INSTITUTE OF
BIOLOGY

NATIONAL CENTRE FOR SCIENTIFIC RESEARCH "DEMOKRITOS"

INSTITUTE OF BIOLOGY

2008
ANNUAL REPORT

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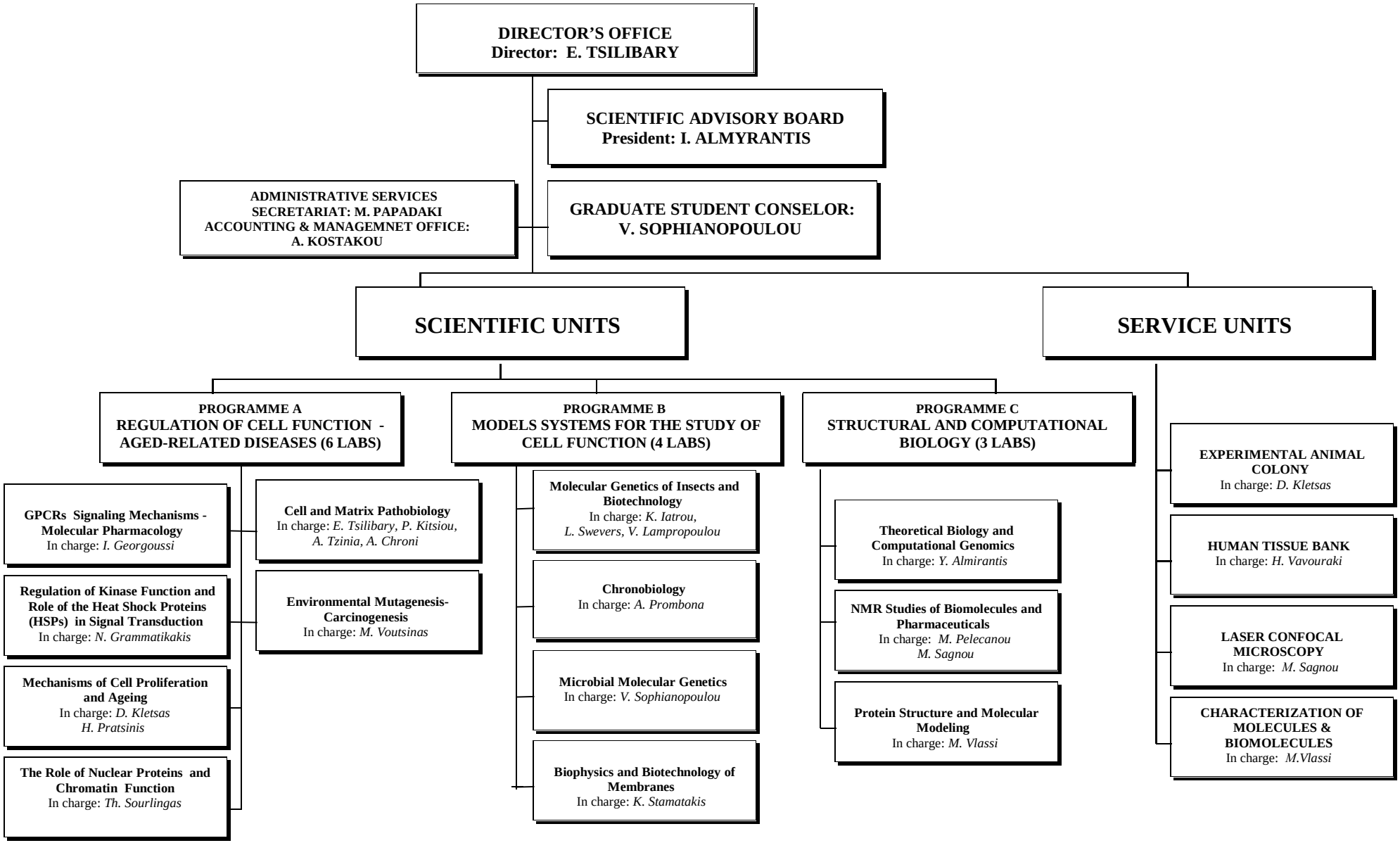
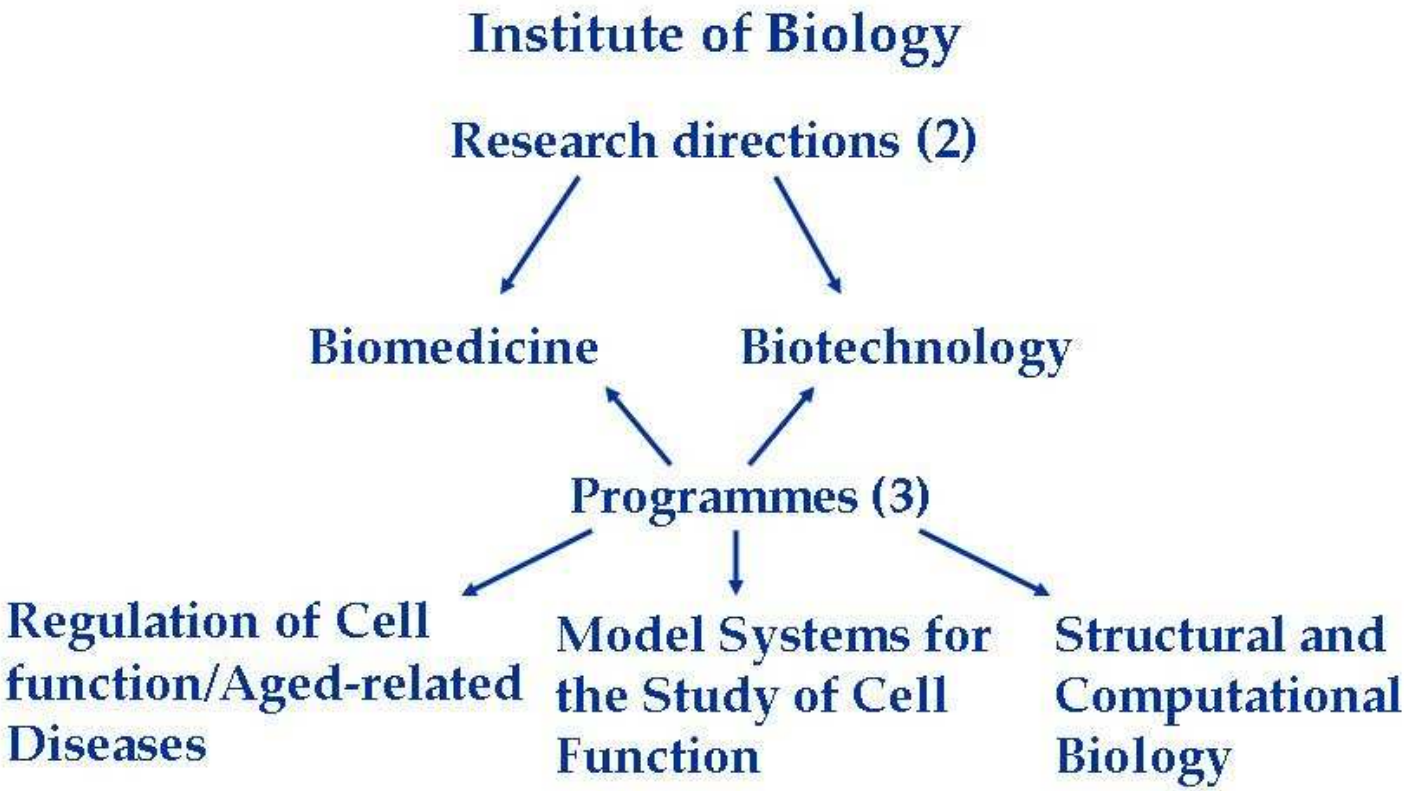
ORGANISATION CHART




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 (24)

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

Georgoussi Zafiroula-Iro	Biochemist
Grammatikakis Nikolaos	Cell Biologist
Prombona Anastasia	Biologist
Stamatakis Konstantinos	Biologist
Swevers Luc	Biologist

Researchers

Chroni Aggelika	Biologist
Kitsiou Paraskevi	Biologist
Konstantopoulou Maria	Biologist
Labropoulou Vassiliki	Biochemist
Sourlingas Thomae	Biologist
Tzinia Athina	Biochemist
Vavouraki Helen	Radiopharmacist
Voutsinas Gerassimos	Biologist

Lecturers

Pratsinis Haris	Chemist
Sagnou Marina	Biologist/ Chemist

Technical Specialists

Panagiotopoulou Aggeliki	Biochemist
Stefanou Dimitra	Agronomist

RESEARCH TECHNICIANS (9)

Avgeris Socrates
Doulgeridis George
Kakkos Stilianos
Kalokiri-Stilianidi Kalliope
Kopanelis Dimitrios
Kotsopoulou Eleni
Pantazi-Mazomenou Anastassia
Tsolomiti-Gourgou Areti
Zafiropoulos Ioannis

ADMINISTRATIVE STAFF (2)

Kostakou Athanassia	Accountant
Papadaki Margarita	Secretary

EMERITUS & COLLABORATING SCIENTISTS (7)

Emeritus Scientists	**LLaboratory**
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 (16)

Fellow	**Supervisor**
Agalou Adamantia	Georgoussi I.
Benaki Dimitra	Pelekanou M.
Drossopoulou Garifallia	Tsilibary E.
Efrose Rodica	Iatrou K.
Fourla Danai	Georgoussi I.
Fragouli Apostolia	Tsilibary E.
Kotzia Georgia	Iatrou K.
Koussis Konstantinos	Iatrou K.
Lagos Dimitris	Sophianopoulou S.
Martinou Kelly	Konstantopoulou M.
Nicolopoulos G.	Vlassi M.
Siskos Elias	Konstantopoulou M.
Skamnaki Vassiliki	Chroni A.
Spyropoulou Antonia	Kletsas D.
Tsitoura Panagiota	Iatrou K.
Vlachakis Dimitris	Vlassi M.

GRADUATE STUDENTS (27)

Student	Supervisor
Aliberti Sofia	Grammatikakis N.
Billini Maria	Stamatakis K./ Sophianopoulou V. – *PhD obtained in 2008*
Bouzarelou Dimitra	Sophianopoulou V.
Danil George	Chroni A.
Dafnis Ioannis	Chroni A.
Dimozi Anastasia	Kletsas D.
Georganta Irene	Georgoussi I.
Handris Panagiotis	Kletsas D.
Ioannides Konstantinos	Iatrou K.
Kapodistria Katerina	Tsilibary E.
Karkoulis Panagiotis	Voutsinas G.
Kostomiri Mirto	Tsilibary E.
Leontiadis Leonidas	Georgoussi I.
Magkrioti Christiana	Iatrou K.
Mavrogonatou Eleni	Kletsas D.
Ninios Ioannis	Sourlingas Th.
Papadopoulou Adamadia	Kletsas D.
Papakonstantinou Maria	Georgoussi I.
Repouskou Anastasia	Prombona A.
Roumelioti katerina	Sophianopoulou V.
Salpea Paraskevi	Sourlingas Th.
Talamagas Anargiros	Tsilbary E. – *PhD obtained in 2008*
Tsagaraki Ioanna	Tzinia A.
Tsotakos Nikos	Tsilibary E
Tzanopoulou Stamatia	Pelekanou M. – *PhD obtained in 2008*
Vaggelatos Ioannis	Sophianopoulou V.
Xedous Marios	Sourlingas Th.

GRADUATE RESEARCH ASSOCIATES

Fellow	Supervisor
Makris Konstantinos *(MD)*	Vavuraki H.
Sellis Diamadis *(MSc)*	Vlassi M.

COLLABORATING GRADUATE STUDENTS (15)

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.
Bichtas Nikos (Univ. of Athens, MSc)	Kletsas D.
Chrissouli Stefania (Univ. of Athens, MSc)	Kletsas D.
Gioni Vassiliki (Univ. of Athens, MSc)	Kletsas D.
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.
Lampidonis Antonis (Agricultural Univ. of Athens)	Voutsinas G.

Oikonomopoulos Spiros (Univ. of Athens, MSc)	Kletsas D.
Tsiagas Ioannis (Univ. of Athens, MSc)	Almyrantis I.
Vatsi Stamatia (Univ. of Athens, MSc)	Vlassi M. – *MSc obtained in 2008*
Vestaki Katerina (Univ. of Athens, MSc)	Voutsinas G.

UNDERGRADUATE STUDENTS AND OTHER IN TRAINING (21)

Student (University)	**Supervisor**
Antoniou Dafni (Univ. of Athens)	Prombona A.
Galeou Aggeliki (Univ. of Athens)	Prombona A.
Darvari Maria (Univ. of Ioannina)	Chroni A.
Faidonos Alexia (Univ. of Bath, UK)	Chroni A.
Hassapis Kiriakos (Univ. of Athens)	Chroni A.
Kapi Marianna (Univ. of Athens)	Prombona A. – *undergraduate dissertation completed in 2008*
Kateifidis Andreas (Univ. of Boston, USA)	Tsilibary E.
Kloukina Vaia (Univ. of Crete)	Tsilibary E.
Krassoudaki Eleni (Univ. of Crete)	Tsilibary E.
Laspa Marina (Univ. of Athens)	Sophianopoulou V. - *undergraduate dissertation completed in 2008*
Lira Katerina – Maria (Univ. College London, UK)	Chroni A.
Malliarakis Grigoris (Univ. of Athens)	Sophianopoulou V.
Nikolos Fotis (Univ. of Athens)	Georgoussi I.
Nomikou Irene (Univ. of Athens)	Prombona A. - *undergraduate dissertation completed in 2008*
Pantazopoulou Marina (Univ. of Athens)	Sophianopoulou V. - *undergraduate dissertation completed in 2008*
Petraki Maria (Univ. of Ioannina)	Chroni A.
Pittis Alexandros (Univ. of Athens)	Sophianopoulou V. - *undergraduate dissertation completed in 2008*
Sarris Michalis (University of Athens)	Georgoussi I. - *undergraduate dissertation completed in 2008*
Tsitsekian Vartan (Univ. of Ioannina)	Sophianopoulou V.
Turnatori Rita (Univ. of Katania, Italy)	Georgoussi I.
Zissi Sofia (Univ. of Ioannina)	Tsilibary E.

INTRODUCTION

The Institute of Biology (IB) is one of eight research Institutes of the National Center for Scientific Research "DEMOKRITOS". The Centre is unique in Greece insofar as combination of different scientific disciplines and many inter-institutional research collaborations are concerned. The goal is optimal progress of research and technology in thematic areas related to the research interests of researchers from different institutes.

22 researchers make up the main scientific staff of the IB (total number of personnel: 124), and in December 2008 a new researcher was recruited (Lecturer level), to be hired in 2009. At the same time new research positions are pending for 2009; the hope is that there will be very competitive candidates for this round as well. In 2008, Drs. Chroni, Lambropoulou, Tzinia and Voutsinas were promoted based on merit, to the tenured, Associate rank. Warm congratulations are extended to all of them.

The IB has recently acquired upgraded and new equipment via competitive funding from the General Secretariat for Research & Technology [GSRT] (EPAN-infrastructure funding, E. Tsilibary, co-ordinator). Approximately 250.000 € were sent in 2008 for new and upgraded equipment (total amount granted: 500.000 € from 2006 to 2008). Moreover, the process of ISO certification according to international standards for the Animal House was completed in July, 2008 (the Laboratory of Human Tissues was certified in 2007). Both these laboratories which render services to a vast number of hospitals, institutions, pharmaceutical companies, private users, etc., have managed to achieve a significant number of sales. Sales started rising significantly with competitive funding from GSRT ("AKMON" grant, matching the amount of sales from March 1st, 2006 to June 30th, 2008); even after completion of the grant sales keep going up and the perspectives are quite encouraging for the future. The researchers in charge of these facilities, Dr. D. Kletsas (overseeing the Animal House) and Dr. E. Vavouraki (in charge of the "Human Tissue Bank") are to be gratefully commented for their extremely hard and successful efforts thus far, in promoting services rendered from each of these laboratories. Of course more infrastructure remains to be upgraded in the next 2-3 years, since both these laboratories were established ~ 40-50 years ago without any renovation ever since. Complete renovation is required for maintaining and updating the ISO certification on a yearly basis. Suffice it to mention that upgrading of these facilities for services started in 2004, together with my tenure as Head of the IB.

The financial and more general indexes of the IB are particularly encouraging, a result of the continuous and successful effort of IB researchers.

Retired, collaborating researchers were again proven to be very productive participating in publications, seminars, research efforts, etc, and in general had a valuable contribution for the IB.

I wish to extend my thankfulness to the elected members of the Scientific Advisory Board of the IB for supporting my efforts to upgrade the Institute, and the members of the Committee for Graduate Education. I also wish to thank all the researchers who participated in various committees of the IB and the Deputy Director, Dr. I. Almyrantis who substantially contributed to the smoothest possible operation of the Institute and my administrative tasks.

Despite continuous difficulties and adverse conditions, support by the majority of researchers is a constant source of optimism and gives me confidence that we will successfully achieve our main goal, upgrading of the IB. My belief is that, based on objective factors including competitive funding, number and quality of publications, citations, etc, the IB has already made substantial progress and continues the process of upgrading, getting better every year. This effort will render the IB more competitive internationally: I have confidence in the research potential of all these researchers who prove their productiveness on a yearly basis and keep getting better.

Finally, I wish to express my gratitude to the administrative support of the IB, Ms. A. Kostaskou (accountant) and Ms. M. Papadaki (secretary).

Effie C. Tsilibary, MD, PhD

Director of IB
February 2008

PROGRAMME A:

REGULATION OF CELL FUNCTION

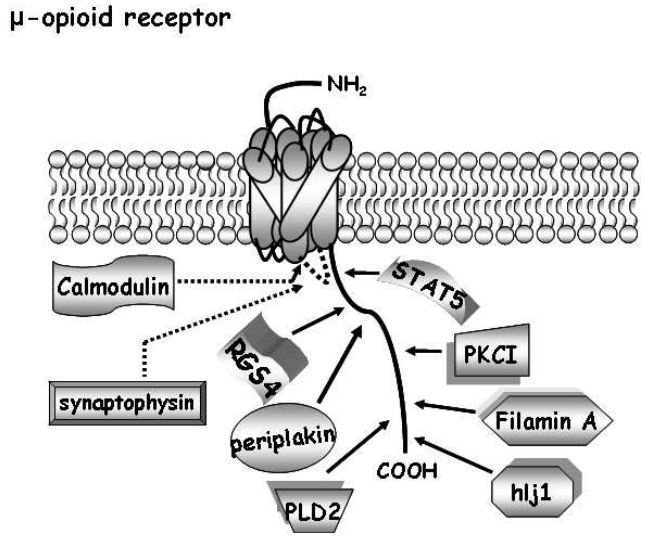
AGED-RELATED DISEASES

Research Group: GPCRs Signaling Mechanisms – Molecular Pharmacology

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
Michalis Sarris, Undergraduate Student – *undergraduate dissertation comleted in 2008*
Fotis Nicolos, Undergraduate Student
Rita Turnatori, Visiting Student

Research Interests

The research interests of our group are focused on the elucidation of the molecular signaling mechanisms mediated by the heptahelical G protein-coupled receptors (GPCRs). 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 drug administration.



