INSTITUTE OF
BIOLOGY

NATIONAL CENTRE FOR SCIENTIFIC RESEARCH "DEMOKRITOS"

INSTITUTE OF BIOLOGY

2006
ANNUAL REPORT

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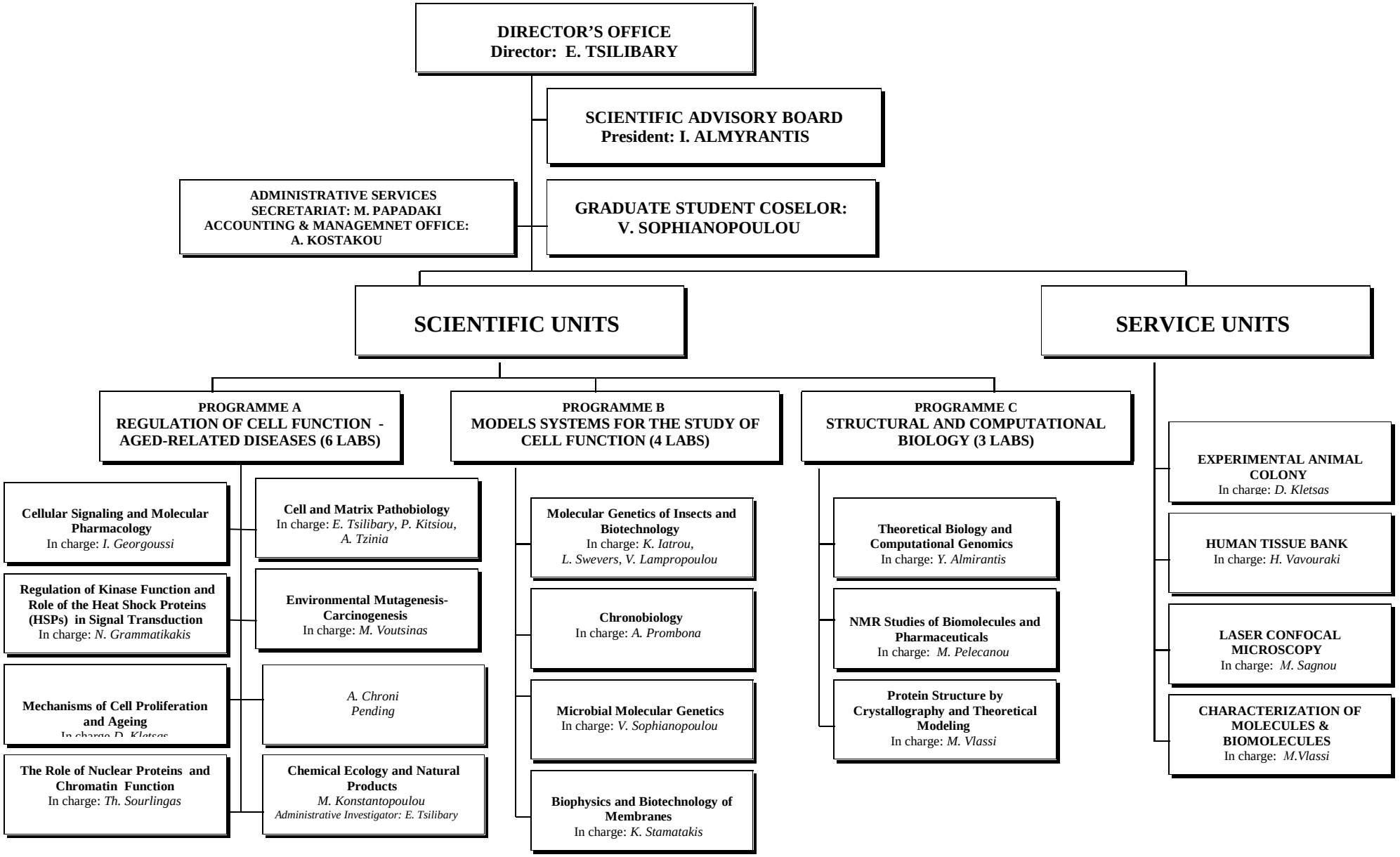
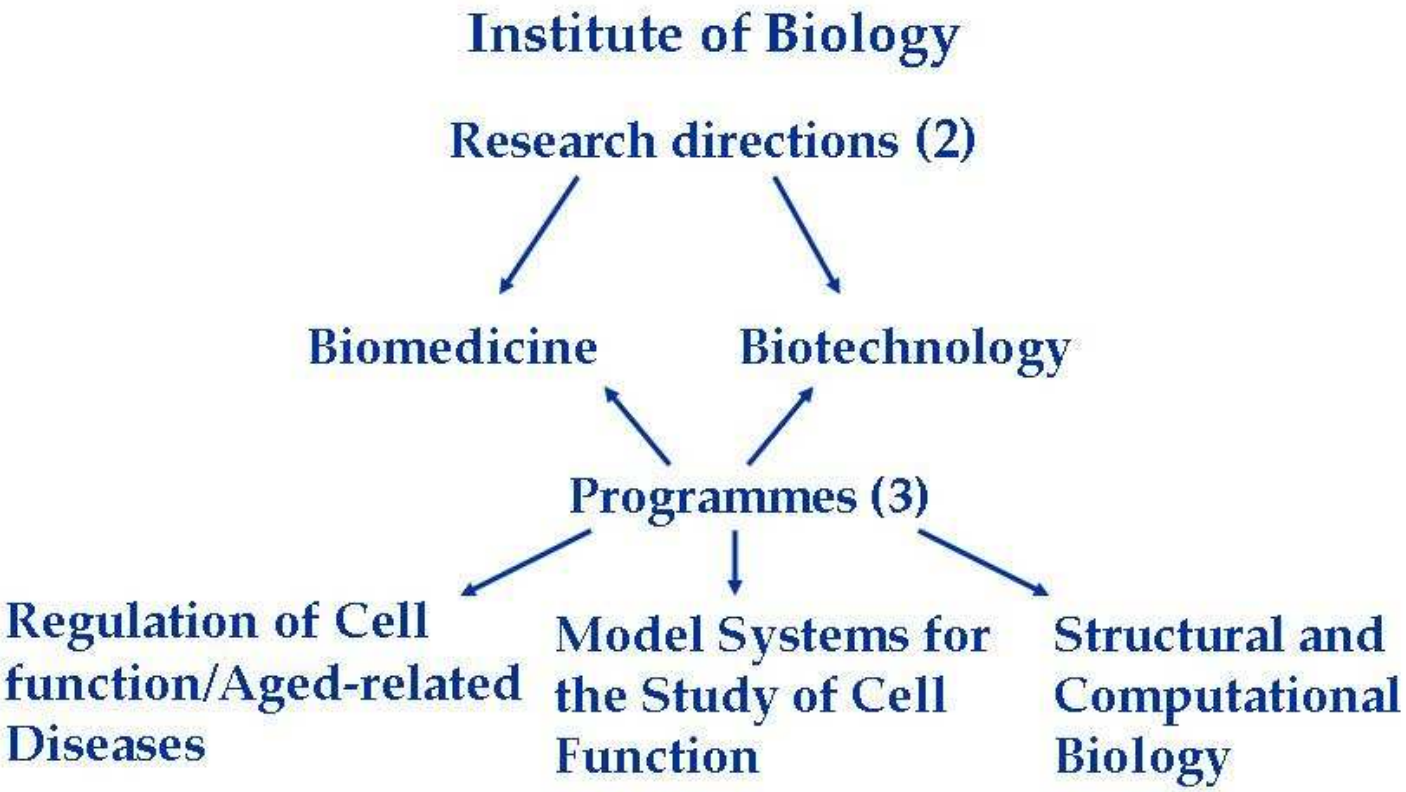
ORGANIZATION CHART




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

Effie Tsilibary	MD, Cell Biologist

ACTING DIRECTOR

Almyrantis Ioannis	Chemist

SCIENTIFIC STAFF

Research Directors

Almyrantis Ioannis	Chemist
Iatrou Kostas	Professor of Biochemistry and Molecular Biology
Sophianopoulou Vassiliki	Biologist
Tsilibary Effie	MD, Cell Biologist

Senior Researchers

Georgoussi Zafiroula-Iro	Biochemist
Grammatikakis Nikolaos	Cell Biologist
Kletsas Dimitris	Biologist
Pelecanou Maria	Pharmacist
Prombona Anastasia	Biologist
Stamatakis Konstantinos	Biologist
Swevers Luc	Biologist
Vlassi Metaxia	Physicist-Chrystallographer

Researchers

Chroni Aggelika	Biologist
Kitsiou Paraskevi	Biologist
Labropoulou Vassiliki	Biochemist
Voutsinas Gerassimos	Biologist
Sourlingas Thomae	Biologist
Tzinia Athina	Biochemist

Technical Specialists

Konstantopoulou Maria	Biologist
Panagiotopoulou Aggeliki	Biochemist
Sagnou Marina	Biologist/ Chemist
Stefanou Dimitra	Agronomist
Vavouraki Helen	Radiopharmacist

RESEARCH TECHNICIANS

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Kalokiri-Stilianidi Kalliope
Kopanelis Dimitrios
Kotsopoulou Eleni
Pantazi-Mazomenou Anastassia
Tsolomiti-Gourgou Areti
Zafiropoulos Ioannis

ADMINISTRATIVE STAFF

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Papadaki Margarita	Secretary

EMERITUS SCIENTISTS

Emeritus Sientists	**LLaboratory**
Ignatiadou Lydia (Dr. Hydrobiologist)- *Emeritus*	Iatrou K.
Mazomenos Vassilios (Dr. Chemical Ecologist)- *Emeritus*	Lab of Chemical Ecology and Natural Products
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.

POSTDOCTORAL FELLOWS

Fellow	**Supervisor**
Andronopoulou Evi	Iatrou K.
Benaki Dimitra	Pelekanou M.
Douris Vassilios	Iatrou K.
Drossopoulou Garifallia	Tsilibary E.
Efrose Rodica	Iatrou K.
Kenoutis Christos	Iatrou K.
Kizis Dimosthenis	Sophianopoulou V.
Metaxatou Aghelina	Ignatiadou L. *(Emeritus)*
Pratsinis Haris	Kletsas D.
Vlachakis Dimitris	Vlassi M.

GRADUATE STUDENTS

Student	**Supervisor**
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Billini Maria	Stamatakis K.
Bouzarelou Dimitra	Sophianopoulou V.
El Hamitie Abraam	Grammatikakis N.
Erpapazoglou Zoi	Sophianopoulou V.. – **PhD obtained in 2006**

Georganta Irene	Georgoussi I.
Georgomanolis Theodoros	Swevers L.
Giannouli Christina	Kletsas D. – **PhD obtained in 2006**
Handris Panagiotis	Kletsas D.
Leontiadis Leonidas	Georgoussi I.
Magkrioti Christiana	Iatrou K.
Manta Maria	Tsilibary E.
Mazarakou Georgia	Georgoussi I.. – **PhD obtained in 2006**
Mavrogonatou Eleni	Kletsas D.
Morou Evaghelia	Georgoussi I.
Nikas Vassilios	Voutsinas V.
Ninios Ioannis	Sourlingas Th.
Papakonstantinou Maria	Georgoussi I.
Repouskou Anastasia	Prombona A.
Roumelioti katerina	Sophianopoulou V.
Sdralia Konstantia	Iatrou K.
Talamagas Anargiros	Tsilbary E.
Tsagaraki Ioanna	Tzinia A.
Tsotakos Nikos	Tsilibary E
Tzanopoulou Stamatia	Pelekanou M.
Venieratos Panagiotis	Tsilibary E.
Xedous Marios	Sekeri K.

GRADUATE RESEARCH ASSOCIATES

Fellow	**Supervisor**
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Sellis Diamantis *(MSc)*	Almirantis I.
Tsikou Daniela *(MSc)*	Iatrou K.

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Student (University)	**Supervisor**
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Chrissouli Stefania (Univ. of Athens, MSc)	Kletsas D.
Fragoulopoulos Evaggelos (Univ. of Rome)	Tsilibary E.
Gioni Vassiliki (Univ. of Athens, MSc)	Kletsas D.
Kachrilas Stafanos (Univ. of Athens)	Voutsinas G.
Karkoulis Panagiotis (Univ. of Athens)	Voutsinas G.
Konstantakatou Evmorphia (Univ. of Athens)	Voutsinas G
Lagopati Nefeli (Athens Polytechnic School))	Tsilibary E.
Lampidonis Antonis (Agricultural Univ. of Athens)	Voutsinas G.
Litsiou Eleni (University of Athens)	Voutsinas G.
Melachrinou Sofia (University of Athens)	Voutsinas G.
Papadopoulou Adamantia (Univ. of Athens, MSc)	Kletsas D.
Salpea Paraskevi (Univ.of Athens)	Sourlingas Th.
Vatsi Stamatia (Univ. of Athens, MSc)	Vlassi M.
Villias Giorgos (Univ. of Athens, MSc)	Vlassi M.

UNDERGRADUATE STUDENTS AND OTHER IN TRAINING

Student (University)	Supervisor
Galeou Aggeliki (Univ. of Athens)	Prombona A.
Giarika Athina (Univ. of Thrace)	Voutsinas G.
Gika Eliana (Univ. of Athens)	Prombona A..
Dragoumani Konstantina (Univ. of Creta)	Vlassi M
Kapi Marianna (Univ. of Athens)	Prombona A.
Lamprakis Christos (Univ. of Thrace)	Vlassi M.
Laspa Marina (Univ. of Athens)	Sophianopoulou V.
Leptidis Stefanos (Univ. of Thrace)	Vlassi M.
Nomikou Irene (University of Athens)	Prombona A.
Pantazopoulou Marina (Univ. of Athens)	Sophianopoulou V.
Pittis Alexandros (Univ. of Athens)	Sophianopoulou V.
Sarris Michalis (University of Athens)	Georgoussi I.
Simos Nikos (Wagenngen University, Holland)	Vlassi M.
Tsitsekian Dikran (Agricultural Univ. of Athens)	Sophianopoulou V.
Varouksi Niki (Univ. of Athens)	Sourlingas Th.
Verouti Sofia (University of Athens)	Sophianopoulou V.

INTRODUCTION

The Institute of Biology (IB) is one of eight Institutes of the National Center for Scientific Research DEMOKRITOS. The Center is unique in that is a multidisciplinary Research Center combining research in different thematic areas and collaborations between different disciplines. The mission of this Institute comprises the following:

1. Internationally competitive research in the areas of Cellular, Structural, and Molecular Biology, as well as Biophysical, Biomedical and Biotechnological Research, in collaboration with the related Institutes of National Center for Scientific Research Demokritos.
2. The training of new scientists / researchers at the graduate and post-graduate levels.
3. The development of research findings aiming at helping and protecting Public Health.

The connection with other Hellenic Public Services and Organizations, and other Hellenic, European and International Scientific and Research Institutions, as well as with the Private Sector, aiming at bilateral transfer.

Research directions of the IB have been partly re-determined, following the retirement of several researchers through 2003, and the adjusted directions are included in the following three programs:

Program A: Regulation of Cellular Function / Age-Related Diseases
Program B: Model biological systems for the study of cellular functions
Program C: Structural and Computational Biology

A pivotal aim of the Institute aim is to contribute to the society with the development of new knowledge, via research projects conducted by IB researchers, and research efforts which can be applied and develop connections with the private sector. This is evident by the development of three programmes type "PRAXE A" that focus on this aspect , as well as by recent patents (one international patent in 2004, and one application to the Greek Patent Office during 2004). As defined by the three major programmes, **research** and **development** areas of the IB during 2004 focused on:

- Cellular function: *gene and chromatin organization, matrix pathobiology, cell senescence, cell signaling, cancer and tumor development*
- Insect and micro-organism molecular genetics / biotechnology
- Environment: *study of the effect of environmental factors on genetic material*
- Structural studies of proteins and bioactive molecules with crystallographic, NMR, and microthermidometry approaches

Infrastructure

For the materialization of research and development activities, the IB possesses specialized equipment, such as: laser confocal microscope, X-ray crystallography system, nuclear magnetic resonance spectrophotometer (NMR) at 500 MHz, circular dichroism and infra-red spectrometers, flow cytometer, equipment for cell cultures, DNA sequencer, systems of liquid and gas chromatography, ultracentrifuges, scintillation counter, etc.

