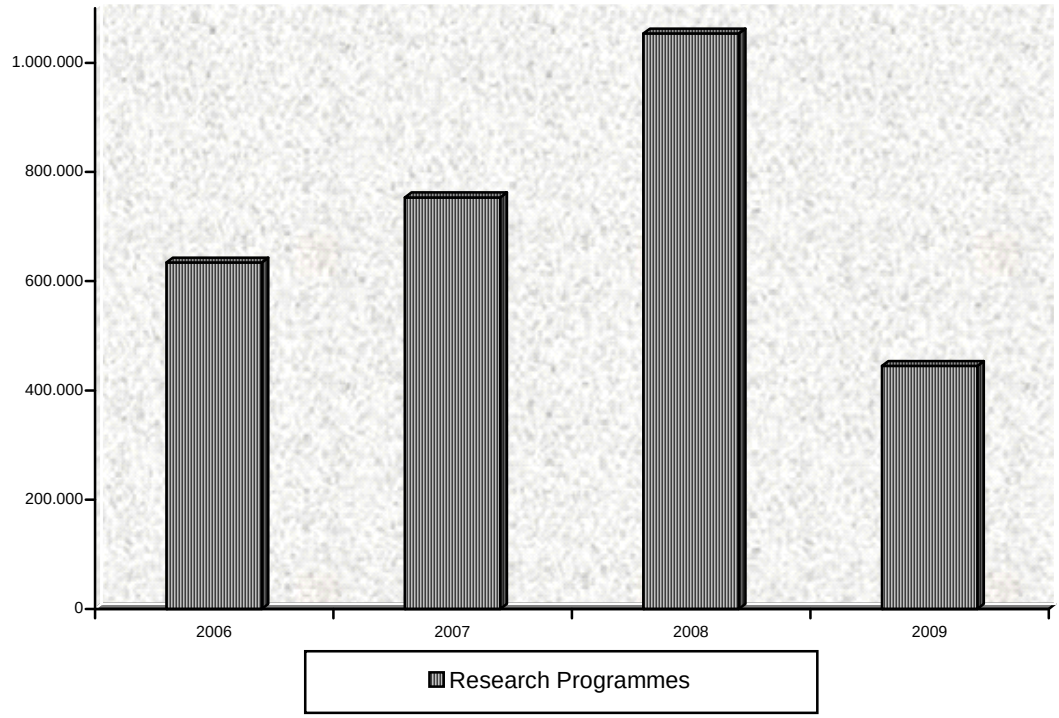
CHANGES OF IB STAFF
DURING 2006-2009



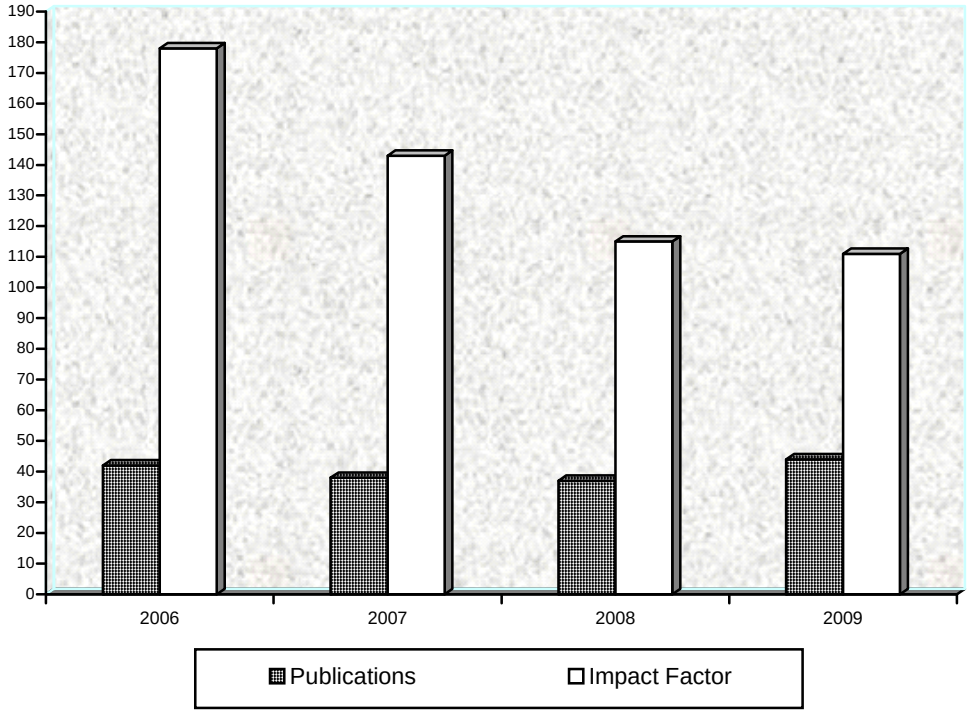


CUMULATIVE EXTERNAL FUNDING OF THE INSTITUTE DURING 2006-2009

(EURO)



PUBLICATIONS IN PEER-REVIEWED JOURNALS AND CUMULATIVE IMPACT FACTOR DURING 2006-2009



CITATIONS OF THE INSTITUTE DURING 2006-2009