More specifically our objectives aim to:

- identify novel opioid receptor-interacting proteins and signaling pathways in an attempt to define novel pharmacological targets of GPCRs,
- identify transcription factors and genes whose action is altered upon activation of the opioid receptors, and finally,
- characterize pharmacologically new compounds, as novel potential analgesics to alleviate chronic pain.

2008 Findings

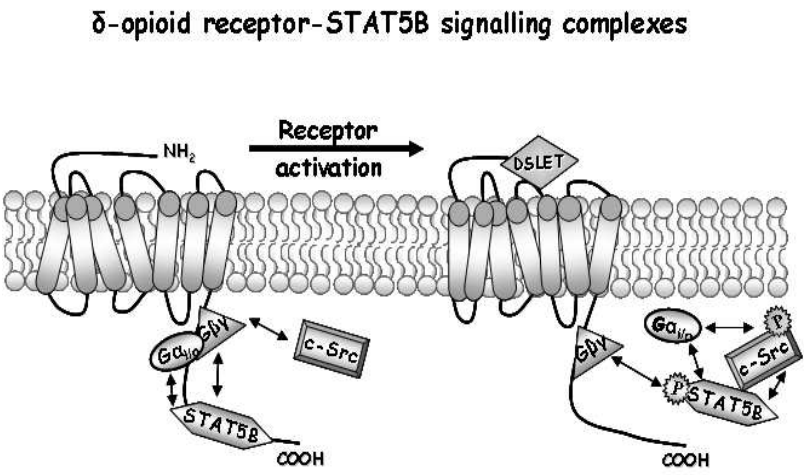
Novel interacting partners of the μ-, δ- and κ- opioid receptors

In an attempt to investigate the signaling mechanisms mediated by μ and δ opioid receptors (μ-OR, δ-OR), we demonstrated for the first time the ability of μ-OR and δ-OR to interact with members of the Regulators of G protein Signaling family, such as RGS2 and RGS4. Further studies indicated that RGS4 promotes selectivity of activated opioid receptors to interact with a specific subset of G proteins. Flow Cytometry Analysis demonstrated that expression of RGS4 in HEK293 cells accelerates the internalization rate of δ-OR, whereas confocal microscopy experiments indicated that RGS4 translocates from the cytoplasm to the cell membrane upon δ-OR activation. Additional studies using truncated forms of RGS4 demonstrated that the N-terminus of the protein is the key functional determinant. The effect of RGS4 and RGS2 in the κ-OR signaling is also investigated.

In parallel, studies related to the role of a new protein such as *spinophilin*, knowing to interact with several heptahelical receptors are carried out. Our results indicated that spinophilin interacts with both μ and δ opioid receptors and alters their signaling.

Transcription factors and functional analysis of genes that are regulated upon opioid receptor activation

In an attempt to elucidate the molecular mechanisms leading to phosphorylation of transcription factors and activation of gene transcription upon opioid receptor-stimulation, we demonstrated the formation of a constitutive multi-component complex comprised of the δ-OR, STAT5B, Gα and Gβγ subunits. Through this dynamic complex STAT5B is phosphorylated and induces transcriptional activation, revealing a novel signaling pathway through which δ-OR may regulate gene transcription and alter synaptosomal plasticity.



Studies using RNA microarrays from SHSY-5Y cells, endogenously expressing μ-OR and treated for different time intervals with morphine, indicated a number of genes that are down- and/or up-regulated. Our interest is focused on the up-regulated proteins, such as the Hsp90 and syntrofin (collaboration with Prof. K. Iatrou, laboratory of insect Molecular Biology and Biotechnology, Institute of Biology, and Dr Mayi Arcellana-Panlilio, microarrays facility group, University of Calgary, Canada).

Pharmacological characterization of new compounds for treatment of chronic pain.

Under the 6th framework we are funded and participate in the EU Consortium "NORMOLIFE" (LSHC-CT2006-037733). Our group characterizes new pharmacological compounds (synthesized by other members of the consortium) with analgesic effect. The results suggested 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).

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, headed by Prof. K. Iatrou, we participate in the scientific research consortium "ENAROMATIC" (FP7-222927, coordinator K. Iatrou). As part of this work, our group has developed a novel assay for screening new ligands for the olfactory receptors OR1 and OR2.

Review Articles

Georgoussi Z. (2008) In **"**Molecular aspects of G protein-coupled receptors: Interacting proteins and function" on "Novel interactive partners regulating opioid receptor signalling beyond the G protein paradigm" (Invited review) Nova Science publishers (eds F. Ciruela and R. Lujan) Chapter 6, 169-206

2008 Proceedings to Conferences

E. Georganta, G. Mazarakou, A. Agalou and Z. Georgoussi (2008). The C-termini of the μ- and δ-opioid receptors differentially bind to Signal Transducers and Activators of Transcription, STAT5A and STAT5B. *Epitheorese Klinikes Farmakologias kai Farmakokinetikes*, Vol. 22 (2), p 152-155.

E. Georganta, A. Agalou and Z. Georgoussi (2008). STAT5B forms tight complexes with the δ-opioid receptor and Gβγ subunits. *The FEBS Journal*, Vol. 275, Supplement 1, p 324.

L.J. Leontiadis and Z. Georgoussi. RGS4: A novel interacting protein essential for μ- and δ- opioid receptor signaling. *The FEBS Journal*, Vol. 275, Supplement 1, p.338

L.J. Leontiadis, M-P. Papakonstantinou, M. Sarris and Z. Georgoussi. RGS4 and RGS2 differentially modulate opioid receptor signaling. *Epitheorese Klinikes Farmakologias kai Farmakokinetikes*, Vol. 22 (2), p 214-216

L.J. Leontiadis, M-P. Papakonstantinou, M. Sarris and Z. Georgoussi. RGS4 protein interacts with μ- and δ- opioid receptors to modulate their internalization fate. *"From Cells to Behavior"*, p. 111

E-M. Georganta, A. Agalou and Z. Georgoussi (2008). Stimulation of the δ-opioid receptor triggers novel signaling pathways leading to transcriptional activation. *"From Cells to Behaviour"*, p 41.

L. J. Leontiadis, E.-M. Georganta, M. Papakonstantinou, D. Fourla, M. Sarris, A. Agalou and Z. Georgoussi (2008) "Chimeric peptides corresponding to intracellular regions of opioid receptors as "baits" for screeining novel receptor-interacting partners". *6th Hellenic Forum on Bioactive peptides* (ed. P. A. Kordopatis) *in press*

2008 Presentations at International Scientific Conferences

E. Georganta, A. Agalou and Z. Georgoussi. Interaction of the δ-opioid receptor with STAT5B and Gβγ subunits reveals novel signaling pathways. European Opioid Conference – European Neuropeptide Club Joint Meeting, April 8-11, 2008, Ferrara, Italy (Oral Presentation)

E. Georganta, A. Agalou and Z. Georgoussi. STAT5B forms tight complexes with the δ-opioid receptor and Gβγ subunits. 33rd FEBS Congress & 11th IUBMB Conference, June 28- July 3, 2008, Athens, Greece

L.J. Leontiadis and Z. Georgoussi. RGS4: A novel interacting protein essential for μ- and δ- opioid receptor signaling. 33rd FEBS Congress & 11th IUBMB Conference, June 28-July 3, 2008, Athens, Greece

Z. Georgoussi "Novel interacting players regulating opioid receptor signaling" European Opioid Conference – European Neuropeptide Club (ENC) Joint Meeting, April 8-11, 2008, Ferrara, Italy (Invited Speaker)

L. Leontiadis, M. Papakonstantinou, M. Sarris and Z. Georgoussi RGS4 regulates opioid receptor signaling, July 13-18, 2008 Charleston, SC, USA

Other Distinctions

Invited speaker in the Institute of Radioisotopes and Radiodiagnostic Products of N.C.S.R. "D" on "Interaction of opioid receptors with cytoplasmic proteins triggers novel signaling pathways", February 14, 2008.

Invited speaker in the Institute for Biological Research and Biotechnology of the NHRF on "Novel interacting partners regulating opioid receptor signaling", April 4, 2008.

Invited speaker in the European Opioid Conference- European Neuropeptide Club (ENC) Joint Meeting on "Novel interacting players regulating opioid receptor signaling", Symposium 5- Opioid receptor and signaling, Ferrara, Italy, April 8-11, 2008.

Invited speaker in the Hellenic Forum on Bioactive peptides on "Chimeric peptides corresponding to intracellular regions of opioid receptors as "baits" for screening novel receptor-interacting partners", Patra, May 18-20, 2008

Invited speaker in the Department of Pharmacology, University of Catania, Sicily, Italy, on "Chimeric opioid peptides as "baits" for screening novel receptor-interacting partner", November 26, 2008

- Gold Metal in the International Warsaw Invention Show (IWIS), on "Chimeric opioid-neurotensin ligands as new prospective analgesics in chronic pain" to Lipkowski A.W., Kleczkowska P., Kosson P., Klinowiecka A**., Georgoussi Z**. and Tourwe D., Poland, June 2008
- The IFIA Scientific Gold Metal of the International Federation of Inventors' Association to: Lipkowski A.W., Kleczkowska P., Kosson P., Klinowiecka A. **Georgoussi Z**. and Tourwe D. on

"Chimeric opioid-neurotensin ligands as new prospective analgesics in chronic pain", October 2008

- Second Hellenic Inventors' Award of the Industrial Property Organisation to: Iatrou K., Swevers L. and Georgoussi Z. during the Science and Technology Festival (Zappeion Conference and Exhibition Center) for the work on "Genetically modified lepidopteran cell lines expressing high levels of functional mammalian opioid receptors as highthroughput screening systems for the identification of opioid receptor agonists and antagonists", November 2008

Citations 2008 (without self- citations): 28

Total Citations 2005-2008 (without self- citations): 78

h-factor: 10

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).

2008 Publications

Sabath E., Negoro H., Beaudry S., Paniagua M., Angelow S., Shah j., Grammatikakis N., Yu AS., Denker BM. (2008) Galpha12 regulates protein interactions within the MDCK cell tight junction and inhibits tight-junction assembly. J Cell Sci. 121: 814-24.

Impact Factors (for 1 publication): 6,42

Citations 2008 (without self- citations): 99

Total Citations 2005-2008 (without self- citations): -

h-factor: 14

Research Group: Mechanisms of Cell Proliferation and Ageing

Research Staff
Dimitris Kletsas, Research Director
Haris Pratsinis, Lecturer
Dimitrios Stathakos, Emeritus Scientist
Antonia Spiropoulou, Postdoctoral Fellow
Panagiotis Handris, Graduate Student
Eleni Mavrogonatou, Graduate student
Adamantia Papadopoulou, Graduate Student
Anastasia Dimozi, Graduate Student
Vassilki Gioni, Collaborating Graduate Student *(MSc)*
Stafania Chrissouli, Collaborating Graduate Student *(MSc)*
Spiros Econopopoulos, Collaborating Graduate Student *(MSc)*
Andreas Armatas, Collaborating Graduate Student *(MSc)*
Nikos Bichtas Collaborating Graduate Student *(MSc)*

Research Interests

We are focusing on the role of growth factors, and especially of TGF-β, in tissue repair during development and ageing. Their action 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 focus 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. Finally, the characteristics of centenarians, as an example of successful ageing, are also studied.

Aim of these 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.

2008 Findings

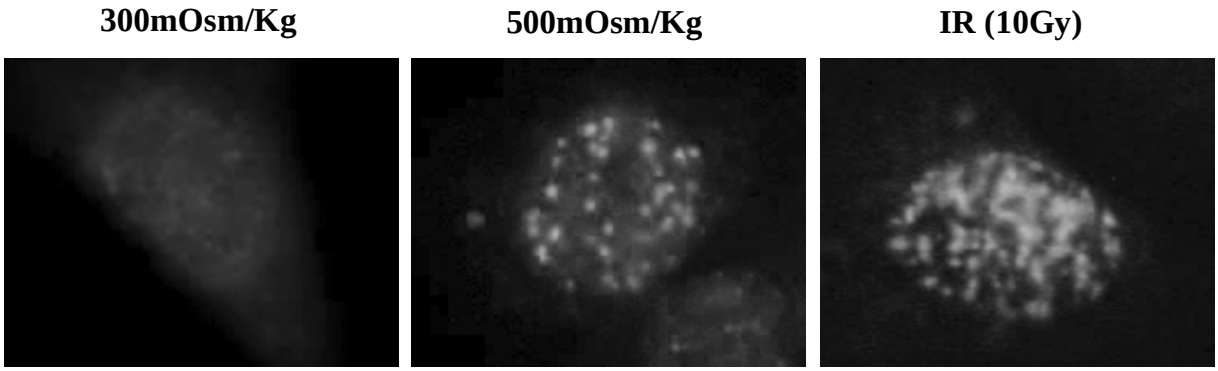
We have continued our studies on the role of growth factors in tissue repair and - having in mind the different repair strategies between fetuses and adults - we are studying the role of the amniotic fluid, i.e. the physiological environment of embryonic cells, on various parameters of tissue repair. We have found that amniotic fluid stimulates cell proliferation, migration, contraction and collagen synthesis in fetal and adult fibroblasts. The role of the various growth factors in the amniotic fluid and the mechanisms underlying these phenomena are currently under investigation.

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 premarure 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.

Stem cells are currently studied in several biomedical applications. In parallel, there is an increased concern about the safety of their use due to their multipotent character. We, as part of a large EU-funded network, are aiming at the use of stem cells in autologous cell replacement therapies and are studying the characteristics of in vitro senescent human mesenchymal stem cells and the consequences of their use in vivo.

One of the tissues that is severely affected by ageing is the intervertebral disc. We have studied intervrtebral 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. p38 MAPK was found to participate in G2 arrest under these conditions, since inhibition of its activity releases the cells from G2 into mitosis. High osmolality resulted also in the ATM-mediated phosphorylation of p53 on Ser15, the up-regulation of $p21^{WAF1}$ and the hypophosphorylation of the retinoblastoma protein, in accordance to the observed G1 arrest. As we have shown with siRNA experiments, this arrest is controlled by p53. Furthermore, histone H2A.X phosphorylation (Figure 1) and comet assay revealed the presence of DNA damage after hyperosmotic treatment, possibly attributed to the abrupt alterations in chromatin configuration observed early after exposure of the cells to this stress. In contrast to previous studies in renal cells, we have shown that disc cells exert a DNA damage response similar to the one expressed after ionizing radiation. Even more, disc cells residing within a hyperosmotic environment retained their ability to respond to newly introduced DNA damage, and possess an increased DNA repair efficiency.



Histone H2A.X phosphorylation due to high osmolality in nucleus pulposus cells from intervertebral disc. Cells exposed to gamma irradiation were used as positive controls

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. In immunohistochemical experiments on patients' samples, we have found, especially in cell clusters (characterisaed by enhanced proliferation), an increased activation of these signalling pathways.

In parallel, we have investigated the effect of anticancer agents on the homeostasis of stromal cells. In particular, we have shown that the known anticancer drug imatinib mesylate (STI571 or Glivec) has an antifibrotic effect on human breast fibroblasts, by inhibiting their proliferation and the synthesis of collagen I and III. These findings indicate that this compound may be suitable for the inhibition of desmoplasia, a crucial parameter in breast tumour development.

In addition, we have continued our studies on the cytostatic/cytotoxic, anti-ageing and the would healing activity of natural products and new synthetic compounds. Finally, we have studied the role

of exogenous stresses (mechanical stimulation or blue light radiation) on the proliferation and differentiation of normal cells.

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Gioni, V., Karampinas, T., Voutsinas, G., Roussidis, A.E., Papadopoulos, S., Karamanos, N.K., Kletsas, D. (2008). Imatinib mesylate inhibits proliferation and exerts an antifibrotic effect in human breast stroma fibroblasts. Mol. Cancer Res. 6, 706-714.

Israilides, C., Kletsas, D., Arapoglou, D., Philippoussis, A., Pratsinis, H., Ebringerová, A., Hríbalová, V., Harding, S.E. (2008). *In vitro* cytostatic and immunomodulatory properties of the medicinal mushroom *Lentinula edodes*. Phytomedicine 15, 512-519.

Taoufik, K., Mavrogonatou, E., Eliades, T., Papagiannoulis, L., Eliades, G., Kletsas, D. (2008). Effect of blue light on the proliferation of human gingival fibroblasts. Dent. Mater. 24, 895-900.

Chondrogianni, N., Trougakos, I.P., Kletsas, D., Chen, Q.M., Gonos, E.S. (2008). Partial proteasome inhibition in human fibroblasts triggers accelerated M1 senescence or M2 crisis depending on p53 and Rb status. Aging Cell 7, 717-732.

Pratsinis, H., Kletsas, D. (2008). Growth factors in intervertebral disc homeostasis. Connect. Tissue Res. 49, 273-276.

Tenta, R., Pitulis, N., Tiblalexi, D., Consoulas, C., Katopodis, H., Konstantinidou, E., Manoussakis, M., Kletsas, D., Alexis, M.N., Poyatzi, A., Koutsilieris, M. (2008). Mechanisms of the Action of Zoledronic Acid on Human MG-63 Osteosarcoma Cells. Horm. Metab. Res. 40, 737-745.

2008 Presentations at International Scientific Conferences

E. Mavrogonatou, D. Kletsas (2008) 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. 33rd FEBS Congress & 11th IUBMB Conference, June 28 – July 3, 2008, Athens, Greece.

A. Papadopoulou, D. Kletsas (2008) Ionizing radiation provokes premature senescence in human lung fibroblasts that enhance the growth of malignant lung epithelial cells *in vitro* and *in vivo*. 33rd FEBS Congress & 11th IUBMB Conference, June 28 – July 3, 2008, Athens, Greece.