The Institute also houses the following **Facility Units / Core Facilities**:

a. Animal facility
b. Tissue bank
c. Laboratory for characterization of molecules/biomolecules
d. Confocal Microscopy

Research and Development Targets

Knowledge obtained from research performed at the Institute of Biology aims at:

- Deciphering at a molecular level of the functional properties of cells and functional changes in age-related diseases, including cell senescence
- Designing effective methods of predicting, diagnosing, and interfering with the development of common hereditary and non-hereditary age-related diseases, such as the metabolic disease of diabetes, neurodegenerative diseases (Alzheimer's, etc.) and cancer
- Localizing reagents with specific pharmacological actions in natural products
- Examining the effects on the cellular function of adverse exogenous factors (such as ionizing radiation, narcotics, water deprivation, increased salt concentrations, sun light, trauma, attack from various pathogens

Fulfilled/perofrmance: During 2006, the Institute of Biology provided seed money for nine (9) inter-institute collaborations; eight were collaborations between two researchers and one represented a collaboration between four researcher and four research projects. These collaborations represent an intense effort of focusing in order to create a competitive cutting edge by uniting efforts from different points of view and expertise. Several efforts (in particular the pre-existing) already yielded publications or are in the process of publishing. The Head of the Institute encourages further development of research collaborations between researchers with the aim to achieve excellence in discrete thematic areas and becoming a reference centre for these areas in Greece. Examples represent neurodegenerative diseases with emphasis on Alzheimer, Cell Senescence, thematic areas in the filed of Cancer related to cell proliferation, the biological clock, apoptosis, mutations, etc, thematic areas related to basic research in Diabetes Mellitus, such as diabetic nephropathy and pancreatic β-cell apoptosis, research in Biotechnology included but not limited to the environment (e.g. development of novel pharmaceuticals to deal with chronic pain and addiction to narcotics, etc).

It is important to also note a recently-started collaboration between the institutes of Biology, Material Science and Physical Chemistry which is focused on the development of novel nanomaterials for imaging, targeted drug delivery and also possibly for the treatment of tumors. This collaboration is supported by two major programs from GSRT on the total amount of 2.3 million € (EPAN for infrastructure since 2005, and peripheral programs "PEP" for Attica in 2006), is ongoing since 2005 and involves several research groups from the three different institutes, with a perspective to expand and involve more researchers.

Frankly, I can hardly realize that three years elapsed since I was appointed Head of the IB. The never-ending, extremely intense effort for improvement and further development of the IB "pushed" the time forward with great speed. Several of my original aims were fulfilled in part (fund raising, renovation of office furniture and equipment, re-allocation of the available space, etc.), others are in the process of materialization (equipment upgrade and renewal, more fund raising), and some more are still in the planning (new positions for researchers, renovation of building infrastructure, etc). Indeed, the intensity and great burden of this ongoing effort as Head of the IB, somewhat "removed" me from direct contact with my research projects and this has been a source for major frustration, which was moderated by the positive results for this institute thus far, for which support from the researchers has been and will be instrumental. **This positive outcome** i**s reflected in the objective parameters of the progress report such as funding, number of publications and citations, impact factors, presentations in meetings, etc. All these were substantially improved since 2005, and reliably document progress and improvement in effect, thus demonstrating that we are moving in the right direction. This fact is by itself a major source of pride and satisfaction, simply because reality proves wrong previous negative comments which were expressed during the last evaluation**. This fact is my consolation prize as a researcher who does not have enough time for an appropriate endeavor in her research interests….Thus I would like to express my gratitude to all the members of my research team who continually support my research activities.

I sincerely hope that these encouraging objective factors documenting improvement and stature should be a source of confidence for all members of the IB, and a starting point for more intense and numerous efforts. I extend to all of you my warmest and most sincere congratulations for a remarkably successful outcome of your research efforts, and wish further success for all!

The process of accreditation according to the European standards of two major service laboratories, the Human Tissue Laboratory and the Experimental Animal Colony is almost complete, with certification verifying that all European S.O.Ps are followed for their processes. The first Accreditation certificate for the Human Tissue Laboratory was issued this month (May 2007), and the other is pending for later in 2007. For fulfilling this aim, Drs. H. Vavouraki and D. Kletsas respectively, have been extremely helpful despite interim problems which were successfully overcome.

During 2006 Drs. Constantopoulou, Sagnou and Vavouraki joined the ranks of researchers after the appropriate evaluation procedure. The IB welcomes the three scientists, newly appointed as researchers, wishes success in their new careers, and hopes that they will successfully meet the demanding criteria pf their new positions. Additionally, in 2006 Dr. Pelecanou was promoted based on merit to Full Researcher (Researcher A'). I extend my warm congratulations and very best wishes for successfully continuing and expanding her remarkable research efforts.

In 2006, three new research programs (STREP) were granted by the EEC to Drs. Chroni, Georgoussi and Kletsas (innovative grant). Warm congratulations are extended to all three successful contenders, together with the wish to multiply the grant number during 2007.

Once again the emeritus researchers proved more that active with publications, monographs, books and books chapters, participation in research projects, alignment with the aims of the IB etc., and in general had a valuable contribution for the outcome of the IB efforts.

In my continuous effort all the members of the Advisory Committee proved extremely helpful advisors: Drs Almyrantis, Kletsas, Pelecanou Sophianopoulou, and Vlassi, all worked hard and with exemplary dedication for helping me in fulfilling my administrative responsibilities and contributed to the positive outcome and solution of arising problems as well as for decision making. In particular, I would like to express my most sincere thankfulness to the Vice-Director, Dr. Almyrantis, who despite the intense and worsening health problems in his immediate family environment contributed to an optimal operation of the IB and stood by me in difficult moments of which there have been plenty, but were overcome with his participation, impartial advice, and wisdom.

The IB research seminars for which Dr. Iatrou was this initial organizer were successfully continued, starting September 2006, with Dr. Chroni in charge, and have been extremely informative thus far. Furthermore, I wish to extend my thankfulness to all members of the IB who promptly participated in various committees fulfilling different duties and helped for further upgrading of the IB.

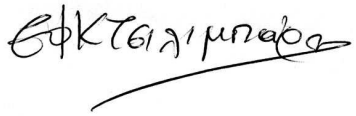
Despite the innumerable difficulties, problems, and adversities I face on a daily basis, the confidence demonstrated to me by the majority of researchers is my major source of support and optimism regarding success in fulfilling the aims of improving and further developing the IB. Sustained effort and coherence have already contributed to an improved "image" of the IB, thus rendering it more competitive internationally. I am asking you all to dedicate more efforts to collaborative research and escalate the effort to participate in local or European funding programs, raise funds from the private sector, etc., so that there are sufficient funds for optimal performance of your competitive research. I have confidence in the research potential of all of you who daily prove your contribution to the cause and continue upwards. Perhaps we should leave aside the

few, repeated complaints for insignificant matters of concern and strive united for achieving our central aim, the recognition of the IB a "live" and competitive nucleus of basic research.

I cannot finish without thanking our competent accountant, Ms. Athanasia Kostakou who efficiently fulfills most financial aspects of the IB operation, and regularly updates me on all accounting and financial matters of this institute. From the long list of performers I could not possibly omit the… general coordinator of our efforts, Ms. Margarita Papadaki, s substantial factor for the smooth operation of the IB and a flawless operation of the secretariat: she successfully performs her task as the IB secretary and a mother as of April 2006 and is always enthusiastic and extremely efficient.

My best wishes to all of you for continued success and more!

Effie C. Tsilibary, MD, PhD



Director of IB
February 2007

PROGRAMME A:

REGULATION OF CELL FUNCTION

AGED-RELATED DISEASES

Research Group: Pending

Research Staff
Aghelika Chroni, Researcher
Giorgos Michas, Collaborating Graduate Student (MSc)

Research Interests

Study of the role of lipids and lipid metabolism proteins in cells or circulation and in pathological conditions, such as atherosclerosis and Alzheimer's disease. 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 (A-I, E), enzymes, lipid transfer proteins, lipoprotein receptors (SR-BI) and lipid transporters (ABCA1) 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. Except from their role in the circulation, lipoproteins participate in lipid homeostasis in brain as well. Various studies have indicated that the disturbance of homeostasis of lipids (and particularly of cholesterol) and lipoproteins in the brain can contribute to the onset or progression of Alzheimer' disease.

Our goals are:

1) Study of the composition and functions of HDL from humans with disturbances of HDL metabolism and from animal models. Correlation of variation in the composition and functions of HDL to the presence of atherosclerosis in humans and animals with disturbances of HDL metabolism.

2) Study of the mechanisms by which cholesterol and proteins (such as apolipoprotein E, lipid transporter ABCA1, lipoprotein receptor SR-BI) that are involved in cholesterol homeostasis in brain cells, affect the pathogenesis of Alzheimer's disease.

2006 Findings

Structure-function relationship of apoA-I in vitro and in vivo.
ApoA-I promotes ABCA1-mediated lipid efflux that results in the initial lipidation of apoA-I that after a series of steps leads to the formation of HDL particles. In vitro data showed that the C-terminus deletion mutants apoA-I[Δ(185-243)] and apoA-I[Δ(220-243)] had diminished ABCA1-mediated cholesterol efflux, while the apoA-I[Δ(232-243)] deletion mutant and the apoA-I[E191A/H193A/K195A] point mutant had normal cholesterol efflux. Following adenovirus-mediated gene transfer in apoA-$I^{-/-}$ mice we found that mice expressing the apoA-I[Δ(185-243)] and the apoA-I[Δ(220-243)] didn't form HDL, while mice expressing the WT apoA-I, the apoA-I[Δ(232-243)] or the apoA-I[E191A/H193A/K195A] formed HDL normally. The findings indicate that a) the C-terminal region 220-231 of apoA-I is important for the formation of HDL in vivo and b) mutations in apoA-I that diminish its functional interactions with ABCA1 diminish the formation of HDL in vivo.

Structure-function relationship of apoE.
We investigated the ability of C-terminal truncated forms of apoE4 to activate the enzyme LCAT that is necessary for the formation of HDL and HDL-like lipoproteins in circulation and brain, respectively. Recently, apoE4 was found to undergo proteolytic cleavage in Alzheimer's disease brains, resulting in neurotoxic C-terminal-truncated fragments. ApoE interacts with amyloid peptide β in brain, although the exact effect of this interaction is not known. We found that the LCAT activation capacity of all the C-terminal truncated forms of apoE4 was 13-19% of the LCAT activation capacity of WT apoE4. This finding indicates that the C-terminal-truncated fragments of apoE4 cannot be lipidated properly and may have altered interactions with amyloid peptide β in brain.

2006 Publications

Gorshkova, I. N, Liu T., Kan H. Y., Chroni A., Zannis V. I and Atkinson D. (2006). Structure and stability of apolipoproteinA-I in solution and in discoidal High-Density Lipoprotein probed by double charge ablation and deletion mutation. Biochemistry 45, 1242-1254

Singaraja R. R., Visscher H., James E. R., Chroni A., Coutinho J. M., Brunham L. R., Kang M. H., Zannis V. I., Chimini G., and Hayden M. R. (2006) Specific mutations in ABCA1 have discrete effects on ABCA1 function and lipid phenotypes both in vivo and in vitro. Circ. Res. 99, 389-397

Zannis, V.I., Chroni, A., and Krieger, M. (2006) Role of apoA-I, ABCA1 and SR-BI in the biogenesis of HDL. J. Mol. Med. 84 276-294

2006 Presentations at International Scientific Conferences

Chroni, A., Duka, A., Koukos, G. and Zannis, V. I. (2006) The carboxy-terminal region of apoA-I is important for the biogenesis of HDL in vivo. 31st FEBS Congress, June 24-29, 2006, Istanbul, Turkey

Impact Factors (for 3 publications): 18,236

Citations 2006 (without self- citations): 39

Total Citations 2004-2006 (without self- citations): 84

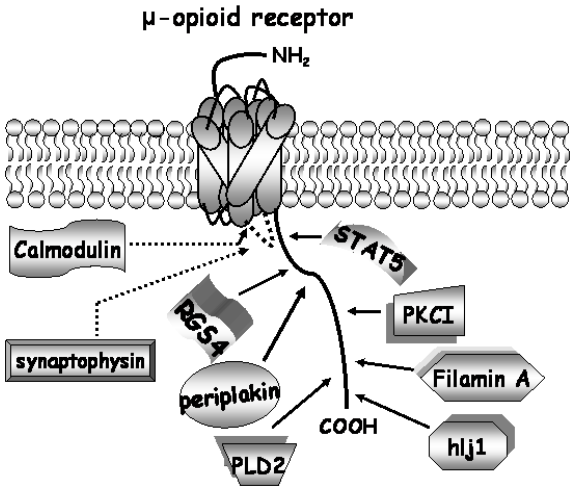
h-factor: 6

Research Group: Cellular Signalling and Molecular Pharmacology

Research Staff
Iro Georgoussi, Senior Researcher
Georgia Mazarakou, Graduate Student
Evangelia Morou, Graduate Student
Leonidas LeontiadisGraduate Student
Irene Georganta, Graduate Student
Maria Papakonstantinou, Graduate Student
Michalis Sarris, Undergraduate Student

Research Interests

Our research interests are focused on the elucidation of the molecular mechanism governing the G protein coupled receptors (GPCRs) using as a model system the opioid receptors. More specifically our efforts aim:



a) In the elucidation of the signalling pathways mediated by the three opioid receptor subtypes (μ, δ and κ)

b) In the identification of novel interactive partners of GPCRs

c) In the identification of transcription factors and genes that are activated or suppressed upon opioid administration and finally

d) In the development of High Throughput Screens (HTS) for mammalian GPCRs, and for antennal mosquito odorant receptors that lead in the identification of new pharmacological targets and new mosquito disruptors host seek response using functional reference systems in insect cell lines (*collaboration Prof. Kostas Iatrou group & STREP SP6th EU " Normolife" consortium*).

2006 Findings

a) Identification of novel interacting partners of opioid receptors

In an effort to define distinct signal transduction pathways that occur in the proximity of the opioid receptors we were able to dissect out the functional determinants of the δ- and μ-opioid receptors responsible for Gβγ, Gα and particularly RGS4 protein binding. With the use of GST-fusion peptides encompassing parts of the C-terminal domain of the δ and μ-opioid receptors demonstrated that tight complexes between Gα subunits of G proteins, activated RGS4 and helix VIII of the C-terminal domain of the opioid receptors are formed. In parallel we have shown that expression of RGS4 in HEK293 cells alters ERK1,2 phosphorylation upon activation of the δ and μ-opioid receptors with their specific ligands.

b) Phosphorylation of transcription factors: Alterations of cellular signalling and synaptosomal plasticity

In an attempt to define the molecular mechanisms that lead to alteration of transcription upon opioid administration of the opioid receptors in HEK293 cells, we demonstrated that DSLET-activation of the δ-receptor leads to STAT5B (Signal Transducers and Activators of Transcription) phosphorylation, mediated by a Src tyrosine kinase. Furthermore, we identified that STAT5B interacts with the carboxyl-tail of δ-opioid receptor and forms tight complexes with the δ-receptor, Gβγ dimers and Src kinase. Based on these data and our previous findings

(Mazarakou and Georgoussi 2005) in regard to STAT5A/B phosphorylation by the opioid receptors our current studies are focused in the identification of genes that are activated upon morphine administration of SHSY-5Y cells using gene microarrays. For that reason hybridization of differentially fluorescent labeled cDNA probes generated from control, acutely or chronically-treated human SH-SY5Y neuronal cell RNA are isolated (*Collaboration with Prof. Kostas Iatrou* & the *Gene Microarray Facility of the University of Calgary*). These results will provide insights on which genes are induced upon opioid receptor (acute or chronic) mediated STAT activation and open new possibilities for the identification of additional targets for pharmacological intervention.

c) Signal transduction mechanisms mediated by olfactory receptors of *Anopheles gambiae*

In collaboration with Professor K. Iatrou and Dr L. Swevers *(Laboratory of Insect Molecular Genetics and Biotechnology, IB)* we have successfully expressed two olfactory receptors of *Anopheles gambiae* such as OR1 and OR2 together with the promiscuous Gα16 protein in lepidopteran Bm5 cells. Activation of these ORs with specific ligands raises intracellular Ca^{2+} release. The pharmacological characterization and the identification of the G protein signalling pathways mediated upon activation of the two cloned mosquito antennal odorant receptors (OR1 and OR2) is in progress.

d) Development of new actve substances for pain therapy of cancer patients.