H. Pratsinis, G. Sapkas, D. Kletsas (2008) Growth Factor-Stimulation of Intervertebral Disc Cell-Proliferation: Involvement of Pivotal Signalling Pathways. 33rd FEBS Congress & 11th IUBMB Conference, June 28 – July 3, 2008, Athens, Greece.

O. Kousidou, A. Berdiaki, D. Kletsas, G. Tzanakakis, N. Karamanos (2008) Genistein as a key factor affecting the expression of matrix proteoglycans and metalloproteinases implicated in breast cancer. 33rd FEBS Congress & 11th IUBMB Conference, June 28 – July 3, 2008, Athens, Greece.

O.C. Kousidou, A. Berdiaki, D. Kletsas, G.N. Tzanakakis, N.K. Karamanos (2008) The importance of estrogen receptors in mediating the effect of estradiol in the expression of matrix proteoglycans in breast cancer. 33rd FEBS Congress & 11th IUBMB Conference, June 28 – July 3, 2008, Athens, Greece.

P.A. Kokkinos, I.K. Zarkadis, D. Kletsas, D.D. Deligianni (2008) Release of mitogenic factors and expression of differentiation marker genes resulting from physiological mechanical stimulation of human osteoblasts growing on an orthopedic alloy of titanium. 33rd FEBS Congress & 11th IUBMB Conference, June 28 – July 3, 2008, Athens, Greece.

A. Koryllou, T.G. Sourlingas, H. Pratsinis, M. Patrinou-Georgoula, V. Pletsa. p53-dependent differential sensitivity of lung cancer cell lines to the chemotherapeutic agents N-methyl-N-nitrosourea and trichostatin A. 33rd FEBS Congress & 11th IUBMB Conference, June 28 – July 3, 2008, Athens, Greece.

I.P. Trougakos, M. Lourda, M.M. Antonelou, D. Kletsas, V.G. Gorgoulis, I.S. Papassideri, Y. Zou, L.H. Margaritis, D.A. Boothman, E.S. Gonos (2008). Clusterin binds to the cytoplasmic Ku70-Bax nexus and

suppresses Bax activation and relocation to mitochondria. 33rd FEBS Congress & 11th IUBMB Conference, June 28 – July 3, 2008, Athens, Greece.

N. Fokialakis, N. Aligiannis, X. Alexi, M.N. Alexis, H. Pratsinis, E. Kalpoutzakis, A.L. Skaltsounis (2008). Isolation of bioactive compounds from Genista halacsyi (Leguminosae) and evaluation of their estrogenic activity. 7th Joint Meeting of AFERP, ASP, GA, PSE & SIF, August 3-8, 2008, Athens, Greece.

K. Metwally, H. Pratsinis, D. Kletsas (2008). Pyrimido[4,5-c]quinolin-1(2H)-ones as a novel class of antimitotic agents: Synthesis and in vitro cytotoxic activity. 7th Joint Meeting of AFERP, ASP, GA, PSE & SIF, August 3-8, 2008, Athens, Greece.

M. Anastasiadi, H. Pratsinis, D. Kletsas, A.L. Skaltsounis, S.A. Haroutounian (2008). Determination of biologically interesting polyphenols from grapes, wines and vinification byproducts of Greek origin—assessment of their in vitro antioxidant activity. 7th Joint Meeting of AFERP, ASP, GA, PSE & SIF, August 3-8, 2008, Athens, Greece.

M. Anastasiadi, H. Pratsinis, D. Kletsas, S.A. Theotokatos, S.A. Haroutounian (2008). Determination and quantitative analysis of the principal polyphenolic compounds present in stem extracts of native Greek islands grape varieties—assessment of their antioxidant activity in vitro. 7th Joint Meeting of AFERP, ASP, GA, PSE & SIF, August 3-8, 2008, Athens, Greece.

M. Anastasiadi, H. Pratsinis, D. Kletsas, A. Papras, A. Panagiotou, S.A. Haroutounian (2008). Changes during ripening in the content of the principal bioactive polyphenols in five Greek native Vitis vinifera cultivars. 7th Joint Meeting of AFERP, ASP, GA, PSE & SIF, August 3-8, 2008, Athens, Greece.

D. Kletsas (2008) Cellular senescence and tissue homeostasis. European Tissue Research Society Annual Meeting, September 10-12, St. George's Bay, Malta. (invited speaker)

D. Kletsas (2008) Cellular senescence and cancer development. 8th International Conference on Anticancer Research, October 17-22, Kos, Greece. (invited speaker)

D. Kletsas, A. Papadopoulou (2008) Human lung fibroblasts prematurely senescent sfter exposure to ionizing radiation enhance the growth of malignant lung epithelial cells in vitro and in vivo. 6th European Congress of Biogerontology. November 30 - December 3, Noordwijkerhout, The Netherlands.

D. Kletsas (2008) Mechanisms of cell senescence and its role in age-related loss of homeostasis. LINK-AGE Satellite Working Research Group Meeting. November 29-30, Noordwijkerhout, The Netherlands. (invited speaker)

Impact Factors:
D. Kletsas (for 6 publications): 18,317
H. Pratsinis (for 2 publications): 2,902

Citations 2008 (without self- citations):
D. Kletsas: 363
H. Pratsinis: 58

Total Citations 2005-2008 (without self- citations):
D. Kletsas: 1091
H. Pratsinis: 161

h-factor:
D. Kletsas: 18
H. Pratsinis: 9

Research Group: The Role of Nuclear proteins and Chromatin Function

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Research Interests

1) Studies 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 heterochromatic regions of chromatin and/or in the reorganization of euchromatic/heterochromatin regions of chromatin during ageing and apoptosis.

2) Concomitant to the above, the effects of histone deacetylase inhibitors in 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.

3) We are also studying the role of epigenetic histone modifications, such as acetylation and methylation, in changes in gene expression levels of age-related genes in peripheral blood leucocytes.

4) The acetylation of promoters of genes of the biological clock (circadian rhythm) of mammalian cell systems and how the products of these circadian genes affect cell cycle-related gene expression and carcinogenesis is also being studied.

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.

2008 Findings

1) We studied the phosphorylation levels of the DNA linker H1 histone somatic subtypes in human peripheral blood lymphocytes as a function of donor age and found a significant reduction of the mono-phosphorylated forms of the H1.4 and H1.5 subtypes in the elderly donors. This result was found to be related with the observed increase in the heterochromatin protein, HP1α, from lymphocyte lysates of the same elderly donors. These results for the first time associate the dephosphorylation of two histone H1 subtypes with an increase in a heterochromatin protein and the possible role that this specific epigenetic change may have in the reorganization or increase in heterochromatic regions of chromatin during ageing.

2) 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, two leukemic cell lines were selected from which chromatin was isolated 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 only cuts 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. Previous work of others, have shown that H1.5 and H1^{o} are localized in heterochromatic regions of chromatin (condensed chromatin). It is known that during the last stages of apoptosis condensed chromatin bodies are formed. Experiments are under way using an antibody for the heterochromatic protein, HP1α, so as to ascertain whether there is an increase in heterochromatin during apoptosis in our cell system.

3) Study of the epigenetic changes that occur around DNA loci of 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*) from peripheral blood from donors of different age group. Results from a limited number of donors 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 an elderly donor versus those from a young donor.

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

4) 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 and acetyltransferase 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 and curcumin, an inhibitor of the histone acetyltransferase, p300. With qPCR, we found that the expression levels of the clock genes *per1, per2,* and *cry1* as well as the genes *c-myc* and *wee1* change differentially with respect to the specific agent used. Moreover, we found that these changes are also influenced by the specific circadian time that the cell cultures were treated with these inhibitors.

The general aims of this study are being carried out within the framework of a research collaboration with the laboratory of Dr. Anastasia Prombona of the Institute of Biology N.C.S.R. "D".

5) 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. Preliminary experiments have shown a decrease in the H1.5 subtype, 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 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.

2008 Publications

Happel, N., Doenecke, D., Sekeri-Pataryas, K.E., Sourlingas, T.G. (2008). H1 Histone subtype constitution and phosphorylation state of the ageing cell system of human peripheral blood lymphocytes. Exp. Gerontol., 43, 184-199

Porras, A., Kozar, S., Russanova, V., Salpea, P., Hirai, T., Sammons, N., Mittal, P., Kim J.Y., Ozato, K., Romero, R. Howard, B.H. (2008). Developmental and epigenetic regulation of the human TLR3 gene. Mol. Immunol., 46, 27-36

2008 Presentations at International Scientific Conferences

Y. Ninios, K. E. Sekeri-Pataryas, T. G. Sourlingas,.Differential sensitivity of leukemic cell lines to trichostatin A. 33rd FEBS Congress. Athens, Greece, 2008.

P. Salpea, V. R. Russanova, K. E. Sekeri-Pataryas, B. H. Howard, T. G. Sourlingas. Age - related changes at the histone $H1^0$ gene region. 33rd FEBS Congress. Athens, Greece, 2008.

M. Xidous, K. E. Sekeri-Pataryas, A. Prombona, T. G. Sourlingas. Effects of the histone deacetylase inhibitor, trichostatin A, on clock and cell cycle-related gene expression levels as a function of circadian time in NIH3T3 mouse fibroblasts. 33rd FEBS Congress, Athens, Greece. 2008.

A. Repouskou, K. E. Sekeri-Pataryas, T. G. Sourlingas, A. Prombona. Resetting of the mammalian clock by TSA has phase-dependent effects on the expression levels of *c-myc* and *wee1* genes in N2A murine cells. 33rd FEBS Congress. Athens, Greece. 2008.

A. Koryllou, T. G. Sourlingas, H. Pratsinis, M. Patrinou-Georgoula, V. Pletsa. P53-dependent differential sensitivity of lung cancer cell lines to the chemotherapeutic agents *N*-methyl-*N*-nitrosourea and trichostatin A. 33rd FEBS Congress. Athens, Greece. 2008.

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Research Group: Cell & Matrix Biochemistry/Pathobiology

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Research Interests

- Transcriptional regulation of podocalyxin in cultured immortalized Human Glomerular Epithelial Cells (HGEC) under diabetic conditions.
- Biological activities of innovative nanoparticles for diagnostic and therapeutic use
- Effect of high glucose concentration on insulin survival signalling in pancreatic beta-cells
- Examination of the role of the enzyme MMP-9 in neurodegenerative conditions of the CNS/Alzheimer
- Differentiation of PC12 cells in the presence of NGF / the role of MMP-9
- Molecular mechanisms of atherosclerosis. Structure function relationship of proteins involved in lipoprotein metabolism pathways
- Role of lipids and lipoproteins in Alzheimer's disease. Insights on the relationship between apolipoprotein E4 and A_β metabolism in brain
- Regulation of glutamate transporters in physiological and neurodegenerative conditions
- Mechanisms of osteoblastic cell survival and apoptosis in conditions of inflammation

2008 Findings

1. Transcriptional regulation of podocalyxin in cultured immortalized Human Glomerular Epithelial Cells (HGEC) under diabetic conditions.
The anti-adhesin podocalyxin-like protein (PCLP) represents a differentiation marker for the glomerular epithelium. Binding of WT1 to conserved elements within the podocalyxin gene promoter

results in transcriptional activation. WT1 may participate in the induction of target genes directly, as well as through interactions with other transcriptional regulators, such as CBP. Prolonged exposure of HGEC to high (25mM) glucose results in suppression of PCLP expression compared to HGEC cultured in low (5mM) glucose. Immunoblotting, RT-PCR analysis and flow cytometry demonstrated that exposure of HGEC not expressing PCLP, to 5mM glucose for 24 weeks did not restore PCLP expression. Nuclear WT1 protein levels exhibited no significant differences in low vs. high glucose. In the presence of 5mM glucose, cross immunoprecipitation of WT1 and CBP from total cell lysates, indicated that cellular CBP co-precipitated with WT1. However, CBP co-precipitating with WT1 in the presence of 25mM glucose was decreased by 40%. We conclude that high glucose irreversibly impairs the ability of WT1 to initiate transcription in part by decreased association of WT1 with CBP. In the mature kidney, 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 ZO-1, catenins, and CD2AP. In vitro culturing of HGEC, in the presence of high glucose levels, also results in irreversible downregulation of nephrin expression. On the other hand, downregulation of ZO-1, CD2AP and podocin expression are completely reversible. In the presence of 5mM glucose levels both WT1 and Sp1 can bind to the podocalyxin and nephrin promoter regions as shown by ChIP analysis. HGECs continuously cultured in the presence of physiological glucose levels begin exhibiting reduced WT1 and Sp1 binding to both the podocalyxin and nephrin promoter regions, after being cultured in the presence of 25mM glucose. Reduced WT1 and Sp1 binding to the nephrin promoter region precedes the observed reduction on the podocalyxin promoter region. We have therefore concluded that Sp1 is a transcriptional co-regulator for WT1, translating changes in slit diaphragm structure into altered gene expression.

2. Mechanisms of pancreatic beta-cell apoptosis in type 2 diabetes

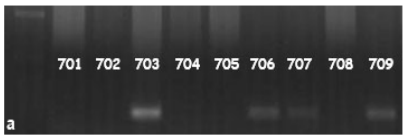
A) Role of insulin survival signalling. Type 2 diabetes is characterized by beta-cell dysfunction and apoptosis. Insulin signaling plays an important role in the growth and survival of β-cells. We demonstrated that chronic exposure of βTC-6 cells to increased glucose concentrations resulted in a significant inhibition of insulin signalling, including the phosphorylation of the insulin receptor and IRS-2 as well as the activation of IRS-2-associated PI3-kinase. These changes were also reflected in severe impairment of the sequential activation of Akt. Glucose-induced suppression of Akt signalling resulted in increased FoxO activation which was accompanied by enhanced caspase-3 activation and increased susceptibility of βTC6-cells to apoptosis. We found that glucose-induced downregulation of IRS-2 -mediated signalling was associated with increased IL-1β and SOCS-1 expression. These results suggest that glucose-induced IL-1β expression may attenuate IRS-2/Akt/FoxO signalling through the induction of SOCS-1 expression leading to increased susceptibility of β-cells to apoptosis. These data indicate an important role of insulin signaling in β-cell survival and suggest that glucose-induced defects in early steps of insulin signaling may contribute to β-cell apoptosis caused by chronic hyperglycemia in the pathogenesis of type 2 diabetes.

B) Role of cell-surface megalin: Megalin, a high-molecular-weight (~600 kDa) member of the low-density lipoprotein receptor family, is a major endocytic receptor. At clathrin-coated pits, megalin internalizes its ligands into endocytic compartments and is recycled to the cell surface. Megalin plays a critical role in vitamin, lipid and hormone homeostasis. In addition it has been proposed that megalin may regulate or involved in signalling pathways. The mechanisms regulating megalin expression have been largely unknown to date. We demonstrated that chronic exposure of βTC-6 cells to increased glucose concentrations resulted in a significant increase in both mRNA and protein levels of megalin. We hypothesized that glucose regulates megalin expression via the insulin pathway. The involvement of insulin signalling in the regulation of megalin expression is under investigation.

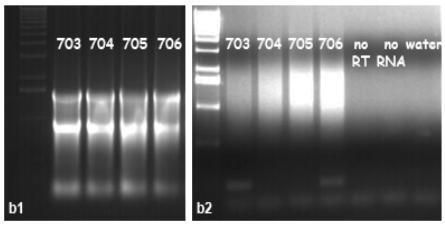
3. Examination of the role of the enzyme MMP-9 in neurodegenerative conditions of the CNS/Alzheimer

In vivo studies of the protective role of MMP-9 against amyloid plaque formation in an animal model of Alzheimer's disease. Experimental data showed that MMP-9 eliminates Ab peptide by processing

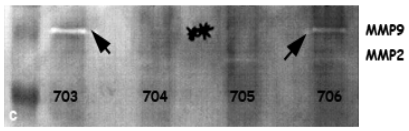
APP towards a-secretase cleavage. To study in vivo the effect of MMP-9 on reducing amyloid load in the brains of an Alzheimer's disease mouse model, we generated constructs containing the cDNA coding for wild type or auto activated forms of MMP-9. The constructs, were tested in a cell line and they were used to generate MMP-9-transgenic mice (fig 1a). MMP-9 transgenic mice will be intercrossed with single-transgenic human APP (hAPP) mice to generate double-transgenic animals (fig 1b). The overall strategy is to study the effects of MMP-9 on amyloid plaque formation in vivo.



a. PCR analysis using DNA isolated from tail tissue



b. RNA isolated from brain tissue (b1), used for reverse transcription and PCR with human MMP9 specific primers (b2)



c. Zymographic analysis using proteins isolated from brain tissue

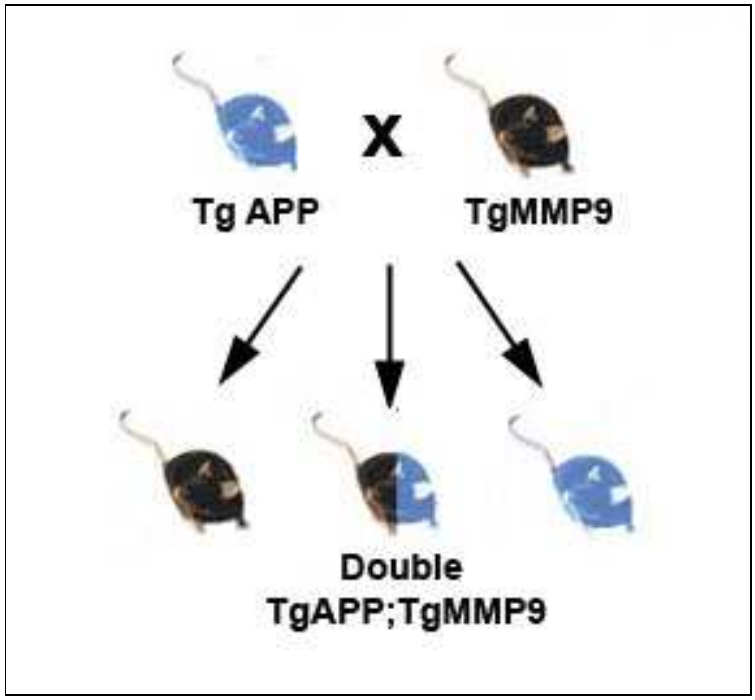


Figure 1a) subloning of the MMP-9 transgene in to mice DNA and detection of MMP-9 on brain homogenates by gelatin zymography. 1b) generation of double Tg mice (MMP-9+/APP+) to study the effect of MMP-9 on amyloid deposition

4. Differentiation of PC12 cells in the presence of NGF / the role of MMP-9: PC12 cells cultured in the presence of the neurotrophic factor NGF showed decreased levels of cellular APP with a concomitant increase in the levels of soluble sAPPα. Additionally, increased expression of the metalloproteinases MMP-2, MMP-9 and ADAM17 (TACE) was observed. The increase was inhibited in the presence of SB-3CT, specific inhibitor of MMPs. Therefore, the role of α-secretases (MMP-9, TACE) in the differentiation of PC12 cells by NGF is under investigation.

5. Mechanisms of osteoblastic cell survival and apoptosis in conditions of inflammation

Study of the protective role of TIMP-1 against TNF-α-induced apoptosis in osteoblastic cells MG63. Results from our study suggest that the specific inhibitor of metalloproteinases TIMP-1 confers protection against TNF-α-induced apoptosis through its interaction with $\alpha v\beta 3$ integrin receptor

6. Molecular mechanisms of atherosclerosis. Structure function relationship of proteins involved in lipoprotein metabolism pathways.

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) Adenovirus-mediated gene transfer in apoA-$I^{-/-}$ mice showed that negatively charged residues of apoA-I Asp89, Glu91 and Glu92 are critical for the normal functions of HDL and when mutated to Ala, cause severe dyslipidemia and accumulation of HDL particles with altered composition which may be ineffective in protecting from coronary heart disease. The discrete phenotypes observed in mice expressing mutations in apoA-I, ABCA1, LCAT or other proteins of HDL metabolism pathway, may help in the diagnosis of similar phenotypes in humans and may be used as new biomarkers for the diagnosis and prognosis of dyslipidemia and/or atherosclerosis.

B) Compositional, structural and functional characterization of HDL from humans, with low HDL cholesterol levels and specific mutations in the cholesterol transporter ABCA1 or the enzyme LCAT, showed a similar phenotype with that observed in mice expressing apoA-I mutations that reduce the apoA-I interactions with ABCA1 or LCAT.

C) The study of apoA-I/ABCG1 interactions showed that ABCG1-mediated cholesterol efflux was greatly reduced by a C-terminal deletion mutant of apoA-I

7. Role of lipids and lipoproteins in Alzheimer's disease. Insights on the relationship between apolipoprotein E4 and A_β metabolism in brain.

A hallmark of Alzheimer's disease is the deposition of plaques containing amyloid β peptide (A_β) in the brain. Brain cells cholesterol levels and proteins involved in cholesterol metabolism can affect the formation 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. 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 development of neuronal degeneration observed in AD.

A) The carboxy-terminal truncated apoE4 form, apoE4[Δ(166-299)], leads to uptake of exogenously added Aβ(1-40) and Aβ(1-42) from human neuroblastoma cells, as compared to WT apoE4. It was found that apoE4[Δ(166-299)] leads to 20% reduction of sphigomyelin levels of cells. 50% of the uptaken Aβ(1-42) remains in cells after 24h and leads to formation of reactive oxygen species (ROS), in contrast to Aβ(1-40) that cannot be detected in cells after 3h and does not lead to ROS formation.

B) Biophysical characterization of progressive C-terminal truncations of apoE4 (residues 166-299) showed that the C-terminal region of apoE4 is important for the structural plasticity of apoE4. The C-terminal deletion of apoE4 does not affect the molecule stability but leads to loss of structural plasticity that may be responsible for altered functionality of truncated apoE4 forms

2008 Publications

Chroni A.*, Pyrpassopoulos S., Thanassoulas A., Nounesis G., Zannis V. I. and Stratikos E. (2008) Biophysical analysis of progressive C-terminal truncations of human apolipoprotein E4: insights into secondary structure and unfolding properties. *Biochemistry*, 47, 9071-9080. * corresponding author

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Zannis, V. I., Koukos, G., Drosatos, K., Vexerides, A., Zanni, E. E., Kypreos, K. E. and Chroni, A. (2008) Discrete Roles of ApoA-I and ApoE in the Biogenesis of HDL Species: Lessons Learned from Gene Transfer Studies in Different Mouse Models. *Ann. Med.,* 40, 14–28.

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Chroni A., Pyrpassopoulos S., Thanassoulas A., Nounesis G., Zannis V.I. and Stratikos E.. Role of the C-terminus in the secondary structure stability and unfolding properties of apolipoprotein E4. *The FEBS Journal*, 275 supplement 1, p. 161, PP3A-17 (2008).

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Petraki M., Chroni A. and Tselepis A. Effect of recombinant lipoprotein-associated phospholipase A2 (Lp-PLA2) on cholesterol efflux from macrophages in culture. *The FEBS Journal*, 275 supplement 1, p. 424, PP8-149 (2008).

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E. C. Tsilibary, P. D. Venieratos, G.I. Drosopoulou and P. V. Kitsiou. Endogenous IL-1β and SOCS-1 induce cell apoptosis through suppression of the insulin signalling pathway in mouse pancreatic β-cells cultured in high glucose. FEBS J. Vol. 275 (S1), p 321. 33rd FEBS Congress and 11th IUBMB Conference Biochemistry of Cell Regulation, June 28-July 3, 2008, Athens, Greece

Dafnis J., Stratikos E., Tzinia A., Tsilibari E.C. and Chroni A(2008). Carboxy-terminal truncated apolipoprotein E4 reduces amyloid peptide beta (Aβ) levels in neuronal cells. FEBS Congress & 11th IUBMB Conference Athens, Greece

I. Tsagaraki, E. Tsilibary, D. Kletsas and A. Tzinia. TIMP-1 association with integrins promotes cell survival by imparting resistance to TNF-α-induced apoptosis in MG63 osteosarcoma cells(2008).. 33rd FEBS Congress & 11th IUBMB Conference Athens, Greece

Drossopoulou G., Tsotakos N, Kotsopoulou E, Tsilibary, EC (2008): Glucose-induced changes of slit diaphragm proteins are reversible and may not result in podocytic foot process effacement. 20th Annual Meeting of the European Renal Cell Atudy Group (ERCSG), 10-13 April, 2008, Gregynog Hall, Powys, Wales

Petraki M. P., Chroni A., Tselepis A. D. (2008) Recombinant PAF-acetylhydrolase enhances the cholesterol efflux from macrophages induced by reconstituted high density lipoprotein or apolipoprotein A-I. *77th EAS Congress*, April 26-29,2008, Istanbul, Turkey

Kateifides A., Koukos G., Chroni A., Duka A. and Zannis V. I. (2008) Adenovirus-Mediated Gene Transfer of ApoAI Mutants in ApoA-I-Deficient Mice Identified Discrete Steps in the Pathway of Biogenesis of HDL and the Role of Specific Residues 89 to 96 Region in Dyslipidemia. *American Society of Gene Therapy*, *11th Annual Meeting*, May 28–June 1, 2008, Boston, MA, USA

Chroni A., Pyrpassopoulos S., Thanassoulas A., Nounesis G., Zannis V.I. and Stratikos E. (2008) Role of the C-terminus in the secondary structure stability and unfolding properties of apolipoprotein E4. *33rd FEBS Congress & 11th IUBMB Conference*, 28 June-3 July, 2008, Athens, Greece

Dafnis J., Stratikos E., Tzinia A., Tsilibary E. C. and Chroni A. (2008) Carboxy-terminal truncated apolipoprotein E4 reduces amyloid peptide beta (Aβ) levels in neuronal cells. *33rd FEBS Congress & 11th IUBMB Conference*, 28 June-3 July, 2008, Athens, Greece

Petraki M., Chroni A. and Tselepis A. (2008) Effect of recombinant lipoprotein-associated phospholipase A2 (Lp-PLA2) on cholesterol efflux from macrophages in culture. *33rd FEBS Congress & 11th IUBMB Conference*, 28 June-3 July, 2008, Athens, Greece

Impact Factors: A. Chroni (for 3 publications): 9,147

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(Kitsiou P): 7
(A. Chroni): 31

Total Citations 2005-2008 (without self- citations): 407
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(Tzinia A, Tzinia AK): 52
(Kitsiou P): 34
(A. Chroni): 167

h-factor: E. Tsilibary:29 , A. Tzinia:7, P. Kitsiou: 5, A. Chroni:10

Research Group: Environmental Mutagenesis -Carcinogenesis

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Research Interests

1. Identification and validation of drug targets for cancer therapy
2. Development and evaluation of biomarkers for diagnosis and prognosis of human diseases
3. Development of protocols for molecular diagnosis of human genetic diseases

2008 Findings

1. Low frequency of the glutathione-S-transferase T1-null genotype in patients with primary myelodysplastic syndrome and 5q deletion

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of acquired clonal hematopoietic stem-cell disorders, characterized by ineffective hematopoiesis and a variable risk of transformation to acute myeloid leukemia. The genes coding for the isoforms glutathione-S-transferase-theta-1 (GSTT1) and -mu-1 (GSTM1), exhibit an inherited homozygous deletion polymorphism (null genotype), resulting in total lack of enzymatic activity. To investigate the association and potential role of GST polymorphisms in MDS pathogenesis within a group of individuals with highly homogeneous ethnic background, we studied the *GSTM1* and *GSTT1* genotypes in a large cohort of Greek 323 patients with primary MDS and 330 healthy individuals. We found that no significant ratio differences of the *GSTT1* and *GSTM1* null genotypes existed between MDS patients and controls. But, when we compared the null genotypes with different karyotype abnormalities, we found that the *GSTM1* null genotype was present in a high percentage (60%) of MDS patients with 7q deletion, whereas the *GSTT1* null genotype was present in only 2/47 cases (4.2%) with a 5q deletion, who also had a number of other cytogenetic abnormalities. This finding suggests a possible causative role of *GSTT1* gene expression in the pathogenesis of this cytogenetic abnormality in primary MDS.

2. Cloning and functional characterization of the ovine Hormone Sensitive Lipase (HSL) full-length cDNAs

Hormone Sensitive Lipase (HSL) is a highly regulated enzyme that mediates lipolysis in adipocytes. HSL enzymatic activity is increased by adrenergic agonists, such as catecholamines and glucagons, which induce cyclic AMP (cAMP) intracellular production, subsequently followed by the activation of Protein Kinase A (PKA) and its downstream signalling cascade reactions. Since HSL constitutes the key enzyme in the regulation of lipid stores and the only enzyme being subjected to hormonal regulation [in terms of the recently identified Adipose Triglyceride Lipase (ATGL)], the ovine Hormone Sensitive Lipase (ovHSL) full-length cDNA clones were isolated, using a Polymerase Chain Reaction-based (PCR) strategy. The two isolated isoforms ovHSL-A and ovHSL-B contain two highly homologous Open Reading Frame (ORF) regions of 2.089 Kb and 2.086 Kb, respectively, the latter having been missed the 688th triplet coding for glutamine (ΔQ
688). The putative 695 and 694 amino acid respective sequences bear strong homologies with other HSL protein family members. Southern blotting analysis revealed that HSL is represented as a single copy gene in the ovine genome, while Reverse Transcription-PCR (RT-PCR) approaches unambiguously dictated its variable transcriptional expression profile in the different tissues examined. Interestingly, as undoubtedly corroborated by both RT-PCR and Western blotting analysis, ovHSL gene expression is notably enhanced in the adipose tissue during the fasting period, when lipolysis is highly increased in ruminant species. Based on the crystal structure of an Archaeoglobus

fulgidus enzyme, a three-dimensional (3D) molecular model of the ovHSL putative catalytic domain was constructed, thus providing an inchoative insight into understanding the enzymatic activity and functional regulation mechanisms of the ruminant HSL gene product(s).

3. A PCR-based integrated protocol for the structural analysis of the 13th exon of the human β-myosin heavy chain gene (MYH7): Development of a diagnostic tool for HCM disease

Familial Hypertrophic Cardiomyopathy (FHC) constitutes a genetic disease of the sarcomere characterized by a Mendelian pattern of inheritance. A variety of different mutations affecting the at least eight sarcomeric gene products has been identified and the majority of them appear to function through a dominant negative mechanism. Family history analysis and genetic counseling have been widely adopted as integral tools for the evaluation and management of individuals with Hypertrophic Cardiomyopathy (HCM). Genetic testing of the disease has been progressively released into the clinical mainstream, thus rendering the development of novel and potent molecular diagnostic protocols an inevitable task. To this direction, we have evolved an integrated PCR-based molecular protocol, which through the utilization of novel "exonic" primers allows, among others, the structural analysis of the 13th exon of the human β-myosin heavy chain gene locus (MYH7) mainly characterized by the critical for HCM Arginine residue 403 (R403). Interestingly, through a DNA sequencing approach, a single nucleotide substitution from "G" to "T" was detected in the adjacent 13^{th} intron, thus divulging the versatile potential of the present molecular protocol to clinical practice.

4. Imatinib mesylate inhibits proliferation and exerts an antifibrotic effect in human breast stroma fibroblasts

Tumor stroma plays an important role in cancer development. In a variety of tumors, such as breast carcinomas, a desmoplastic response, characterized by stromal fibroblast and collagen accumulation, is observed having synergistic effects on tumor progression. However, the effect of known anticancer drugs on stromal cells has not been thoroughly investigated. Imatinib mesylate is a selective inhibitor of several protein tyrosine kinases, including the receptor of platelet-derived growth factor, an important mediator of desmoplasia. Recently, we have shown that imatinib inhibits the growth and invasiveness of human epithelial breast cancer cells. Here, we studied the effect of imatinib on the proliferation and collagen accumulation in breast stromal fibroblasts. We have shown that it blocks the activation of the extracellular signal-regulated kinase and Akt signaling pathways and up-regulates cyclin-dependent kinase inhibitor p21WAF1, leading to the inhibition of fibroblast proliferation, by arresting them at the G0/G1 phase of the cell cycle. Imatinib inhibits more potently the platelet-derived growth factor–mediated stimulation of breast fibroblast proliferation. By using specific inhibitors, we have found that this is due to the inhibition of the Akt pathway. In addition, imatinib inhibits fibroblast-mediated collagen accumulation. Conventional and quantitative PCR analysis, as well as gelatin zymography, indicates that this is due to the down-regulation of mRNA synthesis of collagen I and collagen III—the main collagen types in breast stroma—and not to the up-regulation or activation of collagenases matrix metalloproteinase 2 and matrix metalloproteinase 9. These data indicate that imatinib has an antifibrotic effect on human breast stromal fibroblasts that may inhibit desmoplastic reaction and thus tumor progression.

5. Molecular Genetic Diagnosis of the Tuberous Sclerosis Complex

Tuberous sclerosis (TSC) is an inherited autosomal dominant disorder with an incidence of 1/5.800 births. Diagnosis of the disease is based on clinical findings, while its accuracy is critical in order to detect and treat symptomaticneurological, renal, cardiac and pulmonary lesions, as these are the main causes of death. Since no single feature is unique to the condition, often diagnosis may come after much delay and uncertainty. Genes *TSC1* and *TSC2* were linked to TSC. Both are tumor suppressors, while their products were demonstrated to be involved in the negative regulation of cell growth and proliferation acting upstreamof mTOR. Mutations in TSC1 and TSC2 were shown to be responsible for the development of TSC. This indicates the importance of the genetic test for the disease, not only for fast and accurate diagnosis but also for genetic counselling and pre-natal diagnosis. Finally, while management of TSC largely remains symptomatic, recent discoveries on the upregulation of the mTOR pathway in TSC have allowed clinical trials to begin using targeted drugs for novel therapeutic intervention approaches.

6. Cloning and functional characterization of the 5′ regulatory region of ovine Hormone Sensitive Lipase (*HSL*) gene

Hormone Sensitive Lipase (HSL) catalyzes the rate-limiting step in the mobilization of fatty acids from adipose tissue, thus determining the supply of energy substrates in the body. HSL enzymatic activity is increased by adrenergic agonists, such as catecholamines and glucagons, which induce cyclic AMP (cAMP) intracellular production, subsequently followed by the activation of Protein Kinase A (PKA) and its downstream signaling cascade reactions. HSL constitutes the critical enzyme in the modulation of lipid stores and the only component being subjected to hormonal control in terms of the recently identified Adipose Triglyceride Lipase (ATGL). In order to acquire detailed knowledge with regard to the mechanisms regulating ovine HSL (ovHSL) gene transcription activity, we initially isolated and cloned the 5′ proximal and distal promoter regions through a genome walking approach, with the utilization of the already characterized ovHSL cDNAs. As evinced by BLAST analysis and a multiple alignment procedure, the isolated genomic fragment of 2.744 kb appeared to contain the already specified 5′-untranslated region (5′-UTR), which was interrupted by a relatively large intron of 1.448 kb. Regarding the upstream remaining part of 1.224 kb, it was demonstrated to represent a TATA-less promoter area, harboring several cis-regulatory elements that could be putatively recognized by relatively more general transcription factors, mainly including Stimulating protein 1 (Sp1), CCAAT-box Binding Factors (CBFs), Activator Protein 2 (AP2) and Glucocorticoid Receptor (GR), as well as other cis-acting regions denominated as Insulin Response Element (IRE), Glucose Response Element (GRE), Fat Specific Element (FSE) and cAMP Response Element (CRE), which could likely function in a nourishment (i.e. glucose)-/hormone-dependent fashion. When different genomic fragments were directionally (5′ to 3′) cloned into a suitable reporter vector upstream of a promoter-less luciferase gene and transiently transfected into 3T3-L1 (mouse fibroblasts) as well as T24 (human bladder cancer) cell lines, strong promoter activities were unambiguously detected, with the −140/+18 nucleotide sequence bearing the highest transcriptional response, thus indicating that the 1.224 kb 5′ flanking region, isolated by genome walking, veritably contains the ovHSL gene promoter. Of particular significance are the observations that the functional promoter fragments could trigger the transcriptional activity of luciferase gene only under high concentration of glucose conditions in both cell lines.