In collaboration with ten laboratories of the European Union our team participates in the project named "Normolife" supported by the *SFP6-Life Sciences* -Genomics and Biotechnology for Health (STREP). Our goal in this project is to study the effects of novel compounds and to identify unanticipated interactions and formation of multi-component complexes involved in opioid receptor signalling mechanisms. We have already tested the effect of two newly synthesized multitarget peptide analogs in our cell based assay systems expressing stably the opioid receptors. Current experiments aim at the determination of the signalling mechanisms these analogs initiate upon prolonged administration of the opioid receptors.

2006 Publications

Georgoussi Z., Leontiadis, L., Mazarakou G., Merkouris M., Karren H. and Hamm H. (2006) Selective interactions between G protein subunits and RGS4 with the C-terminal domains of the of the μ-and δ-opioid receptors regulate opioid receptor signalling Cell. Signal. 18 (6): 771-782

Douris V. Swevers, L., Labropoulou V., Andronopoulou E, Georgoussi Z. and Iatrou K. (2006) Stably transformed insect cell lines: tools for expression of secreted and membrane-anchored proteins and high throughput screening platforms for drug and insecticide discovery. Adv. Virus Res.68¨113-156

Georgoussi Z. (2006) In **"**Molecular aspects of G protein-coupled receptors: Interacting proteins and function" on "Novel interacting partners regulating opioid receptor signalling" (Invited review) Nova Science publishers (eds F. Ciruela and R. Lujan) *In press*

2006 Presentations at International Scientific Conferences

Leontiadis L., Hamm H. and Georgoussi Z. The C-terminal domain of the mu-opioid receptor is an anchor domain for direct RGS4 protein binding regulating opioid receptor signalling, Keystone Symposia on Heptahelical resceptor signalling, Colorado February 2006

Andronopoulou E., Tsikou D., Douris V., Labropoulou V., Swevers L., Georgoussi Z., and Iatrou K. Cloning of Anopheles gambiae antennal odorant receptors and functional expression

in silkmoth cells. Fifth International Symposium on Molecular Insect Science, Tucson, AZ. USA, May 20-24, 2006

Leontiadis L., Hamm H. and Georgoussi Z. The carboxyl-terminal tail of the mu opioid receptor – Docking site for RGS4 protein binding. 31st FEBS meeting, Kostandinoupolis, Turkey, 24-29 June 2006

Tsikou D., Douris V., Andronopoulou E., Labropoulou V., Swevers L., Georgoussi Z., and Iatrou K. Functional expression of mosquito antennal odorant receptors in cultured silkmoth cells : toward the development of a cell based high-throughput screening assay for disruptors of the mosquito host seek response. 7th International workshop of the Molecular Biology of Genetics of the Lepidoptera, Kolymbari, Crete, Greece, August 20-26, 2006

Impact Factors (for 2 publications): 9.228

Citations 2006 (without self- citations): 19

Total Citations 2004-2006 (without self- citations): 48

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

Abraam El Hamitie, 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).

2006 Findings

Finally, it would be helpful to outline below my past productivity (until July of 2004 when I transferred my lab to Greece):

Impact factor and number of citations of the PI:

- Total Impact Factor for the work published after 1998 (19 papers): 139,012
- Average Impact Factor (for each of 19 papers from 1998 to present): 7,31
- Total number of citations (for 24 papers, self-references not included): 762
- Average number of citations (for each of 24 papers): 31,75

Impact Factors: 0

Citations 2006 (without self- citations): 99

Total Citations 2004-2006 (without self- citations): -

h-factor: 14

Research Group: Mechanisms of Cell Proliferation and Ageing

Research Staff
Dimitris Kletsas, Senior Researcher
Dimitrios Stathakos, Emeritus Scientist
Haris Pratsinis, Postdoctoral Fellow
Panagiotis Handris, Graduate Student
Christina Giannouli, Graduate Student
Eleni Mavrogonatou, Graduate student
Vassilki Gioni, Collaborating Graduate Student (MSc)
Adamantia Papadopoulou, Collaborating Graduate Student (MSc)
Stafania Chrissouli, Collaborating Graduate Student (MSc)
Anastassios Malakassis, Collaborating Graduate Student (MSc)

Research Interests

We are focusing on the role of growth factors, and especially of TGF-β, in tissue homeostasis 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 stress 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 stress - in comparison to that of the young or the cancer cell are investigated. Especially, we are interested on the role of the senescent cell - due to its pro-inflammatory phenotype - 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 the intervertebral dis,c the degeneration of which provokes severe dysfunctions during ageing. Finally, the characteristics of centenarians, as an example of successful ageing, are also studied.

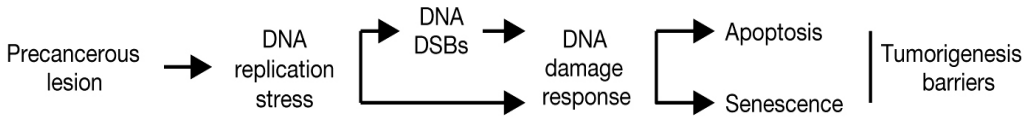
Aim of these studies is the elucidation of the mechanisms underlying the regulation of tissue homeostasis, especially during ageing, and furthermore the contribution in the development of cell replacement therapies. Finally, we study natural products and new synthetic compounds with putative cytostatic/cytotoxic,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

2006 Findings

We have continued our studies on the role of growth factor in tissue repair. Having in mind the different repair strategies between fetuses and adults we have shown that Transforming Growth Factor-β (TGF-β) regulates the proliferation of human fibroblasts according to the developmental stage of the donor: it stimulates the proliferation of adult cells while it inhibits embryonic fibroblasts. TGF-β stimulates adult fibroblasts by induicing the synthesis and release of FGF-2 and ultimately by the activation of the MEK-ERK pathway. In fetal fibroblasts TGF-β inhibits proliferation via the activation of PKA and the subsequent up-regulation of the cyclin-dependent kinase inhibitors $p21^{WAF1}$ and $p15^{INK4}$.The role of TGF-β receptors in this process is under investigation, as well as the role of the amniotic fluid, i.e. the physiological environment of embryonic cells.

Main goal of our studies 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. Besides replicative senescence as a result of telomere erosion, telomere shortening-independent premature senescence can be provoked by exogenous stresses, such as oncogene

overexpression. We have shown, by using cellular systems and human preneoplastic lesions, that oncogene-induced senescence is imposed by DNA damage checkpoints. So it seems that this type of senescence, like apoptosis, serves as a tumorigenesis barrier in preneoplastic lesions (Fig. 1).



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 and that senescent cells promote the growth of adjacent cancer cells. In parallel, we have investigated the effect of anticancer agents on the homeostasis of stroma cells. Finally, we have studied claspin, a nuclear protein involved in DNA replication and DNA damage response and we showed that it is a sensitive marker for aberrant proliferation as its expression coincides with the S phase of the cell cycle.

One of the tissues that is severely affected by ageing is the intervertebral disc. We have shown the presence of a higher percentage of senescent cells in herniated discs, probably contributing to tissue degeneration. We have also 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 degeneration (aiming at tissue repair), we have shown that: 1. various stresses (such as hypertonic stress) activate stress proteins (e.g. p38 MAPK) and induce DNA breaks, leading to cell cycle arrest and 2. disc cells' proliferation by autocrine growth factors is mediated via the ERK and Akt signaling pathways.

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

2006 Publications

Giannouli C.C., Kletsas D. TGF-beta regulates differentially the proliferation of fetal and adult human skin fibroblasts via the activation of PKA and the autocrine action of FGF-2. Cell Signal. 2006 18,1417-29.

Bartkova J., Rezaei N., Liontos M., Karakaidos P., Kletsas D., Issaeva N., Vassiliou L.V.F., Kolettas E., Niforou K., Zoumpourlis V., Takaoka M., Nakagawa H., Tort F., Fugger K., Johansson F., Sehested M., Anderson C., Dyrskjot L., Orntoft T., Lukas J., Kittas C., Helleday T., Halazonetis T.D., Bartek J., Gorgoulis V.G. "Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints" Nature 2006 444, 633-7.

Roberts S, Evans EH, Kletsas D, Jaffray DC, Eisenstein SM. Senescence in human intervertebral discs. Eur. Spine J. Suppl 2006 15, 312-6.

Tsimaratou K, Kletsas D, Kastrinakis N, Tsantoulis P, Evangelou K, Sideridou M, Liontos M, Poulias I, Venere M, Salmas M, Kittas C, Halazonetis T, Gorgoulis V. "Evaluation of claspin as a proliferation marker in human cancer and normal tissues." J. Pathol. 2006 211, 331-339

Rojas-Gil AP, Ziros PG, Diaz L, Kletsas D, Basdra EK, Alexandrides TK, Zadik Z, Frank SJ, Papathanassopoulou V, Beratis NG, Papavassiliou AG, Spiliotis BE. "Growth hormone/JAK-STAT axis signal-transduction defect. A novel treatable cause of growth failure" FEBS J. 2006 273, 3454-66.

Kolokythas G, Pouli N,Marakos P, Pratsinis H, Kletsas D. Design, synthesis and anti-proliferative activity of some new azapyranoxanthenone aminoderivatives. Eur. J. Med. Chem. 2006 41, 71-9.

Skandalis SS, Kletsas D, Kyriakopoulou D, Stavropoulos M, Theocharis DA. The greatly increased amounts of accumulated versican and decorin with specific post-translational modifications may be closely associated with the malignant phenotype of pancreatic cancer. Biochim. Biophys. Acta. 2006 760,1217-25.

Hadjipavlou C, Kostakis IK, Pouli N, Marakos P, Pratsinis H, Kletsas D. "Synthesis and antiproliferative activity of substituted benzopyranoisoindoles: a new class of cytotoxic compounds" Bioorg. Med. Chem. Lett. 2006 16, 4822-5.

Papadimitriou K, Pratsinis H, Nebe-von-Caron G, Kletsas D, Tsakalidou E. "Rapid assessment of the physiological status of Streptococcus macedonicus by flow cytometry and fluorescence probes" Int. J. Food Microbiol. 2006 111, 197-205.

Kostakis IK, Pouli N, Marakos P, Skaltsounis AL, Pratsinis H, Kletsas D. "Design and synthesis of novel amino-substituted xanthenones and benzo[b]xanthenones: evaluation of their antiproliferative activity and their ability to overcome multidrug resistance toward MES-SA/D x 5 cells." Bioorg. Med. Chem. 2006 14, 2910-34.

Kundakovic T, Fokialakis N, Dobric S, Pratsinis H, Kletsas D, Kovacevic N, Chinou I. "Evaluation of the anti-inflammatory and cytotoxic activities of naphthazarine derivatives from Onosma leptantha." Phytomedicine. 2006 13, 290-4.

Papadimitriou K, Pratsinis H, Nebe-von-Caron G, Kletsas D, Tsakalidou E. "Acid Tolerance of Streptococcus macedonicus as Assessed by Flow Cytometry and Single-Cell Sorting." Appl. Environ. Microbiol. 2007 73, 465-76.

2006 Presentations at International Scientific Conferences

Giannouli C.C., Zervolea I., Pratsinis H., Kletsas D. "TGF-β regulates proliferation according to cells' developmental stage and extracellular milieu" 220th FECTS and ISMB meeting, 1-5 July 2006, Oulu, Finland

Pratsinis H., Gioni V., Pilichos K., Yiacoumettis A.M., Tsagarakis S. Kletsas D. "The unexpected anabolic phenotype and extended longevity of skin fibroblatsafter chronic glycocorticoid excess" 5th European Congress of Biogerontology, 16-20 September 2006, Istanbul.

Kletsas D. "Stress and Homeostasis in the Bone and the Intervertebral Disc" Biotechnology Symposium "From Basic Research to Clinical Applications using Biotechnology and Bioengineering" 20-21 October 2006, Lausanne, Switzerland. (invited speaker)

Papadimitriou, K., H. Pratsinis, D. Kletsas, E. Tsakalidou. "Adaptation of Streptococcus macedonicus to acidic environments involves up-regulation of both F-ATPase and glucose specific PEP-PTS transporter". 2nd FEMS Congress of European Microbiologists, 4-8 July 2006, Madrid, Spain

M Anastasiadi, H. Pratsinis, D. Kletsas and S. Haroutounian "Correlation of Antioxidant Activity with the Polyphenolic Content of Extracts From Greek Grapevine Products and Vinification Byproducts" 3rd International Conference on Oxidative Stress in Skin Medicine and Biology, Andros, Greece 21-24 September 2006.

Kletsas D. "Regulation of intervertebral disc cells' proliferation" 1st International symposium "Advances in Spinal Surgery" 16-19 November 2006, Athens. (invited speaker)

Impact Factors (for 12 publications):61.872

Citations 2006 (without self- citations): 233

Total Citations 2004-2006 (without self- citations): 445

h-factor: 13

Research Group: The Role of Nuclear proteins and Chromatin Function

Research Staff
Thomais Sourlingas, Researcher
Kalliopi Sekeri, Emeritus Scientist
Marios Xydous, Graduate Student
Giannis Ninios, Collaborating Graduate Student
Paraskevi Salpea, Collaborating Graduate Student
Niki Varouxi, Undergraduate Student
Kalliopi Kalokyri-Stylianidi, Research Technician

Research Interests

Studies of the expression of histone subtypes and more specifically, linker histone subtypes, as well as the acetylation of nucleosomal histones and their role in chromatin conformational changes during cellular ageing and apoptosis in the in vitro 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 H1o linker histone as well as the acetylated forms of histones H3 and H4 in heterochromatic regions of chromatin and/or in the reorganization of euchromatic/heterochromatin regions of chromatin during ageing and apoptosis. Concomitant to the above, the effect of histone deacetylase inhibitors in the acetylation of non histone target molecules is 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.

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.

2006 Findings

The phosphorylation levels of the DNA linker histone H1 somatic subtypes were studied in the *ex vivo* system of human peripheral blood lymphocytes as a function of donor age. A significant decrease of the mono-phosphorylated forms of the somatic H1.4 and H1.5 subtypes was found in senior and elderly donors. This result can be related to the observed increase of the heterochromatin protein, HP1α, as well as the increased levels of γH2A.X (which is related to telomere shortening and increased DNA damage during ageing) in the same aged donors. Recent *in vitro* studies have shown that histone H1 dephosphorylation is associated with the maintenance of the H1 subtypes in chromatin and the formation or stabilization of condensed chromatin (heterochromatin). Moreover, it has been shown that during ageing heterochromatic foci, known as senescence associated heterochromatic foci (SAHF) are formed in the nucleus. The continuation of this study will involve the elucidation of the functional role of the dephosphorylation of these two H1 subtypes and the increase of HP1α in aged donors in the reorganization or increase of heterochromatic regions and specifically in the formation of heterochromatic foci during ageing and senescence.

A comparative study was undertaken as to the effects of two histone deacetylase inhibitors, trichostatin A and sodium butyrate, on the expression levels of the linker histone subtype, H1o, the acetylation levels of histone H4 and the degree of apoptotic induction in six leukemic cell lines (MOLT-4, U937, NB-4, K562, HL60, Jurkat). The results showed that there is a differential response amongst the different cell lines and the specific inhibitor used with respect to the above mentioned parameters. Specifically, it was found that trichostatin A increases histone H4 acetylation levels to a greater extent that sodium butyrate. However both upregulate histone H1o expression in the cell lines where increased levels of histone H4 acetylations were observed with respect to controls (MOLT-4, U937, NB-4). The degree of

apoptosis was higher in cells where hyperacetylation was induced with trichostatin A treatment as compared to sodium butyrate treatment, which induced lower levels of acetylation. These results show that the histone acetylation/hyperacetylation levels are possibly related to the degree of apoptosis induction and indicate that histone acetylation may be involved in the mode of action of these substances, possibly by activating apoptotic genes. The association of the acetylation levels with the degree of apoptosis found in this work, indicate that histone acetylation levels may be a reliable marker of the activity of deacetylase inhibitors and their use in anticancer regimens.

We are also studying the acetylation of cytoplasmic proteins and their potential involvement in apoptotic signal transduction pathways in normal T lymphocytes and leukemic cells. The results showed that tubulin is acetylated after trichostatin A treatment. Tubulin of normal lymphocytes have stable levels of acetylation which does not change as a function of donor age. On the other hand, the six leukemic cell lines studied (MOLT-4, U937, NB-4, K562, HL60, Jurkat) showed a differential response to the inhibitor with respect to tubulin acetylation levels. Since histone deacetylase inhibitors are used as anticancer agents, whose mode of action in many cases is induction of apoptosis, we studied the apoptosis levels after trichostatin A treatment in relation to the levels of tubulin acetylation. We found a correlation amongst increased levels of tubulin acetylation and apoptosis. Near future experiments, using a deacetylase inhibitor, that does not inhibit the cytoplasmic deacetylase (HDAC6), the only deacetylase that deacetylates tubulin, will show whether tubulin is indeed involved in a cytoplasmic apoptotic signal transduction pathway.

Within the framework of a research project collaboration with the laboratory of Dr. A. Prombona ("Regulation of Transcription of Plants by the Biological Clock"), involving the study of the effects of the levels of histone acetylation in genes which regulate the mammalian biological clock and their effects in cell cycle function and carcinogenesis, circadian rhythm was induced in immortalized fibroblast mouse cell line, NIH3T3 that under normal cell culture conditions does not have a circadian rhythm. At the same time, the effects of curcumin, an inhibitor of the histone acetyltransferase, p300, as well as the histone deacetylase inhibitor, trichostatin A, on the expression levels of circadian clock genes were studied at different circadian times. Preliminary results showed that in cultures where circadian rhythm was induced, curcumin and trichostatin A, decreased and increased the expression levels of clock genes (e.g., *per1*), respectively.

2006 Presentations at International Scientific Conferences

N. Happel, D. Detlef, K.E. Sekeri-Pataryas, T.G. Sourlingas. H1 somatic histone subtype constitution and phosphorylation state of the ageing cell system of human peripheral blood lymphocytes. 5th European Congress of Biogerontology, Istanbul, Sept. 2006.

Citations 2006 (without self- citations): 19

Total Citations 2004-2006 (without self- citations): 36

h-factor: 6

Research Group: Cell and Matrix Pathobiology

Research Staff
Fotini-Effie Tsilibary, Research Director
Paraskevi Kitsiou, Researcher
Athina Tzinia, Researcher
Garifallia Drossopoulou, Postodoctoral Fellow
Argiris Talamaghas, Graduate Student
Panagiotis Vevieratos, Graduate Student
Ioanna Tsagaraki, Graduiate Student
Nikos Tsotakos, Graduiate Student
Maria Manta, Collaborating Graduate Student
Evaggelos Fragopoulous, Collaborating Graduate Student
Nefali Lagopati, Collaborating Graduate Student
Eleni Kotsopoulou, Research Technician

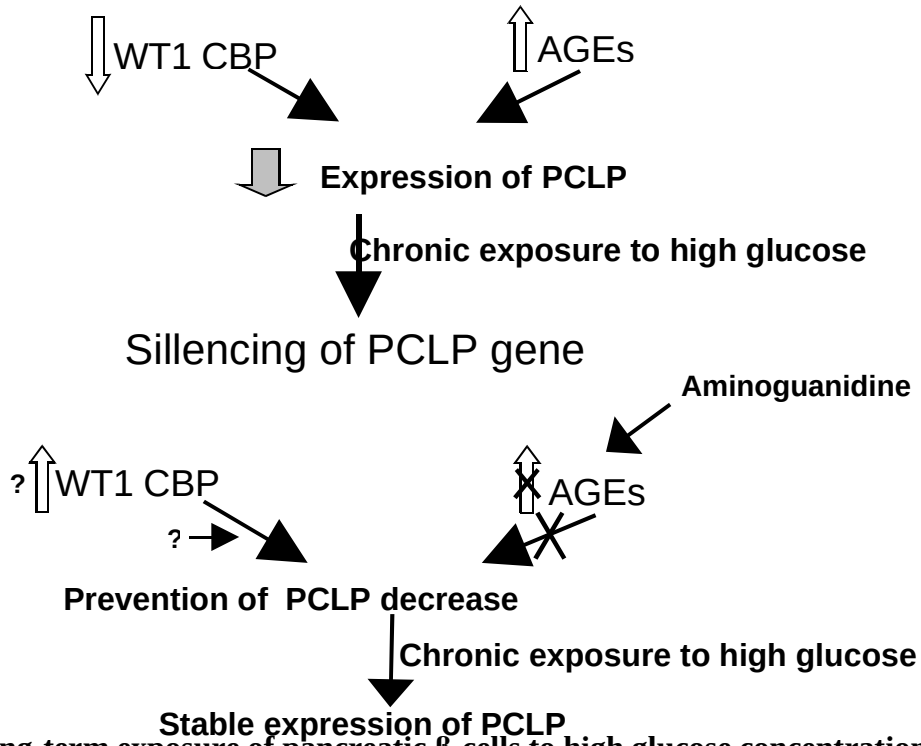
Research Interests

- Regulation of expression of the anti-adhesive sialoprotein podocalyxin, by renal glomerular epithelial cells: Determination of transcription factors involved in the regulation of expression in normal and diabetic conditions
- Elucidation of molecular mechanisms which, in high glucose concentrations and/or in the presence of pro-inflammatory cytokines, impair the insulin signaling survival pathway in pancreatic beta-cells, thus leading to the development of diabetes mellitus
- Examination of functional properties of neuroblastoma cells in Alzheimer's conditions (culture in the presence of amyloid αβ), with the aim to prevent the extracellular accumulation of the amyloid protein which is involved in neuronal degeneration
- Functional properties of glutamate transporters in normal and neuro-degenerative conditions, with the aim to understand the role of these transporters in neurodegenerative conditions, and the regulation of transporter function in these conditions
- Regulation of osteoblastic cell function in inflammatory conditions, with the aim to prevent inflammation-generated modulation of matrix composition and function in conditions such arthritis and osteoporosis.

2006 Findings

1) Regulation of gene expression of the anti-adhesive cell surface sialoprotein podocalyxin in renal glomerular epithelial cells: The basic research goal is to unravel factors involved in the regulation of expression of podocalyxin-like protein (PCLP). These factors were examined in the presence of normal (low: 5mM) and increased (high: 25 mm) glucose levels in the media of cultured human glomerular epithelial cells (HGEC), which do not express PCLP in high glucose.
We have demonstrated thus far that chronic exposure of podocytes to high glucose, a situation resembling diabetes, results in the silencing of the PCLP gene and sequential detachment from their underlying basement membrane. Furthermore we obtained evidence indicating that this effect may be the result of defective association of transcription factor WT1 which regulates PCLP expression, with other transcription factors such as CBP. Defective association between these two factors may impair the transcriptional activity of WT1.
Additionally we have demonstrated that loss of PCLP expression in high glucose is irreversible, since this sialoprotein is not re-expressed if glucose concentrations are reverted to

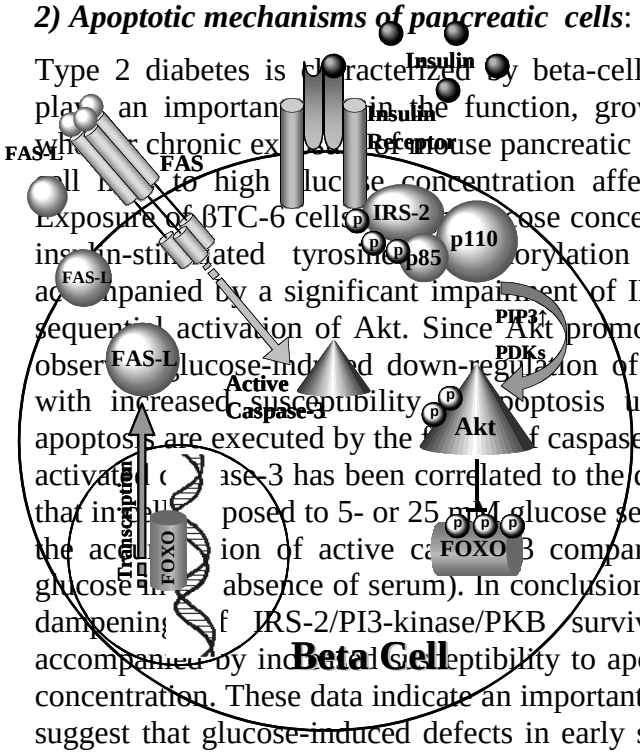
normal levels even for prolonged time intervals. At a cellular level, hyperglycemia may induce damage by the process of non-enzymatic glucosylation and AGE (advanced glycation end-products) formation. Addition of aminoguanidine (AG), and AGE inhibitor, in the culture medium prevents glucose-induced reduction of PCLP expression. Therefore chronic administration of AG on our in vitro system prevents AGE-induced toxicity and perhaps enhances the binding of the WT1-CBP complex on the promoter of the PCLP gene, as shown diagrammatically below.



Long-term exposure of pancreatic β-cells to high glucose concentration resulted in:

2) Apoptotic mechanisms of pancreatic cells:

Type 2 diabetes is characterized by beta-cell dysfunction and apoptosis. Insulin signaling plays an important role in the function, growth and survival of β-cells. We investigated whether chronic exposure of mouse pancreatic β-cells (βTC-6 cells, an insulin-producing beta-cell line) to high glucose concentration affected the insulin-mediated signaling pathway. Exposure of βTC-6 cells to high glucose concentration resulted in a significant impairment in insulin-stimulated tyrosine phosphorylation of IR and IRS-2. These changes were accompanied by a significant impairment of IRS-2-associated PI3-kinase activation, and the sequential activation of Akt. Since Akt promotes cell survival, we investigated whether the observed glucose-induced down-regulation of IRS-2/PkB survival signaling was associated with increased susceptibility to apoptosis upon serum deprivation. The final stages of apoptosis are executed by the family of caspases, including caspase-3. The amount of cleaved/activated caspase-3 has been correlated to the degree of beta-cell apoptosis. We demonstrated that insulin-deprived cells exposed to 5- or 25 mM glucose serum deprivation triggered a 2.5 fold increase in the activation of active caspase-3 compared with control cells (cells grown in 1 mM glucose in the absence of serum). In conclusion our results demonstrated that glucose-induced dampening of IRS-2/PI3-kinase/PKB survival signalling in response to insulin was accompanied by increased susceptibility to apoptosis of βTC6-cells exposed to high glucose concentration. These data indicate an important role of insulin signalling 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.



Reduction of insulin-induced IR phosphorylation.

Reduction of insulin-induced IRS-2 phosphorylation.

Reduction of insulin-induced IRS-2/PI3K association.

Reduction of insulin-induced Akt phosphorylation.

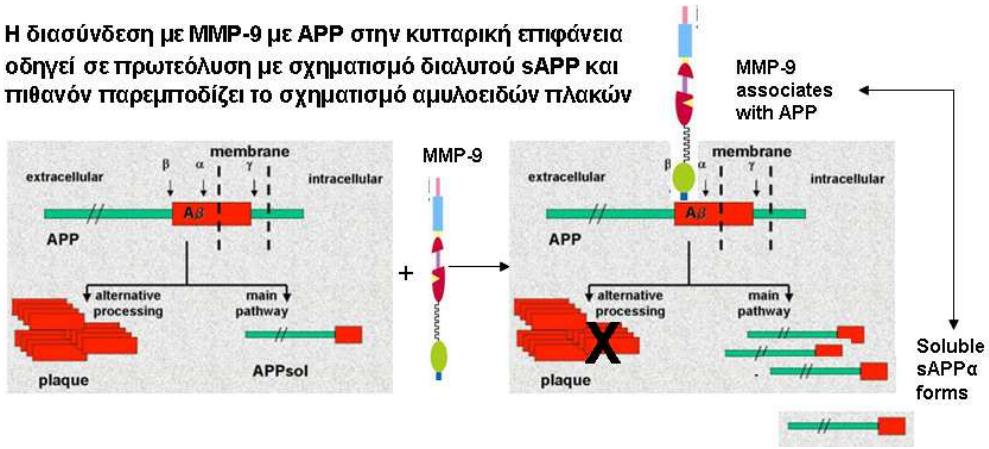
Enhanced FOXO activity.

FAS-L upregulation.

Caspase-3 activation.

The observed downregulation of insulin signaling was accompanied by increased β-cell apoptosis possibly through ca spase-3 activation.

3) Neuroblastoma cell function in conditions simulating Alzheimer (in the presence of amyloid αβ): Previous results of the lab indicated that collagenase MMP-9 expression is induced in SK-N-SH neuroblastoma cells by amyloid Aβ, and acts as neuroprotective α-secretase. More specifically, HEK-293 cells constantly expressing APP695 were transiently transfected with the cDNA for pro-MMP-9. Over expression of MMP-9 increased the amount of sAPPα, an α-secretase cleavage product, with a concomitant decrease in Aβ levels examined by ELISA. Moreover, the processing of APP by MMP-9 in the presence of phorbol ester (PMA), known to enhance the activity of PKC, which modulates the activity of α-secretases, revealed the increase by 2.5x of sAPPa. This increase was inhibited by SB3CT a well-known inhibitor of MMP-9. Finally, by immunoprecipitation and immunofluoresence an association between MMP-9 and APP695 was observed.



4) Regulation of glutamate transporters in physiological and neurodegenerative conditions: To identify functional association between CK1 from *A. nidulans* and human glutamate transporters, a plasmid constructs for CK1 was developed. HEK-293 cells were co-transfected with this plasmid and a plasmid containing the cDNA for human glutamate transporter EAAT3. The expression of the proteins will be examined by western blot analysis.

5) Survival and apoptotic mechanisms of osteoblastic cells in inflammation: Treatment of MG63 cells with TNF-a resulted in a concomitant increase of both MMP-9 and its specific inhibitor TIMP-1. Additionally, increased phosphorylation of both Akt and p38was observed. Under the same experimental conditions no apoptosis was observed, by FACS analysis, due to an apoptotic agent like TNF-a. On the contrary, when cells were forced to apoptosis by adding CHX one hour prior to TNF-α addition the levels of TIMP-1 were decreased by 39%. The anti apoptotic gene bcl-xl was also decreased.

Citations 2006 (without self- citations): 86
(Tsilibary EC, Tsilibary E, Tsilibary PC): 68
(Tzinia A, Tzinia AK): 9
(Kitsiou P): 8

Total Citations 2004-2006 (without self- citations): 304
(Tsilibary, EC. Tsilibary E, Tsilibary PC): 249
(Tzinia A, Tzinia AK): 34
(Kitsiou P): 21

h-factor: Tsilibary:29, Tzinia: 4, Kitsiou: 4

For the following project, Dr. Tsilibary is the Administrative Investigator.

Research Group: Chemical Ecology and Natural Products

Research Staff

Maria Konstantopoulou, Technical Specialist
Vassilios Mazomenos, Emeritus Scientist
Anastassia Pantazi-Mazomenou, Research Technician

2006 Publications

Konstantopoulou M., H. Pratsinis, D. Kletsas and Mazomenos B., 2006. Pheromone binding protein and general odorant binding protein of *Sesamia nonagrioides* (Lepidoptera: Noctuidae): Sex and diel dependent expression. *Entomologia Experimentalis et Applicata* 119(2): 129-136.

M. Konstantopoulou, P. Milonas and B.E. Mazomenos, 2006. Partial Purification and Insecticidal Activity of Toxic Metabolites Secreted by a *Mucor hiemalis* Strain (SMU-21) against Adults of *Bactrocera oleae* and *Ceratitis capitata* (Diptera: Tephritidae) *Journal of Economic Entomology* 99(5): 1657-1664.