2008 Publications

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Lampidonis, A.D., A. Argyrokastritis, D.J. Stravopodis, G.E. Voutsinas, T.G. Ntouroupi, L.H. Margaritis, I. Bizelis and E. Rogdakis (2008) Cloning and functional characterization of the ovine Hormone Sensitive Lipase (HSL) full-length cDNAs: an integrated approach, Gene 416, 30-43. (IF: 2.721)

Stravopodis, D.J., A.Z. Zapheiropoulos, G.E. Voutsinas, L.H. Margaritis and I.S. Papassideri (2008) A PCR-based integrated protocol for the structural analysis of the 13th exon of the human beta-myosin heavy chain gene (MYH7): Development of a diagnostic tool for HCM disease, Exp Mol Pathol 84, 245-250. (IF: 1.377)

Gioni, V., T. Karabinas, G. Voutsinas, A.E. Roussidis, S. Papadopoulos, N.K. Karamanos and D. Kletsas (2008) Imatinib mesylate inhibits the proliferation and collagen synthesis in human breast stromal fibroblasts, Molecular Cancer Research 6, 706-714. (IF: 4.759)

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PROGRAMME B:

MODEL SYSTEMS FOR THE STUDY OF CELL FUNCTION

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Research Interests

1. Regulatory mechanisms controlling insect physiological functions: (i) Oogenesis in lepidopteran insects: a model for differentiation programs induced by ecdysteroid hormones; (ii) 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; (iii) Mechanisms controlling olfactory function in the malaria mosquito *Anopheles gambiae*

2. Molecular Biology and genetic manipulation of insect nuclear polyhedrosis viruses: (i) Viruses expressing proteins harmful to the insect hosts; (ii) Incapacitated viruses as vectors for insect genetic transformation; (iii) Modified viruses as vectors for human gene therapy and cellular immunization.

3. Functional genomics: (i) Systems for production of proteins of economic importance in lepidopteran insect and mammalian cell lines; **(ii**) Cell-based high throughput screening platforms for identification of bioactive substances (activators and inhibitors of pharmacological targets) in synthetic compound libraries and collections of natural products (plants and microorganisms).

2008 Findings

Regulatory control of oogenesis in lepidopteran insects
RNA interference is a very useful technology that may be used for the investigation of gene function in organisms not readily amenable to classical genetic analysis. The feasibility of the approach in the domesticated silkmoth, *Bombyx mori*, was investigated through experiments employing Bm5 cells, a silkmoth-derived cell line. Transfection of double stranded RNA or hairpin RNA-encoding expression constructs has shown that specific RNAi-mediated gene silencing may be induced in Bm5 cells.

Molecular mechanisms of endoparasitoidism in lepidopteran insects
The functions of proteins of the endosymbiotic virus CcBV of the endoparasitoid wasp Cotesia *congregata*, which is injected in the lepidopteran host *Manduca sexta* during its parasitism by *Cotesia*, are studied at two levels. The first one concerns the interaction of viral proteins with proteins of the host and the elucidation of the role of the interactions in the suppression of the immune response that the host is normally capable of mounting. The second level concerns on the elucidation of the functional roles of the members of a family of viral proteins, Vank, which display extensive similarities with the ankyrin repeats of the IκBα inhibitor of the mammalian transcription factor and major regulator of immune response genes NF-κB.

1. The interactions of CcV1 with proteins of the parasitized host
Our previous studies on the functional characterization of CcV1 had shown that the protein interacts with hemolin, an immune protein of host hemocytes, and this interaction influences the physiology of the hemocytes. We expanded our studies by examining the possible role of CcV1 protein in the

melanization process, an important mechanism of cell-mediated insect defence. Our *in vitro and in vivo* results suggest that CcV1 inhibits the melanization reaction. In a parallel line of investigation, we have cloned the open reading frame of *Bombyx mori* hemolin into an expression vector that directs recombinant protein expression in lepidopteran insect cells. The recombinant protein is currently used in the context of a baculovirus infection in order to deduce whether hemolin is capable of mounting an anti-virus response in the infected host.

2. The effect of CcBV Ank proteins on* NF-κB promoter-directed *transcription

To clarify the role of proteins expressed by the *ank* family genes of CcBV, we examined six Ank family members, upon expression in mammalian HEK293 cells, for their ability to inhibit NFκB-dependent transcription. Our results showed that at least two of these proteins are capable of inhibiting the TNFα factor-triggered transcription of a reporter gene (luciferase) placed under the control of an NFκB-responsive promoter. Specifically, we showed that the Ank4 protein had the strongest inhibitory effect on NF-κB-dependent transcription in the mammalian cell line. We also examined the ability of the CcBV Ank proteins to repress or reduce the expression of antimicrobial proteins, another important component of insect innate immunity, by expressing the Ank proteins in different insect cell lines. The assessment of transcriptional induction of reporter genes driven by antimicrobial protein (cecropin and attacin) gene promoters in cells co-transfected with constructs expressing the transcription factors *Bm*RelA, *Bm*RelB and *Bm*Relish1 of *Bombyx* as well as the different Ank proteins, is in progress.

Regulatory control of mosquito olfaction

We continued our studies on the structural and functional characterization of olfactory receptors (ORs) that are present on the surface of the olfactory neurons of the mosquito *Anopheles gambiae.* These receptors recognize and bind, in a specific fashion, small volatiles that act as guiding cues and direct the mosquitoes to their feeding and/or breeding sites. The ORs, members of the superfamily of metazoan heptahelicoidal receptors, are anchored on the plasma membrane of olfactory neurons. To establish the topology of the mosquito ORs on the plasma membranes and deduce the likelihood that they represent members of the G protein coupled receptor (GPCR) family, we generated chimeric receptors containing, at their N- or C-termini, reporter proteins whose function can be monitored only when they are expressed intracellularly. Using this approach, we established that two mosquito ORs that we examined (OR1 and OR2) are anchored on the plasma membrane in a manner opposite to that of GPCRs, i.e., N-terminus in and C-terminus out, irrespective of the presence of their heterodimerization partner OR7 in the cells. This finding casts doubt on the previously assumed fashion of OR signaling and suggests that mosquito ORs may not signal through G proteins. The fashion of OR signaling is under investigation.

In parallel studies, we focussed attention on the functional characterization of the high throughput screening platforms that we developed previously for the detection of ligand mimetics for ORs and odorant binding proteins (OBPs) of *A. gambiae*, because such platforms are expected to contribute in an important way toward the discovery of new agents for the behavioral management of the mosquito populations and a reduction in the frequency of malaria parasite transmission to humans. These activities are described under the «Functional genomics» section below.

Molecular biology and genetic engineering of insect nuclear polyhedrosis viruses

Using transformed Sf21 cells that over-express the viral co-activator IE-1, baculovirus particles in *ie-1* function (IE-1 knockout viruses) were generated. The functional properties of the IE-1 knockout viruses were subsequently studied after infection of insect and mammalian cells. Because dramatically lower levels of baculovirus gene expression were seen in mammalian cells infected with the knockout relative to cells infected with wild type virus, it is predicted that IE-1 knockout baculoviruses will be safer gene transduction vectors relative to their wild-type counterparts.

Baculoviruses that have been modified to incorporate the PiggyBac transposition system in their genome were also generated. Upon infection of mammalian cells, efficient transgene incorporation in the host genome was observed, confirming the potential of baculovirus vectors for stable transgene

expression in mammalian cells and, in the long terms, for gene therapy applications involving stable expression of therapeutic proteins in transduced cells derived from patient donors.

Functional genomics

A high throughput screening system for ecdysteroid analogs specific for coleopteran insects (beetles) was established using cell lines derived from the weevil *Anthonomus grandis*. Interestingly, although dibenzoylhydrazine analogs have been applied in coleopteran pest control, the compounds show little activity in the *in vitro* reporter system (collaboration with Dr. Guy Smagghe, University of Ghent, Belgium). To investigate mechanisms of resistance against insecticides that are based on activation of the ecdysone receptor, lepidopteran and dipteran cell lines that are resistant to growth inhibition by ecdysteroids were analyzed for expression of genes of the ecdysone regulatory cascade. It was found that resistance mechanisms differ between dipteran and lepidopteran cell lines.

Last year we generated a series of cell lines that over-express odorant receptors (ORs) of the malaria mosquito vector, *Anopheles gambiae*, in authentic or tagged forms, and signaling molecules such as Gαs, Gαq or Gα16. The cell lines are characterized with respect to OR signalling properties. Furthermore, a number of *Anopheles* odorant-binding proteins (OBPs) were purified from transformed lepidopteran cell lines that were used for their expression and used in ligand binding experiments. The OR expressing cell lines and the purified OBPs will be used in large scale screening protocols aimed at the isolation of odorant mimetics that could be employed toward the disruption of the mosquito olfactory responses.

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Iatrou, K. and Biessmann, H. (2008). Sex-biased expression of odorant receptors in antennae and palps of the African malaria vector Anopheles gambiae. Insect Biochem. Mol. Biol. 38, 268-274.

Soin, T., Swevers, L., Mosallanejad, H., Efrose, R., Labropoulou, V., Iatrou, K., Smagghe, G. (2008). Juvenile hormone analogs do not affect directly the activity of the ecdysteroid receptor complex in insect culture cell lines. J. Insect Phys. 54, 429-438.

Labropoulou V., Douris, V., Stefanou, D., Magrioti, C., Swevers, L. and Iatrou, K. (2008). Endoparasitoid wasp bracovirus-mediated inhibition of hemolin function and lepidopteran host immunosuppression. Cell. Microbiol. 10, 2118–2128.

Swevers, L., Soin, T., Mosallanejad, H., Iatrou, K. and Smagghe, G. (2008). Ecdysteroid signaling in ecdysteroid-resistant cell lines from the polyphagous noctuid pest *Spodoptera exigua*. Insect Biochem Mol Biol. 38, 825–833.

Mosallanejad, H., Soin, T., Swevers, L., Iatrou, K., Nakagawa, Y. and Smagghe, G. (2008). Non-steroidal ecdysteroid agonist chromafenozide: gene induction activity, cell proliferation inhibition and larvicidal activity. Pesticide Biochem. Physiol. 92, 70-76.

2008 Proceedings to Conferences

Swevers, L. and Iatrou, K. (2009). Ecdysteroids and Ecdysteroid Signaling Pathways During Insect Oogenesis. In: "*Ecdysone, structures and functions*" (G. Smagghe, ed.), Part II: In the Post-genomic Era, Ecdysteroid Genetic Hierarchies in Insect Growth and Reproduction. Springer Science, pp. 129-161.

2008 Presentations at International Scientific Conferences

Efrose, R., L. Swevers, V. Douris, A. Lavdas, R. Matsas and K. Iatrou. (2008). Baculovirus engineering for gene therapy: evaluation of a candidate therapeutic gene for neural disease and trauma and production of vectors devoid of residual viral gene expression. 11th Annual Baculovirus & Insect Cell Culture meeting, Seattle, WA, USA, February 25-27.

Magrioti C, Iatrou K. and Labropoulou V (2008). Interactions between proteins of the symbiotic polydnavirus CcBV of the endoparasitic wasp Cotesia congregate and proteins of the NFkB pathway in insect and mammalian cell lines. 33rd FEBS Congress & 11th IUBMB Conference, June 28 -July 3, 2008, Athens, Greece.

Swevers, L., Soin, T., Mosallanejad, H., Iatrou, K., and Smagghe, G. (2008). Wild-type ecdysteroid receptor signalling in ecdysteroid-resistant cell lines from the polyphagous noctuid pest *Spodoptera exigua*. 17th Ecdysone Workshop, July 20-24, Ulm, Germany.

Mosallanejad, H., Soin, T., Swevers, L., Iatrou, K., Smagghe, G. (2008). Comparative analysis for ecdysteroid receptor functionality in selected lepidopteran and dipteran methoxyfenozide-resistant insect cell lines. 17th International Ecdysone workshop. Ulm University, Ulm, Germany, 20-24 July 2008.

Soin, T., Mosallanejad, H., Martín, D., Iatrou, K., Nakagawa, Y., Smagghe, G., and Swevers, L. (2008). Comparison of the activity of ecdysone agonists in *Bombyx mori* and *Spodoptera littoralis* by *in vitro* reporter assays and *in vivo* toxicity assays. 17th Ecdysone Workshop, July 20-24, Ulm, Germany.

Gotsis-Skretas, O. and Ignatiades, L. (2008). Biodiversity and species rarity of phytoplankton in the Aegean Sea, Eastern Mediterranean. Proceedings of the 43rd "EMBS' – European Marine Biology Symposium, Ponta Delgada, Azores, Portugal, September 2008. (p. 81).

Impact Factors (for 5 publications): 14,719

Citations 2008 (without self- citations): 158

Iatrou K. (Citations by Swevers L. and Lampropoulou V. are included): 79
Swevers L (35 Citations by Iatrou K. are included): 36
Lampropoulou V. (6 Citations by Iatrou K. and Swevers L. are included) : 37
Ignatiades L.: 47

Total Citations 2005-2008 (without self- citations):583
Iatrou K. (Publications by Swevers L. and Lampropoulou V. are included): 248
Swevers L.: 111
Lampropoulou V.: 147
Ignatiades L.: 179

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

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.
- 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.

2008 Findings

Participation in the ENAROMaTIC consortium through collaboration with Prof. Iatrou, project coordinator and head of the Laboratory of "Insect Molecular Genetics and Biotechnology. The project aims to constrain the transmission of malaria by interfering with the mosquitos' ability to detect odors of human origin in their environment. In the framework of ENAROMaTIC a substantial quantity of plant material from over 200 indigenous species has been collected and the volatile secondary metabolites from the plant tissues have been trapped by means of steam distillation. A high throughput system (HTS) assay for the detection of chemical substances capable to interfere with the olfactory perception of mosquitoes is currently under way.

The toxicity of the bioactive secondary metabolites produced by the entomotoxic strain (SMU-21) of *Mucor hiemalis* were tested against insects of the Aphididae family. The toxicity levels and the mode of action of these metabolites were evaluated.

Research for semiochemicals with kairomonal action on parasitoids of the genus *Trichogramma* was continued, under the scope that they will aid substantially in the effectiveness of utilizing such egg parasitoids in integrated pest management programs. Under this scope volatile compounds emanating from olive leaves, flowers and fruits were tested in behavioral bioassays.

The use of the IR 805 Cantronic infra-red thermal photographic camera is a new approach in the detection of palm trees infested by the red palm weevil (*Rhynchophorus ferrugineus*). In the present preliminary study we present thermographic images of palm trees from different urban areas with high infestation records, aiming to develop a reliable high throughput system for the rapid screening of large number of palm trees in areas where conventional monitoring method are either inapplicable or inadequate

In preliminary field studies the mass trapping method in pheromone baited traps against the leopard moth, *Zeuzera pyrina* has been performed in olive orchards in Egypt. Also, a system for mass trapping and monitoring click beetles of the genus *Agriotes* was deployed in three areas of central Greece.

2008 Publications

Siskos, E.P., Mazomenos, B.E,. Konstantopoulou, M.A. (2008). Isolation and Identification of insecticidal components from *Citrus aurantium* fruit peel extract. Journal of Agricultural and Food Chemistry 56(14): 5577-5581.

Hegazi, E.M., Konstantopoulou, M.A., Herz, A., Mazomenos, B.E,. Khafagi, W.E., Agamy, E., Zaitun, A., Abd El-Aziz, G.M., Showiel, S., Abdel-Rahman, S.M., (2008). Is mating disruption effective in controlling the olive moth, *Prays oleae*? Crop Protection DOI: 10.1016/j.cropro.2008.10.005.

Siskos, E.P., Konstantopoulou, M.A,. Mazomenos, B.E., (2008). Insecticidal activity of *Citrus aurantium* peel extract against of *Bactrocera oleae* and *Ceratitis capitata* adults (Diptera: Tephritidae). Journal of Applied Entomology (accepted-in press) DOI: 10.1111/j.1439-0418.2008.01312.x.

Milonas P., Mazomenos, B.E., Konstantopoulou, M., (2008). Kairomonal effect of sex pheromone components of two Lepidopteran olive pests on *Trichogramma* wasps. Insect Science (accepted-in press). DOI 10.1111/j.1744-7917.2009.01245.x.

2008 Presentations at International Scientific Conferences

PG Milonas, A. Martinou, DC Kontodimas, MA Konstantopoulou (2008). Response of *Trichogramma* egg parasitoids toward the synthetic sex pheromone (*Z*)-7-Tetadecenal, of the olive moth *Prays oleae* (Bern). 25th Anniversary International Society of Chemical Ecology Meeting, August 17-22, State college, Pennsylvania, USA, 2008. Abstract: 136.

Impact Factors (for 4 publications): 5,43

Citations 2008 (without self- citations): 18

Total Citations 2005-2008 (without self- citations): 54

h-factor: 5

Research Group: Chronobiology

Research Staff

Anastassia Prombona, Senior Researcher
Anastasia Repouskou, Graduate Student
Marianna Kapi, Undergraduate Student – *undergraduate dissertation completed in 2008*
Dafni Antoniou, Undergraduate Student
Irene Nomikou, Undergraduate Student – *undergraduate dissertation completed in 2008*
Aggeliki Galeou, Training Student

Research Interests

- *Investigation of the biological clock function in plants*

Study of the biological clock function 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 components of the biological clock in mouse fibroblasts and cancer cell lines. Study of the effects of modulated histones' 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 to achieve improved therapy (chronotherapy).

2008 Findings

Investigation of the biological clock function in plants
In resetting experiments of *Phaseolus vulgaris* plants synchronized to different photoperiods, *PvTOC1* exhibits at specific circadian times a light response. This indicates the involvement of PvTOC1 in the differential light induction of *PvLHY* during light synchronization. On the other hand, *PvELF4* seems not to be implicated in this mechanism.

Involvement of the biological clock function in carcinogenesis
Our study focused on gene expression of *per1,* as the representative reporter of the biological clock function and of *c-myc* for its importance in the G_0/G_1 transition of the cell cycle and in carcinogenesis. N2A mouse neuroblastoma cells exhibited oscillating *c-myc* expression levels under conditions of serum deprivation, whereas serum addition to the culture medium leads to a loss of circadian rhythmicity. Moreover, the role of trichostatin A (inhibitor of histone deacetylases) in clock synchronization at different circadian times is under investigation as well as the role of transcription factor CLOCK in the regulation of expression of *c-myc*. This project is carried out in collaboration with Dr. T. Sourlingas ('The Role of Nuclear Proteins and Chromatin Function' group).