Impact Factors (for 5 publications): 2.453

Citations 2006 (without self- citations): 32
V. Mazomenos: 24
M. Konstantopoulou: 8

Total Citations 2004-2006 (without self- citations): 88
V. Mazomenos: 68
M. Konstantopoulou: 20

h-factor:

V. Mazomenos: 11
M. Konstantopoulou: 4

Research Group: Environmental Mutagenesis -Carcinogenesis

Research Staff
Gerassimos Voutsinas, Researcher
Vassilis Nikas, Graduate Student
Dimitra Anastasiou, Collaborating Graduate Student
Stefanos Kachrilas, Collaborating Graduate Student
Sofia Melachrinou, Collaborating Graduate Student
Evmorphia Konstantatou, Collaborating Graduate Student
Panagiotis Karkoulis, Collaborating Graduate Student
Eleni Litsiou, Collaborating Graduate Student
Antonis Lampidonis, Collaborating Graduate Student
Athina Giarika, Undergraduate Student
Sokratis Avgeris, Research Technician

Research Interests

1. Development and evaluation of tumor biomarkers: Genetic and epigenetic alterations in genes involved in cellular metabolism, cell cycle control, DNA repair and apoptosis, and their relation to human disease
2. Identification and validation of drug targets for cancer therapy: Involvement of apoptotic signal transduction in carcinogenesis and drug resistance
3. Development of protocols for molecular diagnosis of human genetic diseases

2006 Findings

Tn-C expression has been observed in papillary and medullary thyroid carcinomas with different staining patterns, accompanied by the prevalence of different mRNA splice variants in cell cultures. It seems possible that Tn-C is rather synthesized by tumor cells than by activated stromal cells.

Fibrillin-1 is strongly expressed by the neoplastic cells of thyroid carcinomas in different degree in the various histologic types and might be implicated in cell-stroma interaction in terms of signaling, attachment and migration

In thyroid carcinomas, the proteolytic cleavage and activation of caspase-8 depends on the balance between expression levels for procaspase-8 and FLIP and correlates with favourable clinical prognosis. Fas may actually stimulate proliferation and confer a survival advantage to thyroid cancer cells.

Thyroid carcinoma cells overall are poorly responsive to clinically relevant concentrations of AEE788 in vitro. The presence of EGFR activating TK domain mutations may identify a small minority of thyroid cancer patients that may benefit from EGFRIs, but additional preclinical evidence of efficacy is needed.

2006 Publications

Tseleni-Balafouta, S., Gakiopoulou H., Fanourakis G., Voutsinas G., Balafoutas D. and Patsouris E. (2006). Tenascin-C protein expression and mRNA splice variants in thyroid carcinoma. Experimental and Molecular Pathology 80, 177-182.

Tseleni-Balafouta, S., Gakiopoulou H., Fanourakis G., Voutsinas G., Litsiou H., Sozopoulos E., Balafoutas D. and Patsouris E. (2006). Fibrillin expression and localization in various types of carcinomas of the thyroid gland. Modern Pathology 19, 695-700.

Mitsiades, C.S., Poulaki V., Fanourakis G., Sozopoulos E., McMillin D., Wen Z., Voutsinas G., Tseleni-Balafouta S. and Mitsiades N. (2006). Fas signaling in thyroid carcinomas is diverted from apoptosis to proliferation. Clinical Cancer Research 12, 3705-3712.

Mitsiades, C.S., Kotoula V., Poulaki V., Sozopoulos E., Negri J., Charalambous E., Fanourakis G., Voutsinas G., Tseleni-Balafouta S. and Mitsiades N. (2006). EGFR as a therapeutic target in human thyroid carcinoma: mutational and functional analysis. Journal of Clinical Endocrinology and Metabolism 91, 3662-3666.

Impact Factors (for 4 publications): 17,25

Citations 2006 (without self- citations): 20

Total Citations 2004-2006 (without self- citations): 47

h-factor: 5

PROGRAMME B:

MODEL SYSTEMS FOR THE STUDY OF CELL FUNCTION

Research Group: Molecular Genetics of Insects and Biotechnology

Research Staff
Kostas Iatrou, Research Director
Luc Swevers, Senior Researcher
Vassiliki Lampropoulou, Researcher
Lydia Ignatiadou, Emeritus Scientist
Evi Andronopoulou, Postdoctoral Fellow
Rodica Efrose, Postodoctoral Fellow
Vassilios Douris, Postdoctoral Fellow
Christos Kenoutis, Postdoctoral Fellow
Aghelina Metaxatou, Postdoctoral Fellow
Konstantia Sdralia, Graduate Student
Theodoros Georgomanolis, Graduate Student
Christiana Magrioti, Graduate Student
Elena Karkoulia, Collaborating Graduate Student
Ioannis Papaethimiou, Graduate Research Assosiate (MSc)
Daniela Tsikou, Graduate Research Assosiate (MSc)
Dimitra Stefanou, Technical Specialist
Dimitrios Kopanelis, Research Technician

Research Interests

1. Silkmoth oogenesis: a model system for the study of differentiation programs induced by the steroid hormone ecdysone.
2. Molecular biology and genetic modification of insect nucleopolyhedrosis viruses.
   a) incapacitated nucleopolyhedrosis viruses as genetic transformation vectors.
   b) modified nucleopolyhedrosis viruses as gene therapy vectors.
3. Functional genomics: high-throughput screening systems for detection of bioactive substances (agonists and antagonists of pharmacological targets) in natural products

2006 Findings

Regulatory factors of lepidopteran oogenesis.
The functional characterization of 3 proteins, NTP1-4, CTP15-1 and BmSH3 interacting with the transcriptional regulator BmGATAβ and the orphan nuclear receptor BmE75C during program of the ovarian follicle differentiation in the silkworm *Bombyx mori* was continued. Western blot and immunolocalization experiments with specific antibodies showed that NTP1-4 is secreted from the follicular epithelial cells toward the oocyte and is incorporated in the vitelline membrane while CTP15-1 is an atypical chorion protein that undergoes N-glycosylation and proteolytic processing before secretion outside the cells of the follicular epithelium. The functional characterization of BmSH3, a putative regulator of the function of the orphan nuclear receptor BmE75C, was also continued through the identification of 3 protein isoforms that are expressed in different compartments of the follicles and the follicular sheath. Immunofluorescence studies suggest that BmSH3 localizes at the basal and apical sides of the epithelial cells of the follicles, a finding compatible with a role in the regulation of cellular shape and adhesion that was proposed for this protein. Co-immunoprecipitation experiments using transfected tissue culture cells confirmed the interaction of BmSH3 with the receptor E75C. Finally, studies on the regulation of oogenesis by prostaglandins were also completed. The studies showed that not only vitellogenic stages but all stages of oogenesis are dependent on prostaglandin signalling. To obtain further insights into the mechanism by which prostaglandin(s) regulates progression of oogenesis at the level of gene expression, DNA microarray hybridization assays will be carried out.

Parasitism of lepidopteran insects by parasitic wasps

To study the function of the endosymbiontic virus CcBV that is introduced in lepidopteran hosts during their parasitism by endoparasitoid wasps, we have cloned and expressed in insect and mammalian cells the CcBV genes that encode Cactuslike proteins. It appears that the viral Cactuslike protein has the capacity to inhibit the function of the factor Rel in silkmoth-derived cells (Bm5 cells) as well as the factor NFκB (mammalian homolog of Rel) in human cells (HEK293). The inhibition of expression of genes encoding antibacterial proteins (attacin and lebocin that are induced by RelA and RelB) by Cactuslike inhibitors in cell lines derived from different insect species is currently under investigation. Furthermore, co-immunoprecipitation experiments performed in order to evaluate the interaction of Cactuslike with hemolin, an insect immune response protein whose interaction with Cactuslike was detected in a yeast two-hybrid interaction assay, failed to confirm the interaction between the two proteins. Finally, to find out to which extent another CcBV protein, CcBV1, affects the physiological function of the hemocytes during parasitism, experiments of phagocytosis of $Gram^-$ bacteria by tissue culture cells (HighFive, S2) and hemocytes of *Bombyx mori* in the absence or presence of CcBV1 were carried out. These experiments showed that CcBV1 is taken up by the hemocytes and the HighFive cells and that the capacity of hemocytes to phagocytose bacteria is concomitantly reduced by 30%.

Regulation of olfactory function in mosquitoes.

After the completion of the initial studies on the functional characterization of mosquito odorant binding proteins (OBPs) that we described last year, our efforts concentrated on the functional expression of two odorant receptors, OR1 and OR2, that are predominantly expressed in the olfactory neurons of female mosquito antennae, as well as a third receptor, OR7, a putative partner of all odorant receptors in this insect. The functional analysis of authentic or tagged versions of these three receptors in silkmoth-derived Bm5 cells showed that the receptors (i) are expressed at high levels in these cells, and (ii) become localized at the plasmamembrane of the cells, as would be predicted for correctly localized GPCR receptors, a class of receptors to which the odorant receptors belong. Immunocytochemistry experiments after co-expression in Bm5 cells showed that OR7 co-localizes with OR1 and OR2, indicative of heterodimerization of OR1/OR7 and OR2/OR7. Further studies that in collaboration with the laboratory of Molecular Pharmacology (Dr. Z. Georgoussi) showed that OR1 and OR2 are activated by their specific ligands, *p*-cresol and *o*-cresol, respectively, in Bm5 cells, that the signalling pathway that is induced occurs primarily through the activation of phospholipase C, and that the co-expression of OR7 with OR1 or OR2 results in modest increases in the activity of the two receptors. Because the activation of phospholipase C causes the release of calcium from subcellular storage sites and the latter may be readily detected by appropriate fluorescent indicators, the Bm5-based expression system of OR1 and OR2 can be used for high-throughput detection of new agonists and antagonists for these receptors.

Molecular biology and genetic modification of nuclear polyhedrosis viruses.

The generation and biochemical characterization of two recombinant insect viruses derived from the baculovirus of the silkworm (BmNPV) that are used as gene transduction vectors for two candidate therapeutic proteins, L1 and F3, in human cells (primary Schwann cell cultures, which support the development of neurons in the peripheral nervous system) was completed. In collaboration with the group of Dr. R. Matsas (Hellenic Pasteur Institute), Schwann cells were infected with the recombinant virus that expresses the L1 protein and were used in *in vitro* bio-assays (scratch assays) that simulate traumas of the nervous system. The experiments showed that the infected Schwann cells that express L1 protein have an increased mobility towards the area of trauma as compared with uninfected cells. Schwann cells infected with recombinant virus that produces F3 did not show this behaviour.

Functional genomics.
Our studies concentrated on the development of high-throughput screening platforms for activators (or inhibitors) of odorant receptors independent of the endogenous signalling pathways that are used by them. For this purpose, we created genetically transformed Bm5 cell lines that over-express silkmoth Gαq or human Gα16 or Gαs. Furthermore, we generated triple transformed cell lines that express the proteins Gα16/OR1/OR7, Gα16/OR2/OR7, Gαq/OR1/OR7 and Gαq/OR2/OR7. These cell lines represent the functional platforms for future screenings of collections of natural products or chemical compounds for the detection of new ligands (agonists or antagonists) for OR1 and OR2 as well as other mosquito odorant receptors .

2006 Publications

Wheelock, C.E., Nakagawa, Y., Harada, T. Oikawa, N., Akamatsu, M., Smagghe, G., Stefanou, D., Iatrou, K., and Swevers, L. (2006). High throughput screening of ecdysone agonists using a reporter gene assay followed by 3-D QSAR analysis of the molting hormonal activity. Bioorg. Med. Chem. 14, 1143-59.

*Kenoutis, C., *Efrose, R., Swevers, L., Lavdas, A., Gaitanou, M., Matsas, R., and Iatrou, K. (2006). Baculovirus-mediated delivery into mammalian cells does not alter their transcriptional and differentiating potential is accompanied by early viral gene expression. J. Vir. 80, 4135-4146.

Douris, V., Swevers, L., Labropoulou, V., Andronopoulou, E., Georgoussi, Z. and Iatrou, K. (2006). Stably transformed insect cell lines: tools for expression of secreted and membrane-anchored proteins and high throughput screening platforms for drug and insecticide discovery. Adv. Virus Res. 68, 113-156.

Andronopoulou, E., Labropoulou, V., Douris, V., Tsikou, Woods, D. F., D., Biessmann, H., and Iatrou, K. (2006). Specific interactions amongst odorant binding proteins of the African malaria vector *Anopheles gambiae*. Insect Mol. Biol. 15, 797-811.

2006 Presentations at International Scientific Conferences

D. Tsikou, V. Douris, E. Andronopoulou, L. Swevers and K. Iatrou. Cloning and functional expression of antennal odorant receptors of Anopheles gambiae in lepidopteran cells. Keystone Symposia on G Protein-Coupled Receptors: Evolving Concepts and New Techniques. Keystone, Colorado, USA, February 12-17, 2006

K. Iatrou. Baculoviruses as transducing vectors for mammalian cells: production solutions for serum-free environments and biological pitfalls and challenges for gene therapy applications. The Williamsburg BioProcessing Foundation 9th Annual Baculovirus & Insect Cell Culture Conference, San Francisco, California, February 2022, 2006.

E. Andronopoulou, V. Labropoulou, V. Douris, D.F. Woods, H. Biessmann and K. Iatrou. Specific interactions amongst classic and Plus-C odorant binding proteins of the African malaria vector *Anopheles gambiae*. Fifth International Symposium on Molecular Insect Science, Tucson, AZ, USA, May 20-24, 2006.

D. Tsikou, V. Douris, E. Andronopoulou, V. Labropoulou, L. Swevers, Z. Georgoussi and K. Iatrou. Cloning of *Anopheles gambiae* antennal odorant receptors and functional expression in silkmoth cells. Fifth International Symposium on Molecular Insect Science, Tucson, AZ, USA, May 20-24, 2006.

K. Iatrou. Assay development for nuclear transcription factors and GPCRs. Workshop on "Approaches for the identification of novel insecticide targets", Fifth International Symposium on Molecular Insect Science, Tucson, AZ, USA, May 20-24, 2006.

Y. Nagakawa, C.E. Wheelock, T. Harada, T. Soin, G. Smagghe, M. Akamatsu, K. Iatrou and L. Swevers. Structure-activity relationship of non-steroidal ecdysone agonists and prediction of ligand binding to the ecdysone receptor of *Bombyx mori*. XVI International Ecdysone Workshop, Ghent, Belgium, July 10-14, 2006.

T. Georgomanolis, K. Iatrou and L. Swevers. Expression analysis of BmSH3, a putative modulator of the function of the ecdysone-regulated orphan nuclear receptor BmE75C during silkmoth oogenesis. XVI International Ecdysone Workshop, Ghent, Belgium, July 10-14, 2006.

K. Van Loocke, Efrose, R., Labropoulou, V., L. Swevers, K. Iatrou and G. Smagghe. Inhibitory effect of the ecdysteroid signaling by the juvenile hormone analogues pryriproxifen, kinoprene and fenoxycarb in a dipteran (S2) and lepidopteran (Bm5) cell line. XVI International Ecdysone Workshop, Ghent, Belgium, July 10-14, 2006.

K. Van Loocke, Y. Nagakawa, B. Watanabe, L. Swevers, K. Iatrou, D. Geelen, D. Reheul and G. Smagghe. Structure-activity relationship of brassicosteroids (BRs) and BR-hybrids in ecdysteroid signaling in a lepidopteran (Bm5) and diupteran (S2) cell line. XVI International Ecdysone Workshop, Ghent, Belgium, July 10-14, 2006.

K. Van Loocke, D. Geelen, L. Swevers, K. Iatrou and G. Smagghe. Developmental defects in the cotton leafworm, Spodoptera littoralis, and inhibition of ecdysteoid signaling effected by administration of saponins. XVI International Ecdysone Workshop, Ghent, Belgium, July 10-14, 2006.

E. Andronopoulou, D. Tsikou, V. Douris, V. Labropoulou, L. Swevers, Z. Georgoussi and K. Iatrou. Functional expression of mosquito antennal odorant receptors in cultured silkmoth cells: toward the development of a cell-based high-throughput screening assay for disruptors of the mosquito host seek response. Seventh International Workshop on the Molecular Biology and Genetics of the Lepidoptera, Kolympari, Crete, Greece, August 20-26, 2006.

V. Douris, E. Andronopoulou, E., Tsikou, N. Sdralia, V. Labropoulou, L. Swevers and K. Iatrou. *LepCell Express*: a modular platform for protein expression in lepidopteran insect cells. Seventh International Workshop on the Molecular Biology and Genetics of the Lepidoptera, Kolympari, Crete, Greece, August 20-26, 2006.

R. Efrose, C. Kenoutis, V. Douris, L. Swevers and K. Iatrou. Towards the development of transcriptionally silent baculovirus-based mammalian gene transduction vectors with improved safety features. Seventh International Workshop on the Molecular Biology and Genetics of the Lepidoptera, Kolympari, Crete, Greece, August 20-26, 2006.

T. Georgomanolis, K. Iatrou, and L. Swevers. BmSH3, an adaptor protein with multiple SH3 and a single SoHo domain: expression analysis during follicle development in the ovary of the silkmoth, *Bombyx mori*. Seventh International Workshop on the Molecular Biology and Genetics of the Lepidoptera, Kolympari, Crete, Greece, August 20-26, 2006.

E. Machado, L. Swevers, and K. Iatrou. Prostaglandin signaling and cAMP production control the rate of follicle progression from vitellogenesis to choriogenesis during silkmoth oogenesis. Seventh International Workshop on the Molecular Biology and Genetics of the Lepidoptera, Kolympari, Crete, Greece, August 20-26, 2006.

V. Labropoulou, V. Douris, D. Stefanou, C. Magkrioti, E. Andronopoulou, L. Swevers and K. Iatrou. The interaction of the *Cotesia congregata* Bracovirus CcV1 protein with *Manduca sexta* hemolin. Seventh International Workshop on the Molecular Biology and Genetics of the Lepidoptera, Kolympari, Crete, Greece, August 20-26, 2006.

L. Swevers, D. Stefanou, T. Soin, K. Van Loocke, G. Smagghe, C. Wheelock, T. Harada, M. Akamatsu, Y. Nakagawa and K. Iatrou. Insect cell-based high-throughput screening systems

for the identification of compounds with ecdysteroid mimetic insecticide activities. Seventh International Workshop on the Molecular Biology and Genetics of the Lepidoptera, Kolympari, Crete, Greece, August 20-26, 2006.

Y. Nakagawa, Wheelock, C.E., Harada, T., Soin, T., Smagghe, G., Akamatsua, M., Iatrou, K., and Swevers, L. (2006). Structure-activity relationship for the activity of non-steroidal ecdysone agonists and the prediction of the ligand binding to the *Bombyx mori* ecdysone receptors. 11th IUPAC International Congress of Pesticide Chemistry. August 6-11, Kobe, Japan.

K. Iatrou. Cell-based functional genomics platforms for discovery of new lead compounds for drug and insect pest control agent development. Third International Biotechnology Forum, October 5-7, 2006, Athens, Greece.

E. Machado, L. Swevers and K. Iatrou. The progression from vitellogenesis to choriogenesis during silkmoth oogenesis requires autocrine/paracrine signaling by prostaglandins. Symosium on the "Impact of Hormone Research on Science and Economy", Annual meeting of the Entomological Society of America, Indianapolis, Indiana, USA, December 10-14, 2006.

Impact Factors (for 4 publications):14,876

Citations 2006 (without self- citations): 106

Iatrou K. (Swevers' publications are included): 124
Swevers L.: 3
Lampropoulou V.: 31
Ignatiades L.: 37

Total Citations 2004-2006 (without self- citations): 361
Iatrou K. (Swevers' publications are included): 124
Swevers L.: 8
Lampropoulou V.: 99
Ignatiades L.: 131

h-factor:
21 (K. Iatrou)
9 (L. Swevers)
5 (V. Lampropoulou)
14 (L. Ignatiadou)

Research Group: Transcriptional Regulation by the Biological Clock

Research Staff
Anastassia Prombona, Senior Researcher
Anastasia Repouskou, Graduate Student
Marianna Kapi, Undergraduate Student
Eliana Gika, Undergraduate Student
Irene Nomikou, Undergraduate Student
Aggeliki Galeou, Training Student
Sokrates Avgeris, Research Technician

Research Interests

- ***Investigation of the biological clock function in Phaseolus vulgaris.***

Regulation by light and the circadian clock of the two putative clock components PvLHY and PvTOC1. Study of *PvLHY* gene regulatory *cis* elements and of PvLHY protein function in order to understand the role of the putative transcription factor in rhythmic transcriptional regulation. Investigation of PvLHY and PvTOC1 roles in the function of the central oscillator in bean. Study of the gating mechanism of the biological clock on input light signals.

- ***Investigation of the 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. Modulation of the histones' acetylation levels and study of its effects on the biological clock function and the cell cycle. Our goal is to study the effects of drugs that control the proliferation of cancer cells in dependence of the circadian time to achieve improved therapy (chronotherapy).

2006 Findings

I.) In the study of the plant biological clock function we proceeded in the production of polyclonal antibodies for factor PvLHY and the study of the plant protein is in progress. Expression levels of *PvELF3* did not exhibit rhythmicity in *Phaseolus* leaves, as is the case in *Arabidopsis thaliana*. For this reason we investigate the role of *PvELF4*, which shows rhythmic expression, in the synchronization of the *Phaseolus* circadian clock to light. The role of these components in the synchronization of the clock is in progress.

II.) Regarding the study of the involvement of the biological clock function in cell proliferation and carcinogenesis, our studies in the mural N2A cell line showed that dexamethasone induces the rhythmic expression of the central oscillator components and thus this malignant cell line possesses a functional biological clock. In experiments, where trichostatin A was applied (inhibitor of the histone deacetylases), we observe an effect on the expression of the central oscillator components *per1* and *per2*, which is differentiated according to the circadian time of application. In opposite, application of curcumin, a p300 inhibitor, has the same effect on the expression of *per1* and *per2*, independently of the circadian time of application. The study of the rhythmically expressed cell cycle genes is in progress. This work is in collaboration with the laboratory of Histone Biochemistry (Dr. T. Sourlingas).

2006 Publications

Kaldis A.-D. and Prombona A. (2006) Synergy between the light-induced acute response and the circadian cycle: a new mechanism for the synchronization of the *Phaseolus vulgaris* clock to light. Plant Mol. Biol. 61, 883-895.

Impact Factors(for 1 publication): 3.328

Citations 2006 (without self- citations): 8

Total Citations 2004-2006 (without self- citations): 32

h-factor: 5

Research Group: Microbial Molecular Genetics

Research Staff
Vassiliki Sophianopoulou, Researcher Director
Eleftherios Sideris, Emeritus Scientist
Dimosthenis Kizis, Postdoctoral Fellow
Zoi Erpapazoglou, Graduate Student
Dimitra Bouzarelou, Graduate Student
Katerina Roumelioti, Collaborating Graduate Student
Anna Bombori, Collaborating Graduate Student (MSc)
Marina Pantazopoulou, Undergraduate Student
Alexandros Pittis, Undergraduate Student
Marina Laspa, Undergraduate Student
Sofia Verouti, Training Student
Dikran Tsitsekian, Trainig 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 and purine transporters b) studies on structure-function relationships of amino acid transporters c) identification of *trans*-acting molecular determinants involved in topogenesis and synthesis / activity of amino acid transport systems

- **Basic research on mechanisms involved in cell wall expansion and phytopathogenicity in fungi**

  Identification and study of non-plant Expansin-like proteins

  a) identification and regulation of the expression of genes encoding expansin-like protein(s) in *Aspergillus nidulans,* b) physiological / functional characterization and cellular localization of the encoded proteins

- **Functional genomics**

  Use of *Aspergillus 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

  Functional expression and structure-function relationships of human NAT homologues, specific for L-αscorbate transport

2006 Findings

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

  i) Studies on ShrA protein function: ShrAp is important for the sorting to the plasma membrane of at least three distinct amino acid transporters in Aspergillus nidulans.

  ii) Preliminary experiments for the isolation of new protein determinants involved in the functional expression and or topogenesis of amino acid transporters.

- **Basic research on mechanisms involved in cell wall expansion and phytopathogenicity in fungi**

  Studies on a non-plant expansin-like protein: Fungal ExpAp is a spore-specific cell wall-enzyme, acting as an endoglucanase. Transcription and translation of the *expA* gene occur at different developmental stages.
- **Functional genomics**:

  Heterologously expressed human Ascorbate transporters SVCT1 and SVCT2, remain stacked to the endoplasmic reticulum of *A. nidulans*.

2006 Publications

Z. Erpapazoglou, P. Kafasla and V. Sophianopoulou, 2006. The product of the SHR3 orthologue of *Aspergillus nidulans* has restricted range of amino acid transporter targets. Fungal Genetics and Biology 43: 222-233

A. Kanapitsas, P. Vartzeli-Nikaki, A.A. Konsta, E.-E. Visvardis and E.G. Sideris, 2006. Dielectric Study of the Programmed Cell Death-Apoptosis, in Human Leukemia *IEEE* Transactions on Dielectr. and Electr. Insul. 13:1057-1062

A. Salem, V. Lumbreras, C. Lopez, D. Kizis and M. Pages, 2006. Maize DBF1-interactor protein 1 containing an R3H domain is a potential regulator of DBF1 activity in stress responses. Plant J. 46(5): 747-757

2006 Presentations at International Scientific Conferences

A. Apostolaki, Z. Erpapazoglou, C. Scazzocchio and V. Sophianopoulou 2006. The *Aspergillus nidulans* apartate/glutamate transporter reveals new mechanisms of amino acid uptake regulation. 31th FEBS Congress (PP-942), Istanbul, Turkey, June 24-29.

D. Bouzarelou, P. Kafasla, St. Frillingos, and V. Sophianopoulou 2006. The proline permease of *Aspergillus nidulans*: functional significance of the native cysteine residues and properties of a cysteine-less transporter. International Meeting on Yeast Transport and Energetics (SMYTE), Prague, Czech Republic, August 31 – September 3

C. Aggelidou, N. Mourtzis, K. Eliadou, V. Sophianopoulou, I.M. Mavridis, K. Yannakopoulou, 2006. Synthesis and characterization of *per* (6-guanidino-6-deoxy) cyclodextrins and studies of their effect on DNA "13th International Cyclodextrin Symposium CD, Torino, Italy, May 14-17.

Impact Factors (for 3 publications): 10,732

Citations 2006 (without self- citations): 37
V. Sophianopoulou: 30
E. Sideris: 8

Total Citations 2004-2006 (without self- citations): 111
V. Sophianopoulou:83
E. Sideris: 28

h-factor: 9(V. Sophianopoulou)

Research Group: Biophysics and Biotechnology of Membranes

Research Staff
Kostas Stamatakis, Senior Researcher
George Papageorgiou, Emeritus Scientist
Maria Billini, Graduate Student

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 Na^+/H^+ antiporters. Studies on Chlorophyll fluorescence induction curves. Studies on the photosynthetic Hydrogen production**.**

2006 Findings

A. Study of sodium proton antiporters in the cyanobacterium *Synechococcus sp. PCC 7942*

Seven genes (*sycnha1*-7) encoding putative Na^+/H^+ antiporters in the cyanobacterium *Synechococcus sp PCC 7942* were cloned and functionally characterized. The transcriptional regulation of all genes was determined under different Na^+ concentrations and pH values. One of them, the *sycnha6* gene, codes for the main antiporter of the organism under salt stress conditions as it was revealed by its inactivation in the cyanobacterium itself.

B. Study of Fluorescence induction curves in higher plants and cyanobacteria

1. Chlorophyll fluorescence induction curves at high and low actinic visible light, post-illumination changes in fluorescence yield and reflectance changes at 820 nm induced by far-red light were used to characterize the state of PSII and PSI and their electron transport capabilities in chlorophyllous twig cortices. We conclude that corticular chlorenchyma may be actively engaged in cyclic at the expense of a linear electron flow in the particular microenvironmental conditions encountered within twigs.

2. We have investigated the slow chlorophyll (Chl) a fluorescence induction (FI) transient of cyanobacterium Synechococcus sp PCC 7942. At room temperature, the transient comprises rise (S → M) and decay (M → T) phases in the s-to-min time window and is a sequel to the fast transient. In cyanobacteria, light-harvesting phycobilisomes (PBS) donate electronic excitation to Chl holochromes of photosystems (PS) II and I in a regulated manner. Here, we examined the phenomenology of the SMT transient at conditions at which the regulation of the PBS → PS I excitation transfer was eliminated. When the PBS → PS I electronic excitation transfer is not regulated, the FI pattern of Synechococcus becomes plant-like.

2006 Publications

Kotakis, Ch.,· Petropoulou, Y.,· Stamatakis, K.,· Yiotis, Ch.,· Manetas, Y. (2006). Evidence for active cyclic electron flow in twig chlorenchyma in the presence of an extremely deficient linear electron transport activity. Planta 225**,** 245-253.

2006 Presentations at International Scientific Conferences

Stamatakis K, Papageorgiou GC and Tsimilli-Michael M (2006) Phycobilisome-to-Photosystem I energy transfer is essential for the slow chlorophyll *a* fluorescence induction in the freshwater cyanobacterium Synechococcus sp PCC 7942. Photosynthesis in the Post-Genomic Era: Structure and Function of Photosystems, 20-26 August 2006, Pushchino, Russia.

Tsimilli-Michael M, Stamatakis K and Papageorgiou GC (2006) Investigation of the full Kautsky transient in *Synechococcus* sp pcc 7942 over the 10^{-5} - 10^{3} s time range: effects

of DCMU, CCCP and hyperosmosis. Photosynthesis in the Post-Genomic Era: Structure and Function of Photosystems, 20-26 August 2006, Pushchino, Russia.

Impact Factors (for 1 publication): 3,053

Citations 2006 (without self- citations): 1

Total Citations 2004-2006 (without self- citations): 11

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
Diamantis Sellis, Collaborating Graduate Student

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 (tenths of nucleotides), 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 other than Zipf's laws 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.

2006 Findings

Chargaff's second parity rule (PR2) states that complementary nucleotides are met with almost equal frequencies in single stranded DNA. This is indeed the case for all bacterial and eukaryotic genomes studied, although the genomic patterns may differ among genomes in terms of local deviations. The behavior of organellar genomes regarding the second parity rule has not been studied in detail up to now. We tested all available organellar genomes and found that a large number of mitochondrial genomes significantly deviate from the 2nd parity rule in contrast to the eubacterial ones, although mitochondria are believed to have evolved from proteobacteria. Moreover, mitochondria may be divided into three distinct sub-groups according to their overall deviation from the aforementioned parity rule. On the other hand, chloroplast genomes share the pattern of eubacterial genomes and, interestingly, so do mitochondrial genomes originating from plants and some fungi. The behaviour of the large majority of the mitochondrial genomes may be attributed to their distinct mode of replication, which is fundamentally different from the one of the eubacteria. Differences between chloroplast and mitochondrial genomes might also be explained on the basis of different replication mechanisms and correlated to differences in the genome size and compaction.

These results may provide some insight into different modes of evolution of genome structure between chloroplasts and mitochondria.

2006 Publications

P.Katsaloulis, T.Theoharis, W.M.Zheng, B.L.Hao, A.Bountis, Y.Almirantis, A.Provata. (2006). Long-range coorelations of RNA polymerase II promoter sequence across organisms. *Physica A* 366, 308-322.

C.Nikolaou & Y.Almirantis. (2006). Deviations from Chargaff's second parity rule in organellar DNA - Insights into the evolution of organellar genomes. *Gene* 381, 34-41.

S. Papageorgiou. (2006). Pulling forces acting on Hox gene clusters cause expression collinearity. *International Journal Of Developmental Biology*. 50, 301-308.

Impact Factors (for 3 publications): 5,986

Citations 2006 (without self- citations): 20

Total Citations 2004-2006 (without self- citations): 52

h-factor: 9

Research Group: NMR Studies of Biomolecules and Parmaceuticals

Research Staff
Maria Pelekanou, Senior Researcher
Dimitra Benaki, Postdoctoral Fellow
Aggeliki Panagiotopoulou, Technical Specialist
Marina Sagnou, Technical Specialist
Stamatia Tzanopoulou, Graduate Student

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
- Complexes of technetium, rhenium and other transition metals designed as potential radiopharmaceuticals

The areas of application of our work is mainly Alzheimer's disease and cancer, but also other diseases of the central nervous system, bacterial infections, etc.
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.

2006 Findings

In the area of technetium and rhenium complexes, the synthesis and characterization of new complexes that carry the anticancer agent 2-(4-aminophenyl)benzothiazole was completed, as part of the effort of development of potential cancer biopharmaceuticals. The biological evaluation of the rhenium complex was accomplished by cell uptake experiments monitored by fluorescence microscopy where it was shown that the fluorescent rhenium complex enters into MCF-7 breast cancer cells but not into normal HFFF-2 and MRC-5 cells (J. Med. Chem. **49**, 5408-5410). These results are encouraging for the continuation of the biological evaluation in tumor bearing mice.