2008 Presentations at Inrernational Scientific Conferences

Repouskou A, Sekeri-Pataryas KE, Sourlingas TG, Prombona A (2008). *Resetting of the mammalian clock by TSA has phase-dependent effects on the expression levels of c-myc and wee1 genes in N2A murine cells*. 33rd FEBS Congress, June 28-July 3, Athens, Greece

Xidous M, Sekeri-Pataryas K, *Prombona A*, Sourlingas T (2008) *Effects of the histone deacetylase inhibitor, trichostatin A, on clock and cell cycle-related gene expression levels as a function of circadian time in NIH3T3 mouse fibroblasts*. 33rd FEBS Congress, June 28-July 3, Athens, Greece

Citations 2008 (without self- citations): 8

Total Citations 2005-2008 (without self- citations): 35

h-factor: 5

Research Group: Microbial Molecular Genetics

Research Staff
Vassiliki Sophianopoulou, Researcher Director
Eleftherios Sideris, Emeritus Scientist
Dimitris Lagos, Postdoctoral Fellow
Mar;ia Billini, Graduate Student – *Phd obtained in 2008*
Dimitra Bouzarelou, Graduate Student
Ioannis Vaggelatos, Graduate Student
Katerina Roumelioti, Graduate Student
Alexandros Pittis, Undergraduate Student – *undergraduate dissertation completed in 2008*
Marina Laspa, Undergraduate Student – *undergraduate dissertation completed in 2008*
Marina Pantazopoulou, Undergraduate Student – *undergraduate dissertation completed in 2008*
Vartan Tsitsekian, Training Student
Grigoris Malliarakis, Training Student
Areti Tsolomiti-Gourgou, Research Technician

Research Interests

- **Research on the molecular mechanisms of recognition and transport of amino acids and nucleobases through cellular membranes via specific transmembrane transporters.**

  Transporters of medical, pharmacological and agricultural importance: amino acid and nucleobase transporters

  a) Identification and regulation of the expression of genes encoding amino acid (proline, glutamate) and purine transporters b) studies on structure-function relationships of amino acid transporters c) identification of *trans*-acting molecular determinants involved in expression, activity, cellular distribution and endocytosis of amino acid transport systems.

- **Basic research on the mechanisms involved in cell wall expansion and phytopathogenicity in fungi. Study of non-plant expansin-like proteins and eisosomal proteins:**

  a) Identification and regulation of the expression of genes encoding non-plant expansin-like protein(s) and investigation of their role in morphogenesis, growth and germination in *Aspergillus nidulans* c) Identification, molecular and functional studies on eisosomal proteins in *A. nidulans*.

- **Functional genomics:** Use of *A. nidulans* as a novel microbial model system for functional expression and biochemical characterization of members of the Nucleobase Ascorbate Transporter (NAT) family from higher organisms (plants and humans).

- **Basic Research the molecular mechanisms involved in salt and pH tolerance of microbial cells**. a) Identification and molecular characterization of Na^+/H^+ antiporters in cyanobacterial cells b) Phenotypic studies in strains deleted in genes encoding Na^+/H^+ antiporters in *Synechococcus elongatus*

2008 Findings

In the context of the project: Basic research on mechanisms involved in cell wall expansion and phytopathogenicity in fungi. Study of non-plant Expansin-like proteins and eisosomal proteins

a) Breakage and reformation of cell wall polymer bonds along with the maintenance of cell wall plasticity during conidia germination, depend upon a range of hydrolytic enzymes whose activities is analogous to expansins, a highly conserved group of plant cell wall proteins with characteristic wall loosening activity. We identified and characterized an expansin-like gene (*eglD*) in *A. nidulans*, the product of which shows strong similarities with bacterial and fungal endo-β1,4-glucanases. *eglD* gene is constitutively expressed in all developmental stages and compartments of *A. nidulans* asexual life. However the EglD protein is exclusively present in conidial cell walls. The role of the EglD protein in morphogenesis, growth and germination rate of conidia was investigated. Our results show that EglD

is a protein with putative endoglucanase activity that possesses cell wall remodeling functions associated with germination.

b) Isolation and studies concerning the topology of the three eisosomal proteins (PilA, PilB and Sur7) in *Aspergillus nidulans.*

In the context of the project: Functional genomics: SCVT1, one of the two human ascorbate transporters of the NAT family was expressed in *Aspergillus nidulans*. Our results showed that SCVT1 remains attached to the endoplasmic reticulum ER (perinuclear distribution).

In the context of the project: Basic Research the molecular mechanisms involved in salt and pH tolerance of microbial cells:

Synechococcus elongatus (strain PCC 7942) is an alkaliphile cyanobacterium that tolerates a relatively high salt concentration as a freshwater microorganism. Its genome sequence revealed seven genes (*nha1-7)*, which's deduced amino acid sequence, is similar to Na^+/H^+ antiporters, and which were characterized at the molecular and functional level. Our results show that among *nha* genes expressed in *Escherichia coli* only *nha3* complemented the deficient Na^+/H^+ antiporter activity of the Na^+-sensitive TO114 recipient strain. Moreover, among cyanobacterial strains disrupted in each of the *nha* genes separately, two present a phenotype different from the wild type (Δ*nhA3* cells showed a high salt and alkaline pH-sensitive phenotype while Δ*nha2* a low salt and alkaline pH-sensitivity). Finally, the transcriptional profile of the *nha1-7* genes, monitored using the real-time PCR technique, revealed that *nha6* gene is up-regulated and *nha1* is down-regulated under certain environmental conditions.

2008 Publications

D. Bouzarelou, M. Billini, K. Roumelioti and V. Sophianopoulou, 2008. An expansin-like protein with putative endoglucanase activity possesses cell wall remodeling function during germination in *Aspergillu nidulans*. Fungal Genet. Biol. 45(6):839-50

M. Billini, K. Stamatakis and V. Sophianopoulou, 2008. Two members of a network of putative Na^+/H^+ antiporters are involved in salt and pH tolerance of the freshwater cyanobacterium *Synechococcus elongatus*. J. Bacteriol. 190(19):6318-6329.

2008 Proceedings to Conferences

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) (in the press)

D. Bouzarelou and V. Sophianopoulou, 2008. EglD, a putative endoglucanase, with an expansin like domain is localized in the conidial cell wall of *Aspergillus nidulans*. Abstract of the 33rd Congress of FEBS "Biochemistry and Cell Regulation" 28 June – 3 July 2008. Athens, Greece. FEBS Journal Supplement 1, PP5-9, page 290.

2008 Presentations at International Scientific Conferences

D. Bouzarelou and V. Sophianopoulou, 2008. EglD, a putative endoglucanase, with an expansin like domain is localized in the conidial cell wall of Aspergillus nidulans. Poster of the 33rd Congress of FEBS "Biochemistry and Cell Regulation" 28 June – 3 July 2008. Athens, Greece

Impact Factors (for 2 publications): 7,5

Citations 2008 (without self- citations): 34

Total Citations 2005-2008 (without self- citations): 104

h-factor: 10

Research Group: Biophysics and Biotechnology of Membranes

Research Staff
Kostas Stamatakis, Senior Researcher
George Papageorgiou, Emeritus Scientist
Meropi Tsimilli – Michael, Collaboratig Scientist
Maria Billini, Graduate Student – *PhD obtained in 2008*

Research Interests

Membrane and cytosolic defense mechanisms mobilized by photosynthetic organisms when provoked by water deficit and salinity. Permeability of plasma membranes to water, ions, and neutral molecules. Critical role of turgor for adaptation to salinity and cell division. Studies on $N\alpha^+/H^+$ antiporters. Studies on Chlorophyll fluorescence induction curves. Studies on the photosynthetic Hydrogen production.

2008 Findings

A. Study of sodium proton antiporters in the cyanobacterium Synechococcus sp. PCC 7942
We conclude the work in Na^+/H^+ antiporters of *Synechococcus* sp PCC 7942 which is an alkaliphilic cyanobacterium that tolerates a relatively high salt concentration as a freshwater microorganism. We focused on molecular and functional characterization of the nha genes encoding Na^+/H^+ antiporters. Our results show that of the nha genes expressed in *Escherichia coli*, only nha3 complemented the deficient Na^+/H^+antiporter activity of the Na^+-sensitive TO114 recipient strain. Moreover, two of the cyanobacterial strains with separate disruptions in the nha genes (Δnha1, Δnha2, Δnha3, Δnha4, Δnha5, and Δnha7) had a phenotype different from that of the wild type. In particular, ΔnhA3 cells showed a high-salt- and alkaline-pH-sensitive phenotype, while Δnha2 cells showed low salt and alkaline pH sensitivity. Finally, the transcriptional profile of the nha1 to nha7 genes, monitored using the real-time PCR technique, revealed that the nha6 gene is upregulated and the nha1 gene is down regulated under certain environmental conditions.

B. 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.

2008 Publications

Billini, M., Stamatakis, K., and Sophianopoulou, V. (2008) Two Members of a Network of Putative Na+/ H+ Antiporters are Involved in Salt and pH Tolerance of the Freshwater Cyanobacterium *Synechococcus elongatus*. *J. Bacteriology 190:6318-6329*

Impact Factors (for 1 publication): 4,009

Citations 2008 (without self- citations): 6

Total Citations 2005-2008 (without self- citations): 19

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)*

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.

2008 Findings

The large-scale features of the spatial arrangement of protein-coding segments (PCS) are investigated by means of the size distribution of their distances. Inter-PCS distances have been found to follow size distributions which may be approximated by power-laws. Linearity in log-log scale extends to several orders of magnitude in the genomes of organisms as disparate as mammals, insects and plants. This feature is also present in the most compact eukaryotic genomes and in half of the examined bacteria, despite their very limited non-coding space. We have tried to determine the sequence of events in the course of genomes' evolution which may account for the formation of the observed size distributions. The proposed mechanism essentially includes two types of events: **(i)** Segmental duplications (and possibly paleopolyploidy), and **(ii)** the subsequent loss of most of the duplicated genes. Such events are well known to occur repetitively for long evolutionary periods. It is shown by computer simulations that the formulated scenario generates power-laws, which remain robust for a variety of parameter choices, and is compatible with the insertion of external sequences, such as viruses or proliferating retroelements. Moreover, it continues to sustain power-laws in the inter-PCS distances' size distribution even under conditions of removal of most of the non-coding DNA, thus explaining the finding of this pattern in genomes as compact as that of *Takifugu rubripes*.

Citations 2008 (without self- citations): 15

Total Citations 2005-2008 (without self- citations): 70

h-factor: 10

Research Group: NMR Studies of Biomolecules and Parmaceuticals

Research Staff
Maria Pelekanou, Research Director
Marina Sagnou, Lecturer
Dimitra Benaki, Postdoctoral Fellow
Aggeliki Panagiotopoulou, Technical Specialist
Stamatia Tzanopoulou, Graduate Student –*PhD obtained in 2008*

Research Interests

Studies of the **structure**, **interactions** and **structure-function relationship** 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
- Organic compounds, labelled or not, designed for targeted action and especially on technetium and rhenium complexes 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.

We use 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.

2008 Findings

In 2008 in the area of peptides, the investigation of the properties of the humanin neuroprotective family of peptides continued with biological and structural studies. The structural results reveal flexible peptides in the aqueous environment able - under certain conditions - to self-associate through the formation of β-sheet structures, a property that is correlated in the literature with their neuroprotective potency. The biological evaluation studies, that were conducted with the use of ^{124}I-radioiodinated derivatives in collaboration with the czech group of Dr. J. Slaninova, reveal marginal entry in the brain, higher stability in serum than in tissue homogenates, and lack of any obvious specific binding in nervous cell membranes or membranes of the brain and stomach tissue. These results are the first report on the *in vivo* biodistribution of this important family of peptides and are setting the base for further investigations. Furthermore, the NMR structural study of complexes of rhenium with derivatives of the neuropeptide bombesin - as part of the process of development of radiodiagnostics for prostate cancer -, as well as with derivatives of the anosopeptide thymosin-alpha -as part of the investigation of its immunoenhancing action - was completed.

In the area of technetium and rhenium complexes, the investigation of the coordination mode of the tricarbonyl $M(CO)_3^+$ core (M= Re, Tc) was continued with new ligands easily derivatized with bioactive molecules for targetted action. The in vivo biological evaluation of $^{99m}Tc(CO)_3^+$ complexes with derivatives of benzothiazole was completed with biodistribution experiments in breast cancer bearing SCID mice. In these experiments, selective uptake of the radiolabelled complex by the tumor was observed, and the results are in the process of publication. Furthermore, in 2008 considerable progress was made in the development of new compounds for application in PET tomography - as radiodiagnostics for Alzheimer' s disease and cancer - as well as in magnetic tomography (MRI) as image enhancers.

2008 Publications

Giglio, J., Patsis, G., Pirmettis, I., Papadopoulos, M., Raptopoulou, C., Pelecanou, M., León, E., González, M., Cerecetto, H., Rey, A. (2008). Preparation and characterization of technetium and

rhenium tricarbonyl complexes bearing the 4-nitrobenzyl moiety as biorreductive diagnostic radiopharmaceuticals. in vitro and in vivo studies. *Eur. J. Med. Chem*. 43, 741-748

Chiotellis, A., Tsoukalas, Ch., Pelecanou, M., Raptopoulou, C., Terzis, A., Papadopoulos, M., Papadopoulou-Daifoti, Z., Pirmettis, I. (2008). A convenient route leading to neutral fac-M(CO)3(NNO) complexes (M= Re, 99mTc) coupled to amine pharmacophores. Inorg. Chem. 47, 2601-2607.

Kunešová, G., Hlaváček, J., Patočka, J., Evangelou, A., Zikos, C., Benaki, D., Paravatou-Petsotas, M, Pelecanou, M., Livaniou, E., and Slaninova, J. (2008) The multiple T-maze in vivo testing of the neuroprotective effect of humanin analogues", Peptides 29, 1982-7; doi:10.1016/j.peptides.2008.06.019

Efthimiadou, E.K., Katsarou, M.E., Fardis, M., Zikos, C., Pitsinos, E.N., Kazantzisa, A., Leondiadis, L., Sagnou. M., Vourloumis, D. (2008). Synthesis and characterization of novel natural product-Gd(III) MRI contrast agent conjugates. Bioorg. Med. Chem. Lett. 18, 6058-6061

Stanica, R.M., Benaki, D., Tsoukatos, D., Tselepis, A., Mikros, E., and Tsikaris, V. (2008). Structure-activity relationships of αIIb 313-320 derived peptide inhibitors of human platelet activation. J. Pept. Sci. 14, 1195-1202

Stamopoulos, D., Bouziotis P., Benaki, D., Kotsovassilis, C., and Zirogiannis, P.N. (2008). Utilization of Nanobiotechnology in Haemodialysis: Mock-Dialysis Experiments on Homocysteine. Nephrol. Dial. Transplant. 23, 3234-3239.

Stamopoulos, D., Manios, E., Gogola V., Benaki, D., Bouziotis, P., Pissas M., and Niarchos, D. (2008). "Bare and Protein-Conjugated Fe_3O_4 Ferromagnetic Nanoparticles for Utilization in Magnetically Assisted Haemodialysis: Biocompatibility with Human Blood Cells. Nanotechnology 19, 505101

2008 Proceedings to Conferences

Benaki, D., Zikos, C., Evangelou, A., Elbert, T., Slaninova, J., Vlassi, M., Mikros, E., Bouziotis, P., Paravatou-Petsotas, M. Papadopoulos, M., Pirmettis, I., Pelecanou, M., and Livaniou, E. "Investigation of the Neuroprotective Action of the Humanin Family of Peptides with in vitro, in vivo, and Structural Studies", FEBS J., 2008, Suppl. 275, 75.

Benaki, D., Zikos, C., Evangelou, A., Slaninova, J., Vlassi, M., Livaniou, E., Mikros, E., and Pelecanou, M., "Comparative structural studies of potent neuroprotective peptides of the Humanin family", J. Peptide Sci., 2008, Suppl. 14, 108.

2008 Presentations at International Scientific Conferences

Benaki, D., Zikos, C., Evangelou, A., Elbert, T., Slaninova, J., Vlassi, M., Mikros, E., Bouziotis, P., Paravatou-Petsotas, M. Papadopoulos, M., Pirmettis, I., Pelecanou, M., Livaniou, E. Investigation of the Neuroprotective Action of the Humanin Family of Peptides with in vitro, in vivo, and Structural Studies. 33rd FEBS Congress & 11th IUBMB Conference, June 28-July 3, 2008, Athens, Greece. (Oral presentation)

Sagnou, M., Tzanopoulou, S., Paravatou-Petsotas, M., Papadopoulos, M., Pirmettis, I., Pelecanou, M. Synthesis and evaluation of novel benzothiazole complexes as potential biochemical, cellular and solid tumour radiotracers. 33rd FEBS Congress & 11th IUBMB Conference, June 28-July 3, 2008, Athens, Greece. (Poster presentation)

Benaki, D., Zikos, C., Evangelou, A., Slaninova, J., Vlassi, M., Livaniou, E., Mikros, E., Pelecanou, M. Comparative structural studies of potent neuroprotective peptides of the Humanin family. 30th European Peptide Symposium, August 31-September 5, 2008, Helsinki, Finland. (Poster presentation)

Impact Factors (for 7 publications): 19,391

Citations 2008 (without self- citations):
M. Pelecanou: 27
M. Sagnou: 12

Total Citations 2005-2008(without self- citations):
M. Pelecanou: 99
M. Sagnou: 43

h-factor:
M. Pelecanou: 12
M. Sagnou: 3

Research Group: Protein Structure and Molecular Modeling

Research Staff
Metaxia Vlassi, Research Director
Dimitris Vlachakis, Postodoctoral Fellow
Giorgos Nikolopoulos, Postodoctoral Fellow
Stamatia Vatsi, Collaborating Graduate Student *(MSc)- MSc obtained in 2008*
Diamadis Sellis, Graduate Associate

Research Interests

Our current research activities focus on structural studies of 1) protein interactions with emphasis on sequence repeat containing protein-protein interaction modules and 2) enzymes and peptides of medical interest with the aim to elucidate structure/stability/function relationships towards a structure-based drug design. The approach we follow includes a combination of bioinformatics techniques (*in silico* 3D-Modelling, docking, Molecular Dynamics simulations) with biochemical and biophysical methods (Circular dichroism (CD), x-ray Crystallography).

2008 Findings

- ✓ Our work on the TPR (tetratricopeptide repeat)-mediated interaction of the Ssn6 and Tup1 proteins (see previous annual reports) is published in ***Proteins*** (see Palaiomylitou/Tartas *et al*, 2008).
- ✓ Using circular dichroism (CD) we found that the DEFL protein (see previous annual reports) has a high α-helical content, in perfect agreement with our previous structure predictions suggesting that DEFL is a HEAT-like repeat containing protein. By a combination of disorder predictions and limited proteolysis mapping we identified a probable structurally stable domain comprising the putative HEAT-repeat region. Construction of a plasmid for over-expression of this domain as a His-tag protein, towards the structural characterization of the putative HEAT region of DEFL, is currently in progress (in collaboration with Dr. W. Warren, James Cook Univ. Australia). This work was funded by a grant from GSRT (05Non-EU-356).
- ✓ By analysing the occurrence of individual aminoacids in the seven positions of heptad-repeats in 4-stranded coiled-coils, we found positional preferences of aminoacids distinguishing between parallel and anti-parallel architectures. This finding was implemented in a computer program to be used as a structure prediction tool for 4-stranded coiled-coils (**MSc thesis** by S. Vatsi, completed: 5/11/2008).
- ✓ We developed a graphical user interface (GUI) to the widely used molecular dynamics simulation computer program, Gromacs. A manuscript describing the tool we developed, Gromita, is submitted **to *Bioinformatics*** (Sellis, Vlachakis and Vlassi, Submitted: Bioinf-2009-0070).
- ✓ Using molecular docking experiments followed by molecular dynamics simulations we showed that a series of synthetic nucleoside analogues can efficiently dock into the active site of the human poly(A)-specific ribonuclease (PARN), a cap-interacting deadenylase that mediates the eukaryotic mRNA turnover. Our findings, in conjunction with *in vitro* experiments, by our collaborators (Balatsos & Stathopoulos), suggest that human PARN is indeed among the molecular targets of such compounds that could therefore, serve as leading compounds for the development of novel inhibitors of PARN with potential use for novel therapeutic approaches. A manuscript describing this work is submitted for publication to ***Biochemistry*** (Balatsos et al., Submitted: Bi-2009-00236k).
- ✓ 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 a combination of a multi-fragment search followed by a virtual combinatorial chemistry approach, docking and molecular dynamics simulations. *In silico* evaluation of the designed compounds is in progress (Vlachakis and Vlassi, *Manuscript in preparation*).

- ✓ Our modeling work on the histone-like DNA-binding protein HU from the thermophile *Thermoplasma volcanium* (HUTvo) and its usage in thermal stability studies of the enzyme was published in ***Extremofiles*** (see Orfaniotou et al., 2009 in press).

2008 Publications

Palaiomylitou*, M., Tartas*, A, Vlachakis, D., Tzamarias, D., Vlassi, M. (2008) Investigating the structural stability of the Tup1 interaction domain of Ssn6: Evidence for a conformational change in the complex. *Proteins* 1;70 (1):72-82.

2008 Presentations at International Scientific Conferences

Nikolopoulos, * G, Vlachakis*, D., Waltenspiel, B., Warren, W.D.., Vlassi, M (2008) Preliminary structural characterization of DEFL, a good candidate for anticancer research. 33rd FEBS Congress & 11th IUBMB Conference, June 28th- July 3rd, 2008. Athens, Greece. Abstract in: *FEBS J*. 275:232.**Shared authorship*

Benaki, D., Zikos, C, Evangelou, A., Slaninova, J., Vlassi, M., Livaniou, E., Mikros, E., Pelecanou, M. (2008). Comparative structural studies of potent neuroprotective peptides of the Humanin family. 30th European Peptide Symposium (30EPS), 31 August – 5 September, 2008. Helsinki, Finland. Abstract in: *J. Peptide Science* 14(S1): 108

Benaki, D., Zikos, C, Evangelou, A., Elbert, T., Slaninova, J., Vlassi, M., Mikros, M., Bouziotis, P., Paravatou-Petsotas, M., Papadopoulos, M., Pirmettis, I., Pelecanou, M., Livaniou, E. (2008). Investigation of the neuroprotective action of the humanin family of peptides with in vitro, in vivo, and structural studies. 33rd FEBS Congress & 11th IUBMB Conference, June 28th- July 3rd, 2008. Athens, Greece. Abstract in: *FEBS J*. 275:75

Impact Factors (for 1 publication): 4,68

Citations 2008 (without self- citations): 28

Total Citations 2005-2008 (without self- citations): 94

h-factor: 11

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
Konstantinos Makris (MD), Graduate Research Assosciate
Stilianos Kakkos, Research Technician

Description

Our permanent task is the continuous search of human tissues from suitable donnors, the effort for the optimization of the production processes, 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.

2008 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 446 cancellous bone grafts, 392 of which were delivered by ORTHOMEDICAL.

As for other scientific collaborations we have prepared either grafts of other tissues, or new processed grafts.

Other Acticities at the IB

Responsible of Quality Assurance Project of the Bank according the ISO 9001/2000 stds,

Students training in the Bank activities

Other Scientific Activities

Representing Greece to the European Regulatory Experts Committee for the Directives 23/2004, 17/2006 and 86/2006 concerning the tissues and cells of human origin.

Member of the European Committee for the establishment of a unique European nomenclature 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

Citations 2008 (without self- citations): 5

Total Citations 2005-2008 (without self- citations): 16

EXPERIMENTAL ANIMAL COLONY

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

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

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

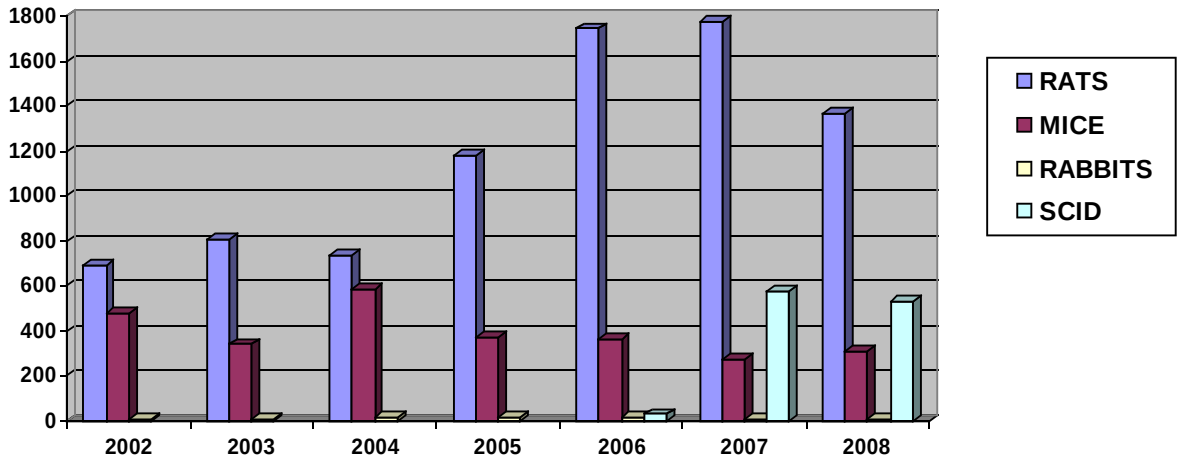
During 2008, the Animal Facility provided the following animals:

Users	*Rats*	*Mice SWR*	*Rabbits NZW*	*SCID mice*
Institute of Biology	44	0	0	31
Institute of Radioisotopes & Radiodiagnostics	60	310	5	259
External Users (research labs, hospitals, pharmaceutical companies, etc.)	1266	0	0	242
Total of animals provided	**1370**	**310**	**5**	**532**

The total income of the Unit for 2008 was 78,447.53 € and these have been used for purchasing of food, cages etc. and for the upgrading of the building and of the services provided. In particular, within this framework of the certification of the Unit according to ISO 9001:2000, a collaboration with a veterinarian has started and the quality of the animals has been tested. In addition, the repair and the upgrading of the building have also started and new instruments have been purchased.

The ΑΚΜΩΝ programme in collaboration with ELPEN Pharma, funded by GSRT, aiming at the upgrading of the animal house has ended. Research projects in collaboration with Thriassio Hospital and Ioannina University are ongoing. A new colony of ETB rats for ELPEN Pharma has been developed.

DISPOSAL OF LABORATORY ANIMALS 2002-2008



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.

2008 Findings

During the year 2008, 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.

2008 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 2008, 16 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 2008, 4 of our graduate students finished their thesis work and became PhDs.

Moreover, 9 students from the University are carrying out their pre-graduate project thesis work at the Institute and 4 students did practical job training. Additionally, 2 students from University abroad did practical lab training in laboratories at the Biology Institute.

In the framework of Graduate Programme, during the year 2008 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:

- *Chromatin Structure and Regulation of gene Expression* [course lecturers: Th. Sourlingas, L. Swevers, course coordinator: V. Sophianopoulou].

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:

- *Teaching in the framework of the postgraduate course: "Biochemistry"* (**Dr. Iro Georgoussi,** Department of Biology, University of Athens)
- *Lecture with title "Cellular signaling for transmembrane recptors and new drugs" in the framework of the postgraduate course "Biochemistry"* (**Dr. I. Georgoussi,** Department of Biology, University of Athens)
- *Lecture with title "Cellular senescence and tissue homeostasis" in the framework of the postgraduate course "Biochemistry***"** (**Dr. D. Kletsas,** Department of Biology, 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 coursee "Cancer Biology", the course "Regulation of Cell Proliferations*" (**Dr. D. Kletsas**, School of Medicine, 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 with title "Molecular mechanisms and theurapeutical interventions in diabetes – mellitus" in the framework of the course"Pathobiochemistry"* (**Dr. E. Tsilibary**, Department of Biology, University of Athens)
- *Lecture with title "Lipoprotein metabolism and plasma lipid homeostasis studies" in the framework of the postgraduate course Biochemistry* (**Dr. A. Chroni**, Department of Chemistry, University of Athens)
- *Lecture with title "Lipoproteins and Atherosclerosis. Atherosclerosis and Alzheimer's disease: common etiology" in the framework of the postgraduate course Biochemistry* (**Dr. A. Chroni**, Department of Chemistry, University of Athens)

- *Lecture with title "Functional Expression and Study of Transmebrane Transporters of Higher Organisms" in the framework of the course "Model Systems of Molecular Microbiology" of the postgraduate programme 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)
- *Teaching in the framework of the postgraduate courses: "Clinical Biochemistry & Molecular Diagnostics"* (**Dr. M. Vlassi**, Department of Biology, University of Athens)
- *Teaching in the framework of the postgraduate courses: "Introduction to Research Methods"* (**Dr. M. Vlassi**, Department of Biology, University of Athens)

During July 2008, 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 (2008) 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 2008

GRADUATE STUDENT	TITLE OF DOCTORAL THESIS	ADVISOR (in Institute of Biology)	UNIVERSITY
Evaggelia Morou	Mapping the sites of interaction of opioid receptors with G proteins and effectors	Z. Georgoussi	Department of Biology University of Athens
Maria Billini	Study of sodium proton antiporters Na^+/H^+ in the cyanobacterium *Synechococcus* sp. PCC 7942	K. Stamatakis & V. Sophianopoulou	Department of Biology University of Athens
Argiris Talamagas	Integrin-mediated regulation of collagenases (MMPS) from β-amyloid protein. Possible role of collagenase B (MMP-9) in the turnover of amyloid recurso protein (APP)	E. Tsilibary & A. Tzinia	Department of Biology University of Athens
Stamatia Tzanopoulou	Synthesis and biological evaluation of technetium and rhenium complexes as potential radiopharmaceuticals	M. Pelecanou	Department of Chemistry University of Athens

LECTURE CONTRIBUTIONS TO
THE 2008 SUMMER SCHOOL
OF THE NCSR "DEMOKRITOS"

(July 2008)

DATE	SPEAKER	TITLE
7/7/08	**Dr. L. Swevers** Institute of Biology, NCSR "Demokritos"	Prevention of malaria transmission through interference with the mosquito olfactory system
8/7/08	**Dr. E. Tsilibary - Dr. G. Drossopoulou** Institute of Biology, NCSR "Demokritos"	Genes and diseases: linking molecular biology with pathology
8/7/08	**Dr. A. Chroni** Institute of Biology, NCSR "Demokritos"	Cardiovascular disease and Alzheimer's disease: common risk factors
8/7/08	**Dr. V. Sophianopoulou** Institute of Biology, NCSR "Demokritos"	Eukaryotic microorganisms as model systems to study transporters of amino acids involved in neurotransmission
8/7/08	**Dr. I. Georgoussi** Institute of Biology, NCSR "Demokritos"	Heptahelical receptors and novel drug development
9/7/08	**Dr. Y. Almirantis** Institute of Biology, NCSR "Demokritos"	Introduction to the study of the genome by means of statistical and probabilistic methods. Is a linguistic approach to the description of "genomic text" possible?
9/7/08	**Dr. M. Sagnou** Institute of Biology, NCSR "Demokritos"	Imaging of Disease
11/7/08	**Dr. Th. Sourlingas** Institute of Biology, NCSR "Demokritos"	Histone subtypes and post translational modifications: fundamental factors for chromatin remodelling during ageing and apoptosis
16/7/08	**Dr. D. Kletsas** Institute of Biology, NCSR "Demokritos"	Cellular senescence and tissue homeostasis

SEMINAR PROGRAMME 2008
INSTITUTE OF BIOLOGY

DATE	SPEAKER	TITLE
7/1/08	**Dr. E. Dermitzakis** The Wellcome Trust Sanger Institute, Cambridge, UK	Causes and patterns of regulatory variation in the human genome
24/1/08	**P. Karkoulis** Institute of Biology, NCSR "Demokritos"	A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion
8/2/08	**Dr. I. Talianidis** BSRC "Al. Fleming"	Transcriptional cross-regulatory circuits controlling liver development
14/2/08	**Dr. I. Georgoussi** Institute of Biology, NCSR "Demokritos" (From the seminars of the Institute of Radioisotopes & Radiodiagnostic Products)	Novel interacting players regulating opioid receptor signaling
15/2/08	**Dr. A. Pintzas** Institute of Biological Research & Biotechnology, National Hellenic Research Foundation	Genomics of cancer mutations and new targeted therapies
29/2/08	**Prof. A. Gravanis** Medical School. Univ. of Creta	Neurosteroids as endogenous modulators of neuronal survival and neurogenesis
14/3/08	**Dr. G. Thireos** IMBB, Foundation for Research & Technology	Life as sensed by a transcriptional factor
26/3/08	**Prof. G. Nikolis** Université Libre de Bruxelles	Nonlinear dynamics and self-organization in the presence of metastable phases
28/3/08	**Dr. P. Syntichaki** IMBB, Foundation for Research & Technology	Genetic basis of ageing in model organisms
4/4/08	**Prof. A. Lazou** Dpt. Of Biology, Univ. of Thessaloniki	Signaling mechanisms in the pathophysiology of heart cells: hypertrophy or apoptosis?
12/4/08	**Prof. I. Martinez** Faculty of Medicine, University of Tromso, Norway	Cartilage physiology and repair
17/4/08	**P. Karkoulis** Institute of Biology, NCSR "Demokritos"	Illumination of signal transduction pathways emanated after the administration of chemotherapeutic factors in human cancer
17/4/08	**K. Ioannides** Institute of Biology, NCSR "Demokritos"	Lepidopteran-specific Nuclear Polyhedrosis Viruses: molecular study and applications in biotechnology
7/5/08	**Prof. J. Herms** Centre for Neuropathology and Prion Research , University of Munich	In vivo 2-Photon imaging in neurodegenerative diseases: Tracking down structural correlates of synaptic failure
8/5/08	**N. Tsotakos** Institute of Biology, NCSR "Demokritos"	Mechanisms that control the suppression of the antiadhesin podocalyxin in human glomerular epithelial cells exposed to high glucose
15/5/08	**E. Salpea**	Histone epigenetic changes in the region of genes

	Institute of Biology, NCSR "Demokritos"	whose expression changes during differentiation and ageing
15/5/08	**A. Repouskou** Institute of Biology, NCSR "Demokritos"	Circadian clock and histone acetylation: their interaction with the cell cycle studied in cultured murine cells
23/5/08	**Dr. P. Sideras** National Foundation of Biomedical Research, Academy of Athens	Role of Activins in respiratory pathophysiology
29/5/08	**M. Xedous** Institute of Biology, NCSR "Demokritos"	The effect of histone acetylation levels in the regulation of the biological clock: consequences for cellular function
3/6/08	**Dr. E. Reboutsika** BSRC "Al. Fleming"	Stem cells pull their SOX up
6/6/08	**D. Kyriakidis** National Hellenic Research Foundation	The way from an inhibitor of polyamine biosynthesis to a transcriptional regulator
19/6/08	**I. Georganta** Institute of Biology, NCSR "Demokritos"	δ- opiod receptor interaction with STATSB and Gβγ subunits reveals novel signaling pathways
26/6/08	**K. Roumelioti** Institute of Biology, NCSR "Demokritos"	Protein factors that control expression, topogenesis and function of acidic amino acids transporters
10/7/08	**S. Aliberti** Institute of Biology, NCSR "Demokritos"	The role of Molecular Chaperons in the regulation of protein kinases
24/7/08	**M. Kostomiri** Institute of Biology, NCSR "Demokritos"	The effect of the antioxidant constituent of oil, Oleuropein, on the amyloid precursor protein (APP) metabolism.
24/7/08	**I. Vaggelatos** Institute of Biology, NCSR "Demokritos"	Investigation of casein cinases type I role in topogenesis of amino acids transpoters in *Aspergillus nidulans*.
11/9/08	**M. Papakonstantinou** Institute of Biology, NCSR "Demokritos"	Identification of novel signaling pathways mediated upon activation of the opioid receptors
25/9/08	**A. Dimozi** Institute of Biology, NCSR "Demokritos"	Expression of IGF-binding proteins in senescent cells
25/9/08	**M. Papadopoulou** Institute of Biology, NCSR "Demokritos"	The Circadian Gene Per1 Plays an Important Role in Cell Growth and DNA Damage Control in Human Cancer Cells
2/10/08	**Dr. M. Verras** Medical School, Univ. of Stanford, USA	The effect of tumor microenvironment on the Wnt signal transduction pathway

COLLECTIVE DATA

FINANCIAL REPORT 2008

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

	PROGRAMMES				
INCOME	**464**	**EPAN** (IB, IPC, IMS**)**	**EXCELLENCE**	**AKMON**	**PEP**
CARRIED OVER FROM 2006	36.900,17	214.152,92	104,36	-31.108,64	3.049,60
FUNDING FROM NCSR "D"	30.000,00	40.000,00	20.000,00	0,00	15.000,00
MATCHING FUNDS	17.553,95	298.080,00	85.952,97	21.046,81	62.933,00
INCOME FROM SERVICES	0,00	0,00	0,00	46.053,92	0,00
DONATIONS FROM COMPANIES	0,00	0,00	0,00	0,00	0,00
TRANSFER FROM OTHER SOURCES	5.994,33	12.703,06	6.417,19	10.600,00	0,00
TOTAL INCOME	**90.448,45**	**564.935,98**	**112.474,52**	**46.592,09**	**80.982,60**