Progress has been also made in the area of the synthesis of a radiodiagnostic targetting the amyloid plaques of Alzheimer's disease based on the structure of the histological dye thioflavin T. Complexation of a suitably modified thioflavin T derivative with the $MO(V)^{3+}$ oxometal core in the presence of aromatic thiols as coligands led to very interesting structures (Inorg. Chem. 45, 902-909), albeit with no binding affinity for the amyloid plaques. By differentiating the design of the complexes in terms of the oxidation state of the metal, the coligands employed, and the distance separating the thioflavin moiety from the metal core, we have succeded in obtaining complexes that selectively bind to amyloid plaques when tested in vivo in slices from Alzheimer's brain. Their biological evaluation continues by testing their ability to cross the blood-brain barrier in mice.

In the area of NMR studies of peptide/proteins of biological interest, the study of the Ser14Gly-humanin a 1000-fold more potent derivative of the neuroprotective peptide humanin was completed (Biochem. Biophys. Res. Commun. 349, 634-642). As expected by the presence of glycine in its structure, this peptide is more flexible than humanin in aqueous solution while in the presence of 30% TFE the α-helix formed extends over a shorter part of the sequence compared to humanin. In agreement with the NMR results, existing structure-function relationship literature data on humanin derivatives bring forth flexibility as a important feature for the neuroprotective activity of this class of peptides.

2006 Publications

Tzanopoulou, S., Pirmettis, I.C., Patsis, G., Raptopoulou, C., Terzis, A., Papadopoulos, M., Pelecanou, M. (2006). Oxorhenium(V) and oxotechnetium(V) [NN][S]$_3$ complexes of 2-phenylbenzothiazole derivatives. Inorg. Chem. 45, 902-909

Chiotellis, A., Tsoukalas, Ch., Pelecanou, M., Papadopoulos, A., Raptopoulou, C., Terzis, A., Pirmettis, I., Papadopoulos, M., Chiotellis, E. (2006). Synthesis and characterization of novel "3 + 2" oxorhenium complexes, ReO[SNO][NN]. Inorg. Chem. 45, 5635-5640

Tzanopoulou, S., Pirmettis, I., Patsis, G., Paravatou-Petsotas, M., Livaniou, E., Papadopoulos, M., Pelecanou, M. Synthesis, characterization, and biological evaluation of M(I)(CO)$_3$(NNO) Complexes (M = Re, ^{99m}Tc) conjugated to 2-(4-aminophenyl)benzothiazole as potential breast cancer radiopharmaceuticals (2006). J. Med. Chem. 49, 5408-5410

Benaki, D., Zikos, C., Evangelou, A., Livaniou, E., Vlassi, M., Mikros, E., Pelecanou, M. Solution structure of Ser14Gly-humanin, a potent rescue factor against neuronal cell death in Alzheimer's disease (2006). Biochem. Biophys. Res. Commun. 349, 634-642

2006 Presentations at International Scientific Conferences

O. Karagiorgou, G. Patsis, M. Pelecanou, C.P. Raptopoulou, A. Terzis, I Pirmettis, M. Papadopoulos. Synthesis and characterization of novel neutral M(CO)$_3$(NSO) complexes (M=Re, ^{99m}Tc). 7th International Symposium on Technetium in Chemistry and Nuclear Medicine, 6-9 September, 2006, Bressanone, Italy.

P. Kyprianidou, A. Chiotellis, D. Papagiannopoulou, C. Tsoukalas, A. Panagiotopoulou, M. Pelecanou, I. Iakovou, M. Papadopoulos, I Pirmettis. New Technetium-99m complexes with fluoroquinolones for the differentiated diagnosis of infection against aseptic inflammation. 7th International Symposium on Technetium in Chemistry and Nuclear Medicine 6-9 September 2006, Bressanone, Italy

A. Chiotellis, C. Tsoukalas, M. Pelecanou, I Pirmettis, M. Papadopoulos, E. Chiotellis. Novel ^{99m}Tc(CO)$_3$(NSO) complexes as potential 5-HT$_{1A}$ receptor imaging agents. 7th International Symposium on Technetium in Chemistry and Nuclear Medicine 6-9 September, 2006, Bressanone, Italy

S. Tzanopoulou, G. Patsis, M. Sagnou, I Pirmettis, M. Papadopoulos, M. Pelecanou. Synthesis and characterization of M(I)(CO)$_3$(NNO) complexes (M=Re,^{99m}Tc) conjugated to 6-methyl-2-(4'-aminophenyl)benzothiazole towards the development of Alzheimer's disease radiodiagnostics. 7th International Symposium on Technetium in Chemistry and Nuclear Medicine 6- September, 2006, Bressanone, Italy.

A. Chiotellis, C. Tsoukalas, M. Pelecanou, A. Papadopoulos, C.P. Raptopoulou, A. Terzis, I Pirmettis, M. Papadopoulos, E. Chiotellis. "Isomerism in a mixed-ligand oxorhenium complex, ReO[SNO][NN]". 7th International Symposium on Technetium in Chemistry and Nuclear Medicine 6-9 September, 2006, Bressanone, Italy.

N. Margaritis, A. Bourkoula, A. Chiotellis, A. Papadopoulos, A. Panagiotopoulou, C. Tsoukalas, C.C. Fernandes, M. Pelecanou, M. Paravatou, E. Livaniou, I. Santos, M. Papadopoulos, I. Pirmettis. A new technetium-99m biomarker for EGFR-TK. 7th International Symposium on Technetium in Chemistry and Nuclear Medicine 6-9 September, 2006, Bressanone, Italy

Stanica, R.M., Benaki, D., Tsoukatos, D., Tselepis, A., Mikros, E., Tsikaris, V., Structure activity relationship of peptide analogues derived from integrin subunit GPIIb. 29th European Peptide Symposium, September 3-8, 2006, Gdansk, Poland

Impact Factors (for 4 publications): 15,628

Citations 2006 (without self- citations): 30

Total Citations 2004-2006 (without self- citations): 59

h-factor:11

Research Group: Protein Structure by Crystallography and Theoretical Modeling

Research Staff
Metaxia Vlassi, Senior Researcher
Dimitris Vlachakis, Postodoctoral Fellow
Giorgos Villias, Collaborating Graduate Student (MSc)
Stamatia Vatsi, Collaborating Graduate Student (MSc)
Christos Labrakis, Undergraduate Student
Stefanos Leptidis, Undergraduate Student
Konstantina Dragoumani, Undergraduate Student
Nikos Simos, Training Student

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).

2006 Findings

- ✓ Using molecular dynamics simulations *in silico*, we investigated the structural stability of the Tup1 interaction domain of Ssn6 towards the elucidation of this TPR-mediated protein interaction mechanism. Analysis of the MD trajectory was performed through a package of computer programs we developed for this purpose.
- ✓ The crystal structure of a ROP variant designed with the aim to elucidate the role of heptad-repeats in the stability of the 4-α-helix bundle structural motif *(see previous reports)* (in collaboration with M. Kokkinidi's group) is published in *Biochemistry* (Publication 1).
- ✓ Using advanced bioinformatics techniques, we detected protein-interaction-like sequence repeats in a protein potential target of structure-based design of anticancer drugs. Experimental work on this protein is in progress. This study is funded by a grant from GSRT/05-Non-EU356.
- ✓ Our work on the elucidation of the structure/thermal stability/function relationship of the β-barrel motif-containing thermophilic enzyme, Chi40 (*see previous reports*) (in collaboration with G. Nounes's group, IRRP) is published in *Proteins* (Publication 2).
- ✓ The 3D-model of a modified humanin – a peptide with neuroprotective function against Alzheimer's (collaboration with M. Pelecanou, IB) and a prediction of T-cell epitopes of the La/SSB autoantigen (collaboration with M. Sakarellou, Univ. of Ioannina) (*see previous reports*) are published in *Biochem Biophys Res Commun* and *J Comput Chem*, respectively (Publications 3 & 4).

2006 Publications

Glykos NM*, Papanikolau Y*, Vlassi M*, Kotsifaki D, Cesareni G, Kokkinidis M. (2006) Loopless Rop: structure and dynamics of an engineered homotetrameric variant of the repressor of primer protein. Biochemistry. 45(36), 10905-19. *Shared first authorship*

Pyrpassopoulos S, Vlassi M, Tsortos A, Papanikolau Y, Petratos K, Vorgias CE, Nounesis G. (2006) Equilibrium heat-induced denaturation of chitinase 40 from Streptomyces thermoviolaceus. Proteins 64(2), 513-23.

Benaki D, Zikos C, Evangelou A, Livaniou E, Vlassi M, Mikros E, Pelecanou M. (2006) Solution structure of Ser14Gly-humanin, a potent rescue factor against neuronal cell death in Alzheimer's disease. Biochem Biophys Res Commun. 349(2), 634-42. Epub 2006 Aug 23.

Kosmopoulou A, Vlassi M, Stavrakoudis A, Sakarellos C, Sakarellos-Daitsiotis M. (2006) T-cell epitopes of the La/SSB autoantigen: prediction based on the homology modeling of HLA-DQ2/DQ7 with the insulin-B peptide/HLA-DQ8 complex. J Comput Chem. 27(9), 1033-44.

Impact Factors (for 4 publications): 15,318

Citations 2006 (without self- citations): 20

Total Citations 2004-2006 (without self- citations): 58

h-factor: 10

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, Technical Specialist, Ph.D
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.

Grafts Production 2006

During last year, we prepared the accreditation of the activities of the Bank according the ISO 9001/2000 stds. So the production of grafts was about 370 including bone grafts, dura mater, and cranium bone.

Other Acticities at the IB

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

Application of the ISO stds to the major Bank activities, like new labeling format, technical equipment supply and functioning, data recording e.t.c.

Other Scientific Activities

Scientific collaboration with the Dentistry Dpt of the University of Athens, on the following topic: PRP (platelet rich pegma) influence on the osteoconductivity of DBM (deminarilised bone matrix)

Representative of Greece to the European Regulatory Experts Committee for the Directives 23/2004 ,17 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.

EXPERIMENTAL ANIMAL COLONY

Research Staff

Dimitris Kletsas, Senior Researcher
Ioannis Zafiropoulos, Research Technician
George Doulgeridis, Research Technician

Description

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

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

The number and species of animals are dictated by the needs of research programs within the Institutes of "NCSR DEMOKRITOS", mainly the Institutes of Biology and Radioisotopes-Radiodiagnostic Products. In addition, strains of experimental animals are determined by demand from research labs outside the Center. Whenever there is surplus of animals, it is sold to research labs, hospitals, pharmaceutical companies, etc., according to demand. Within 2006 the personnel of the laboratory has developed strain of immunocompromised mice (SCID) and all the available infrastructure (room with sterile conditions, etc.).

During 2006, the Animal Facility made available the following animals:

Users	***Rats***	***Mice***	***Rabbits***	***SCID***
Institute of Biology	7	8	5	5
Institute of Radioisotopes & Radiodiagnostics	105	355	9	22
External Users	1634	-	-	-
Total of animals provided	**1764**	**363**	**14**	**27**

The certification of the Unit according to ISO, as well as the elaboration of the ΑΚΜΩΝ project funded by GSRT and in collaboration with the ΕΛΠΕΝ Company, are under process.

LASER CONFOCAL MICROSCOPY

Research Staff

Marina Sagnou, Technical Specialist

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.

2006 Findings

During the year 2006, 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, Senior Researcher
Maria Pelecanou, Senior Researcher
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.

2006 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 2006, 10 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 2006, 3 of our graduate students finished their thesis work and became PhDs.

Moreover, 12 students from the University are carrying out their pre-graduate project thesis work at the Institute and 4 students did practical job training. Additionally, 1 student from University abroad did practical lab training in laboratories at the Biology Institute.

In the framework of Graduate Programme, during the year 2006 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 courses were given by scientists of the Biology Institute:

- *Cell Signalling* [course lecturers: I. Georgoussi (course coordinator), and D. Kletsas].
- *Structural Biology and Theoritical Approaches* [course lecturers: M. Vlassi (course coordinator), and I. Almyrantis].

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:

- *Training Seminar in the framework of 11th cycle of seminars of the Greek Society of Nuclear Medicine and Biology* ***(Dr. G. Voutsinas)***
- *Teaching in the framework of the postgraduate course: "Biochemistry"* (**Dr. Iro Georgoussi,** Department of Biology, University of Athens)
- *Lecture with title "Heptahelical receptors coupled to G proteins" in the framework of the postgraduate course "Biochemistry" "* (**Dr. Iro Georgoussi,** 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 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 "The role of growth factor receptors, proteoglycans and integrins" in the framework of the postgraduate course "Physiology – Immunology"* (**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)

- *Training Seminar with title "Techniques in Cellular Biology and their application in Medicine" in the framework of 11th cycle of seminars of the Greek Society of Nuclear Medicine and Biology* ***(Dr. P. Kitsiou)***
- *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: "Bioinformatics" (***Dr. M. Vlassi**, 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 2006, 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 (2006) 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 2006

GRADUATE STUDENT	TITLE OF DOCTORAL THESIS	ADVISOR (in Institute of Biology)	UNIVERSITY
Georgia Mazarakou	*Molecular signaling mechanisms of the μ-opioid receptor responsible for STAT5A phosphorylation*	Iro Georgoussi	Biology Dpt., University of Athens
Christine Giannouli	*Study of TGF-β- mediated signal transduction in human cells*	Dimitris Kletsas	Biology Dpt., University of Athens
Zoi Erpapazoglou	*Transmebrane transporters in Aspergillus nidulans: Molecular mechanisms involved in sorting and topogenesis*	Vassiliki Sophianopoulou	Biology Dpt., University of Athens

LECTURE CONTRIBUTIONS TO
THE 2006 SUMMER SCHOOL
OF THE NCSR "DEMOKRITOS"
(July 2006)

DATE	SPEAKER	TITLE
11/7/06	**Dr. I. Almyrantis** Institute of Biology, NCSR "Demokritos"	Organisms, genomes and evolution: The understanding of the origin of life and of biological function through the interaction of biology with other sciences
11/7/06	**Dr. I. Almyrantis** 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?
11/7/06	**Dr. M. Pelecanou** Institute of Biology, NCSR "Demokritos"	Applications of Nuclear Magnetic Resonance in Medicine
17/7/06	**Dr. G. Voutsinas – V. Nikas** Institute of Biology, NCSR "Demokritos"	Cancer Genetics – Targeted therapies
18/7/06	**Dr. A. Chroni** Institute of Biology, NCSR "Demokritos"	The disturbances of lipids and lipoproteins metabolism can affect the development and progression of both atherosclerosis and Alzheimer's disease
18/7/06	**Dr. V. Sophianopoulou – Z. Erpapazoglou** Institute of Biology, NCSR "Demokritos"	Amino acid transporters of lower eukaryotes as models to study regulation of gene expression.
20/7/06	**Dr. E. Tsilibary** Institute of Biology, NCSR "Demokritos"	Examples of studies in cell-matrix pathobiology in order to enravel molecular mechanisms of aged-related diseases
18/7/06	**Dr. K. Iatrou** Institute of Biology, NCSR "Demokritos"	From basic research to biotechnology for Health and the Environment: «red» and «green» biotechnological applications from the NCSR «Demokritos»
19/7/06	**Dr. D. Kletsas** Institute of Biology, NCSR "Demokritos"	Cellular senescence and tissue homeostasis
19/7/06	**Dr. Th. Sourlingas** Institute of Biology, NCSR "Demokritos"	Histone subtypes and post translational modifications: fundamental factors for chromatin remodelling during ageing and apoptosis
19/7/06	**Dr. I. Georgoussi** Institute of Biology, NCSR "Demokritos"	The past, the present and the future of heptahelical receptors in health, disease and drug development
19/7/06	**Dr. L. Swevers** Institute of Biology, NCSR "Demokritos"	The developing ovariole of the silkmoth *Bombyx mori* as a model for the study of long term cellular differentiation programs