EXPENSES		**I.B.**			
EQUIPMENT	9.727,11	287.600,18	13.779,62	30.934,44	1.923,43
SUPPLIES	2.547,63	0,00	79.664,96	22.329,87	68.016,72
SALARIES	14.710,00	0,00	0,00	27.850,33	6.000,00
TRAVELS	620,91	0,00	4.022,45	0,00	0,00
OTHER EXPENSES	23.557,38	0,00	0,00	3.028,32	0,00
COMMITTED	899,64	0,00	0,00	645,36	0,00
TRANSFER FROM OTHER SOURCES	6.163,15	0,00	0,00	18.200,00	0,00
TOTAL EXPENSES	**58.225,82**	**287.600,18**	**97.467,03**	**102.988,32**	**75.940,15**

2. GOVERNMENTAL FUNDING .

LIQUID NITROGEN	6.332,01
ANIMAL CHOW	4.987,84
TOTAL GOVERNMENTAL FUNDING	**11.319,85**

2. *EXTERNAL FUNDING FROM THE PROGRAMMES OF THE INSTITUTE*

SOURCE OF FUNDING (number of programmes)	**FUNDING (in EUROS)**			
	Programme A	**Programme B**	**Programme C**	**INSTITUTE**
European Union (5)	261.931	179.456		441.387
General Secretariat for Research & Technology (15)	492.462	68.068	15.500	576.030
Ministry of health & Social Solidarity (2)	11.983	-	-	11.983
International Atomic Energy Ageny (IAEA) (1)	-	-	500	500
Aspis Bank (1)	-	-	5.000	5.000
National Scholarship Foundation (1)	-	-	500	500
Greek Society of Oncologists (1)	3.000	-	-	3.000
Bristol Myers Squibb (1)	15.000	-	-	15.000
TOTAL	**784.376**	**247.524**	**21.500**	**1.053.400**

COLLECTIVE DATA ON PRODUCTIVITY OF SCIENTIFIC PROGRAMMES

	PROGRAMME			
	A	**B**	**C**	**INSTITUTE**
Researchers	10	7	4	22*
Technical Specialist	-	1	1	2
Emeritus & Collaborating Scientists	2	4	1	7
Postdoctoral Fellows	6	7	3	16
Graduate Students	19	6	2	27
Collaborating Graduate Students	11	-	4	15
Graduate Research Associates	-	-	1	2[£]
Undergraduate Students	12	10	-	22
Research Technicians	2	4	-	9 [@]
Administrative Staff	-	-	-	2
Total Personnel	**62**	**39**	**16**	**124**
Publications in Peer-Reviewed Journals	**17**	**12**	**8**	**37**
Publications (Average) in Peer-Reviewed Journals per Scientist	1.6	1.71	2	1.68
Cumulative Impact Factor in Peer-Reviewed Journals (number of publications)	**59.384 (17)**	**31.118 (12)**	**24.071 (8)**	**114.573 (37)**
Average Impact Factor in Peer-Reviewed Journals	3.493	2.593	3.008	3.096
Cumulative Impact factor per Scientist	5.938	4.445	6.017	5.207
Proceedings to Conferences	**14**	**4**	**2**	**20**
Proceedings (Average) per Scientist	1.4	0.571	0.5	0.909
Total Publications	**31**	**16**	**10**	**57**
Publications (Average) per Scientist	3	2.285	2.5	2.545
Citations	**596**	**224**	**71**	**896***
International Patents	-	-	-	-
Greek Patents	-	-	-	-
Presentations to International Conferences	**36**	**10**	**4**	**50**
Presentations (Average) per Scientist to International Conferences	3.6	1.428	1	2.272
Presentations to Greek Conferences	**20**	**3**	**4**	**27**
Presentations (Average) per Scientist to Greek Conferences	2	0.3	1	1.227
Total Presentations to Conferences	**56**	**13**	**8**	**77**
Presentations (Average) per Scientist to Conferences	5.6	1.857	2	3.5

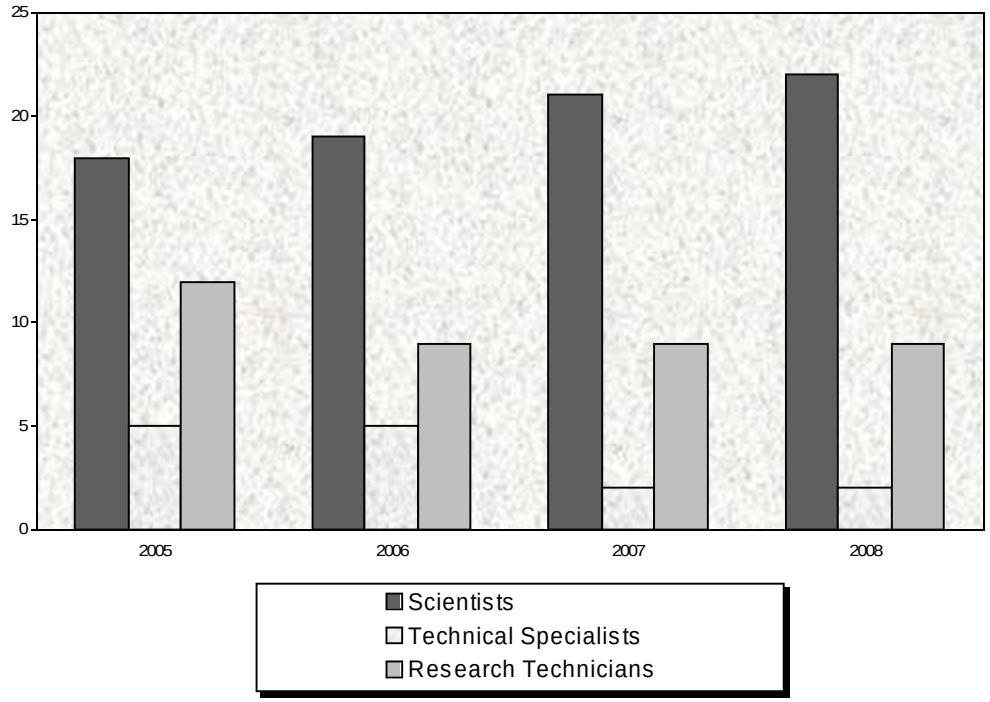
** 1 Scientist of Human Tissue Bank is included*

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

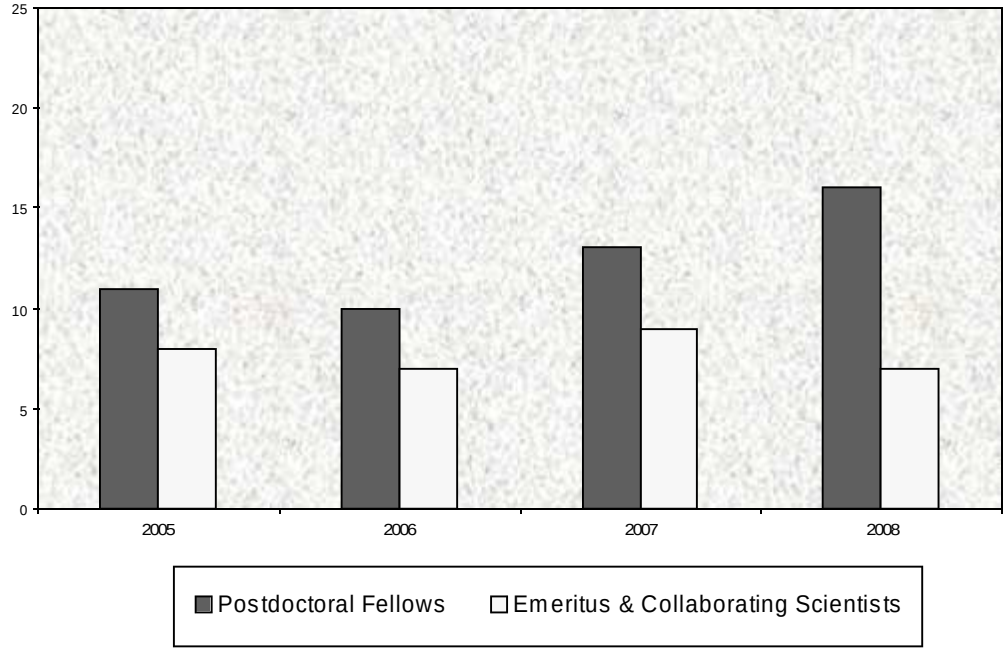
£ 1 GraduateResearch Associate of Human Tissue Ban kis included

CHANGES OF IB STAFF
DURING 2005-2008

"TENURED EMPLOYEES"

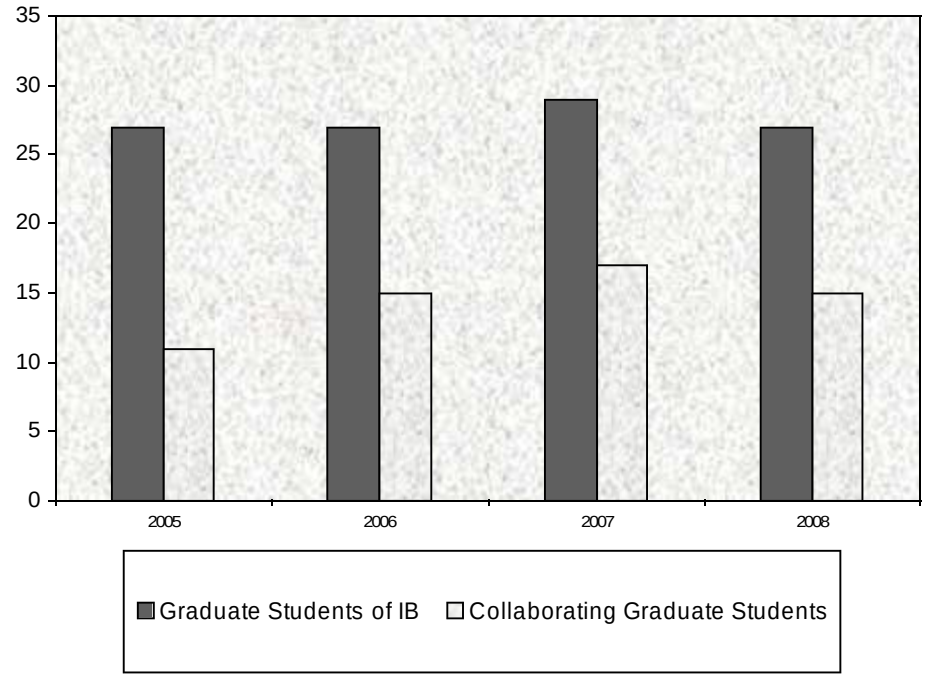


"POSTDOCTORAL FELLOWS and EMERITUS & COLLABORATING SCIENTISTS"

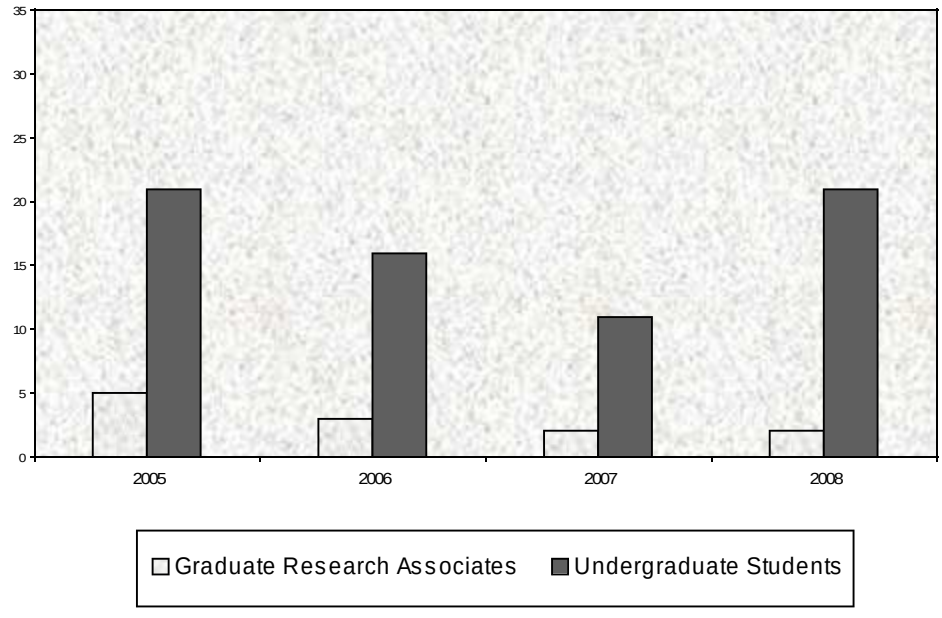


CHANGES OF IB STAFF
DURING 2005-2008

"GRADUATE STUDENTS"

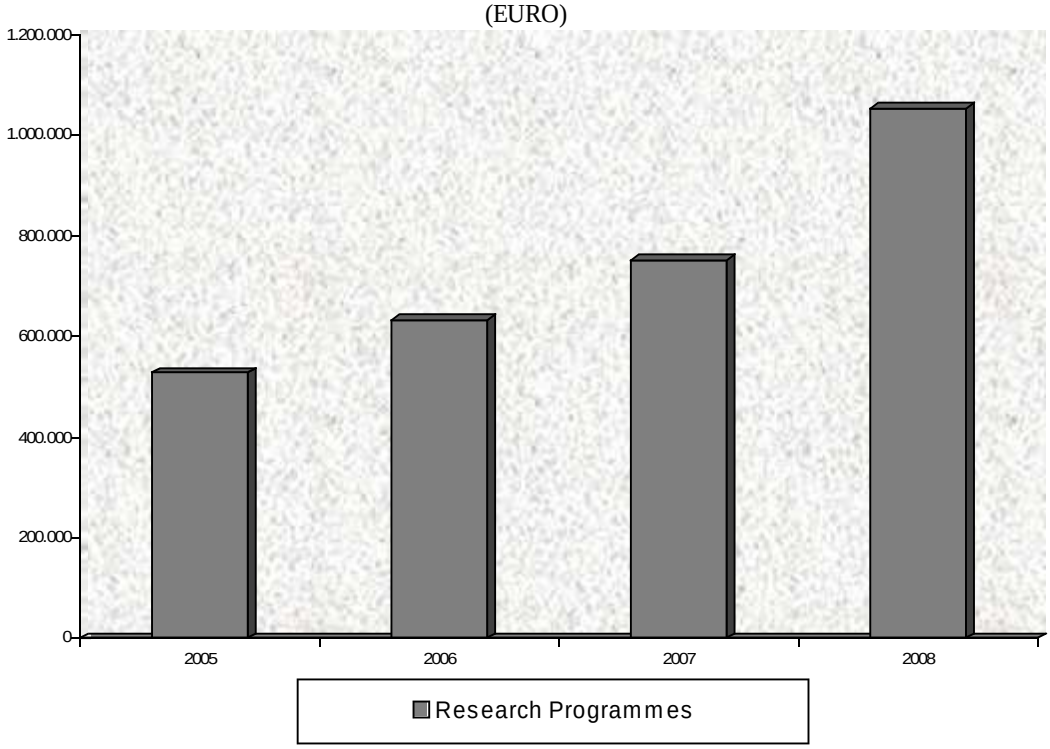


"GRADUATE RESEARCH ASSOCIATES AND UNDERGRADUATE STUDENTS "



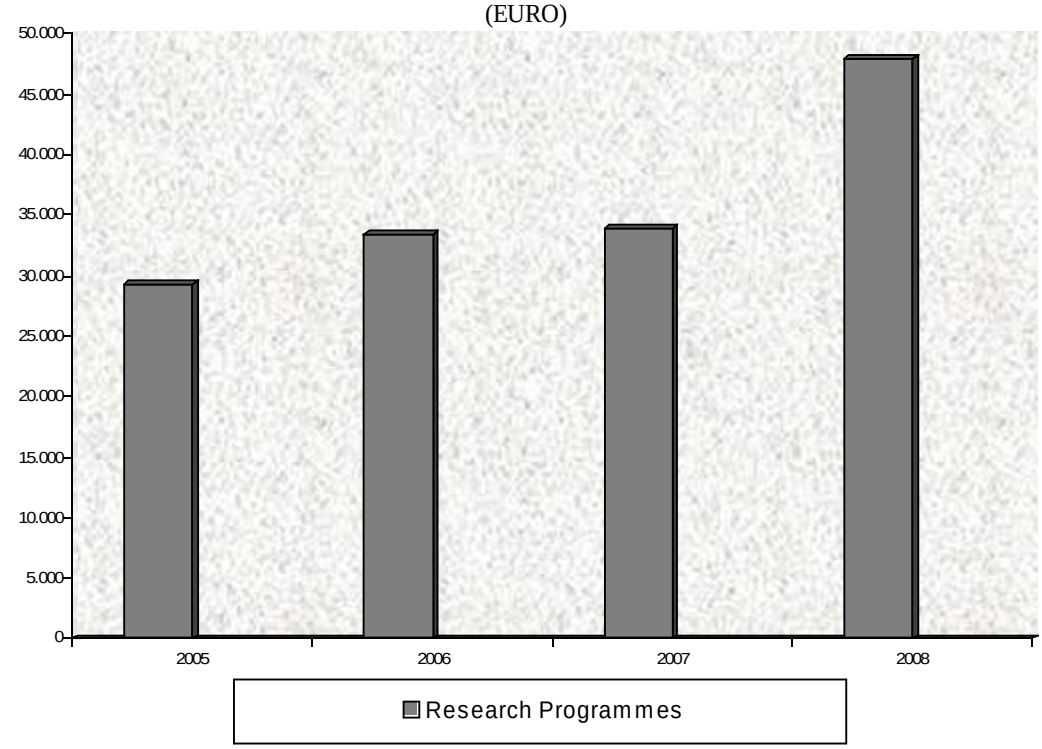
CUMULATIVE EXTERNAL FUNDING OF THE INSTITUTE DURING 2005-2008

(EURO)



EXTERNAL FUNDING OF THE INSTITUTE PER SCIENTIST DURING 2005 - 2008

(EURO)



PUBLICATIONS IN PEER-REVIEWED JOURNALS
AND CUMULATIVE IMPACT FACTOR DURING 2005-2008