SEMINAR PROGRAMME 2006
INSTITUTE OF BIOLOGY

ΗΜΕΡ.	ΟΜΙΛΗΤΗΣ	ΤΙΤΛΟΣ
13/1/06	**Prof N. Moschonas** Dpt of Biology, Univ of Creta	The complex role of neuralized –like protein (Neurl) of mammals
19/1/06	**G. Ninios** Institute of Biology, NCSR "Demokritos"	Homo- and heterodimerization of APP family members promotes intercellular adhesion
19/1/06	**D. Anastassiou** Institute of Biology, NCSR "Demokritos"	The involvement of Hsp90 in cell signaling and PDK1 stability
20/1/06	**Dr. S. Tzartos** Hellenic Pateur Institute	Acetylcholine receptors in helath and disease
3/2/06	**Prof. Th. Sklaviadis** Dpt of Pharmacology, Univ. of Thessaloniki	Prion diseases: Pathogenesis ans therapeutic approaches
10/2/06	**Dr. R. Matsa** Hellenic Pateur Institute	Mechanisms of neuronal stem cells differentiation: perspectives for the treatment of neurodegenerative diseases
17/2/06	**Dr. A. Pintzas** National Hellenic Research Foundation, IBRB	Ras and Trail: Targeting the apoptotic process in cancer
24/2/06	**Prof. A. Athanassiadou** Medical School of Univ. Of Patras	Gene transfer in primary cell cultures with episomatic transporters for gene therapy
10/3/06	**Prof. M. Hatzopoulou - Kladara** Dpt of Biology, Univ. of Thessaloniki	Molecular mechanisms of action of hepatic factor HNF-4
17/3/06	**Dr. P. Mavromara** Hellenic Pateur Institute	Molecular biology of hepatic C virus
7/4/06	**Dr. G. Panayiotou** Biomedical Sciences Research Center "Al. Fleming"	JNK-mediated signalling: the double role of bifunctional phosphateses
12/4/06	**G. Ninios** Institute of Biology, NCSR "Demokritos"	Study of the Activation of the DNA Fragmentation Factor after the Induction of Apoptosis by Histone Deacetylase Inhibitors
12/4/06	**A. Repouskou** Institute of Biology, NCSR "Demokritos"	Circadian clock and histone acetylation: Study of their interaction with the cell cycle in *in vitro* mouse cell systems
14/4/06	**Dr. G. Mossialos** Biomedical Sciences Research Center "Al. Fleming"	The role of onco-repressing protein CYLD in normal and pathological conditions
19/4/06	**V. Nikas** Institute of Biology, NCSR "Demokritos"	Targeted therapy in ovarian cancer
19/4/06	**D. Anastassiou** Institute of Biology, NCSR "Demokritos"	Study of signal transduction pathways in urinary bladder cancer
3/5/06	**A. El Hamitie**	Molecular Chaperons / "Heat Shock Proteins"

	Institute of Biology, NCSR "Demokritos"	(HSPs) as regulators of Signal Transduction under Stress
3/5/06	**S. Alimperti** Institute of Biology, NCSR "Demokritos"	The role of novel Molecular Chaperones in the regulation of protein kinases in mammals
10/5/06	**M. Xedous** Institute of Biology, NCSR "Demokritos"	The Effect of Histone Acetylation Levels in the Regulation of the Biological Clock: Consequences for Cellular Function
10/5/06	**E. Mavrogonatou** Institute of Biology, NCSR "Demokritos"	The effect of hyperosmotic stress on the proliferation and senescence of nucleus pulposus intervetrtebral disc cells
24/5/06	**I. Tsagaraki** Institute of Biology, NCSR "Demokritos"	Apoptotic and anti-apoptotic mechanisms in MG63 cells
26/5/06	**Dr. V. Zachariou** Ιατρική Σχολή Παν/μιου Κρήτης	RGS9: Από την κυτταρική Βιολογία στη Συμπεριφορά
31/5/06	**D. Bouzarelou** Institute of Biology, NCSR "Demokritos"	Cloning and molecular characterization of a gene encoding an expansin-like protein in *Aspergillus nidulans*.
7/6/06	**M. Billini** Institute of Biology, NCSR "Demokritos"	Study of antitransporters Na^+/H^+ in the cyanobacterius *Synechococcus elongatus*
7/6/06	**P. Venieratos** Institute of Biology, NCSR "Demokritos"	Glucose-induced defects in insulin signalling pathway in mouse pancreatic β- cells
15/6/06	**L. Leontiadis** Institute of Biology, NCSR "Demokritos"	Functional interactions of the opioid receptors with various proteins
21/6/06	**T. Tzanopoulou** Institute of Biology, NCSR "Demokritos"	Benzothiazoles and radiodiagnosis: A relationship with intriguing prospectives!
4/9/06	**Dr. Ch. Nicolaou** Bioinformatics and Genomics, Center of Genomic Regulation, Barcelona, Spain	Old and new codes: Determination of the nucleosomal positioning in genomic scale
6/10/06	**Dr. N. Kyrpides** DOE Joint Genome Institute, Walnut Creek, California, USA	The future of genomics
17/10/06	**M. Xedous** Institute of Biology, NCSR "Demokritos"	Circadian gene expression in individual fibroblasts: cell-autonomous and self-sustained oscillators pass time to daughter cells
19/10/06	**Dr. N. Mastellos** IRRP, NCSR "Demokritos"	Protein interactions mediated by complement in developmental and regenerative processes
24/10/06	**V. Nikas** Institute of Biology, NCSR "Demokritos"	SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity
24/10/06	**D. Anastassiou** Institute of Biology, NCSR "Demokritos"	Role of phosphatidylinositol-3 kinase/Akt pathway in bladder cancer cell apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand
30/10/06	**Prof. V. Zannis**	The role of apoE in lipid homeostasis, in HDL

	Boston University Medical School, Boston, Massachusetts, USA	biogenesis and in atherosclerosis
31/10/06	**G. Ninios** Institute of Biology, NCSR "Demokritos"	Curcumin induces caspase-3-dependent apoptotic pathway but inhibits DNA fragmentation factor 40/caspase-activated DNase endonuclease in human Jurkat cells
31/10/06	**A. Repouskou** Institute of Biology, NCSR "Demokritos"	Early ageing and age-related pathologies in mice deficient in BMAL1, the core component of the circadian clock
7/11/06	**A. El Hamitie** Institute of Biology, NCSR "Demokritos"	Raised plasma nerve growth factor levels associated with early-stage romantic love
7/11/06	**S. Alimperti** Institute of Biology, NCSR "Demokritos"	Cdc37 is essential for chromosome segregation and cytokinesis in higher eukaryotes
14/11/06	**N. Tsotakos** Institute of Biology, NCSR "Demokritos"	WT1 induces apoptosis through transcriptional regulation of the proapoptotic Bcl-2 family member
14/11/06	**I. Georganta** Institute of Biology, NCSR "Demokritos"	Alteration in 5-HT1B receptor function by p11 in depression-like states
21/11/06	**K. Roumelioti** Institute of Biology, NCSR "Demokritos"	Isolation and characterisation from phytopathogenic fungi, genes that encode ammonium transporters and their role in dimorphism
21/11/06	**M. Papakonstantinou** Institute of Biology, NCSR "Demokritos"	Adenonosine A2a blockade prevents synergy between μ-opiate and cannabinoid CB1 receptors and eliminates heroin-seeking behavior in addicted rats
24/11/06	**Dr. D. Magoura** Academy of Athens, IIBEAA	Signalosomes as developmental molecular switches
1/12/06	**Prof. Ch. Savakis** Medical School of Univ. of Creta	Transposon-based transgenesis by using mobile elements for functional genomic analysis.
8/12/06	**Prof. A. Georgopoulos** University of Minessota Medical School, Minneapolis, USA	Multidisciplinary studies of brain function, cognition and behavior
12/12/06	**V. Kachrilas** Institute of Biology, NCSR "Demokritos"	PIK3CA gene mutations in endometrial carcinoma: correlation with PTEN and K-RAS alterations
15/12/06	**Dr. I. Kiaris** Meical School of Univ. of Athens	P53/p21/Notch and heterotypic interactions in cancer

COLLECTIVE DATA

FINANCIAL REPORT 2006

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

	PROGRAMMES				
INCOME	**464 IB**	**1240 EPAN**	**1269 EXCELLENCE**	**1333 AKMON**	**1334 AKMON**
CARRIED OVER FROM 2006	78.820	299.941	99.934	20.000	0
FUNDING FROM NCSR "D"	40.000	0	0	0	0
MATCHING FUNDS	1.439	0	0	0	0
INCOME FROM SERVICES	0	0	0	0	11.593
DONATIONS FROM COMPANIES	0	0	0	0	0
TRANSFER FROM OTHER SOURCES	20.057	352.640	100.489	0	5.600
TOTAL INCOME	***140.315***	***652.581***	**200.424**	**20.000**	**17.193**
EXPENSES					
EQUIPMENT	12.235	222.156	19.238	0	13.479
SUPPLIES	10.926	0	0	0	1.376
SALARIES	36.462	0	2.000	0	5.000
TRAVELS	4.864	0	3.245	0	0
OTHER EXPENSES	13.629	0	29.114	0	14.090
COMMITTED	4.455	0	3.638	18.000	1.734
TRANSFER FROM OTHER SOURCES	60.295	0	0	15.600	0
TOTAL EXPENSES	***142.867***	***222.156***	**57.235**	**33.600**	**35.678**

2. GOVERNMENTAL FUNDING

ANIMAL CHOW	2.987
TOTAL GOVERNMENTAL FUNDING	***2.987***

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 (4)	101.497	-	-	101.497
General Secretariat for Research & Technology (19)	329.188	142.288	11.500	482.976
Ministry of health & Social Solidarity (2)	14.650	-	-	14.650
International Atomic Energy Ageny (IAEA) (1)	-	-	500	500
National Bank of Greece (1)	-	-	4.500	4.500
AO Foundation (1)	30.000	-	-	30.000
TOTAL	**475.335**	**142.288**	**16.500**	**634.123**

COLLECTIVE DATA ON PRODUCTIVITY OF SCIENTIFIC PROGRAMMES

	PROGRAMME			
	A	**B**	**C**	**INSTITUTE**
Researchers	10	6	3	19
Technical Specialist	1	1	2	5*
Emeritus Scientists	3	3	1	7
Postdoctoral Fellows	2	6	2	10
Graduate Students	18	8	1	27
Collaborating Graduate Students	13	-	2	15
Graduate Research Associates	-	2	1	3
Undergraduate Students	3	9	4	16
Research Technicians	4 #	2	-	9 @
Administrative Staff	-	-	-	2
Total Personnel	**54#**	**37**	**16**	**113**
Publications in Peer-Reviewed Journals	**23+**	**9+**	**11**	**42**
Publications (Average) in Peer-Reviewed Journals per Scientist	2.3	1.5	3.6	2.21
Cumulative Impact Factor in Peer-Reviewed Journals (number of publications)	**109.019 (23)**	**31.629 (9)**	**36.932 (11)**	**177.58 (42)**
Average Impact Factor in Peer-Reviewed Journals	4.739	3.514	3.357	4.228
Cumulative Impact factor per Scientist	10.901	5.271	12.310	9.346
Proceedings to Conferences	**13$**	**4$**	**9**	**25**
Proceedings (Average) per Scientist	1.3	0.66	3	1.315
Total Publications	**36**	**13**	**20**	**67**
Publications (Average) per Scientist	3.6	2.166	6.666	3.526
Citations	**547**	**152**	**70**	**769**
International Patents	-	-	-	-
Greek Patents	-	2	-	2
Presentations to International Conferences	**16**	**25**	**9**	**50**
Presentations (Average) per Scientist to International Conferences	1.6	4.166	3	2.631
Presentations to Greek Conferences	**24**	**5**	**6**	**35**
Presentations (Average) per Scientist to Greek Conferences	2.4	0.833	3	1.842
Total Presentations to Conferences	**40**	**30**	**15**	**85**
Presentations (Average) per Scientist to Conferences	4	5	5	4.473

** 1 Technical Specialist of Human Tissues Bank is included*

 # 1 Research Technician who is occupied in other programme also is included

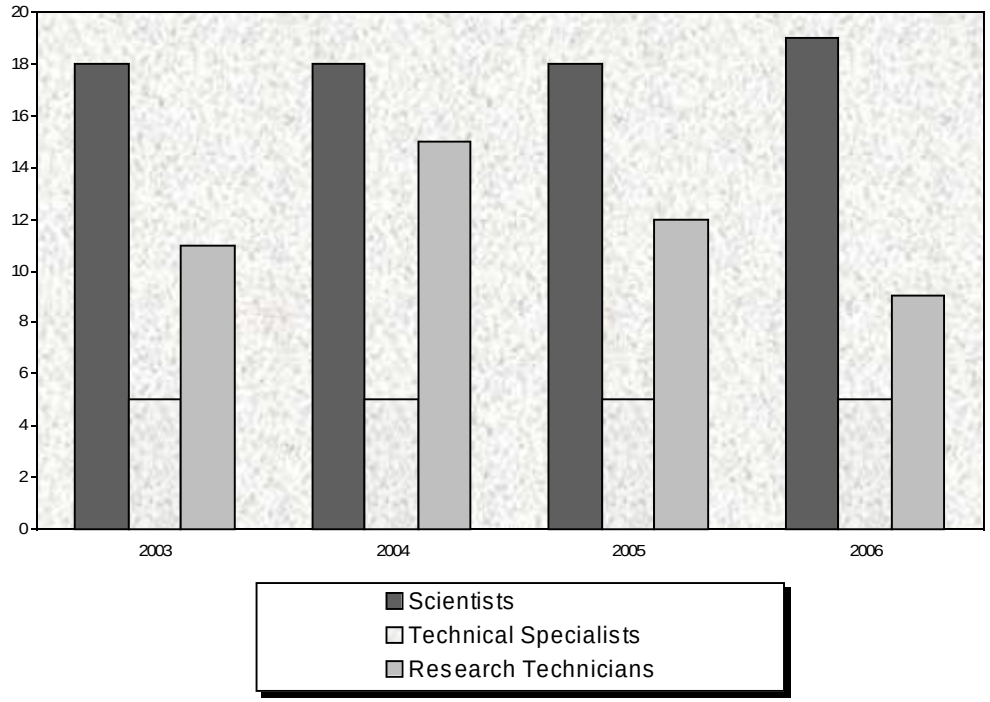
@ 2 Research Technicians who are occupied in Experimental Animal Colony and 1 Research Technician who is occupied in Human Tissue Bank are included

+1 publication common to A and B programme is included

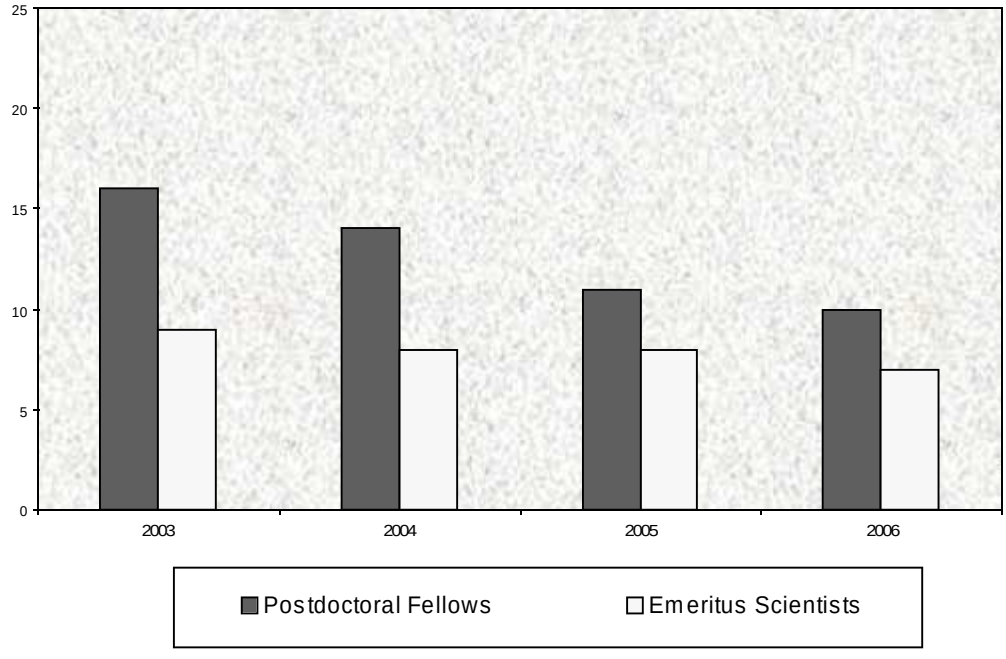
$ 1 publication in proceedings of international conference common to A and B programme is included

CHANGES OF IB STAFF DURING 2003-2006

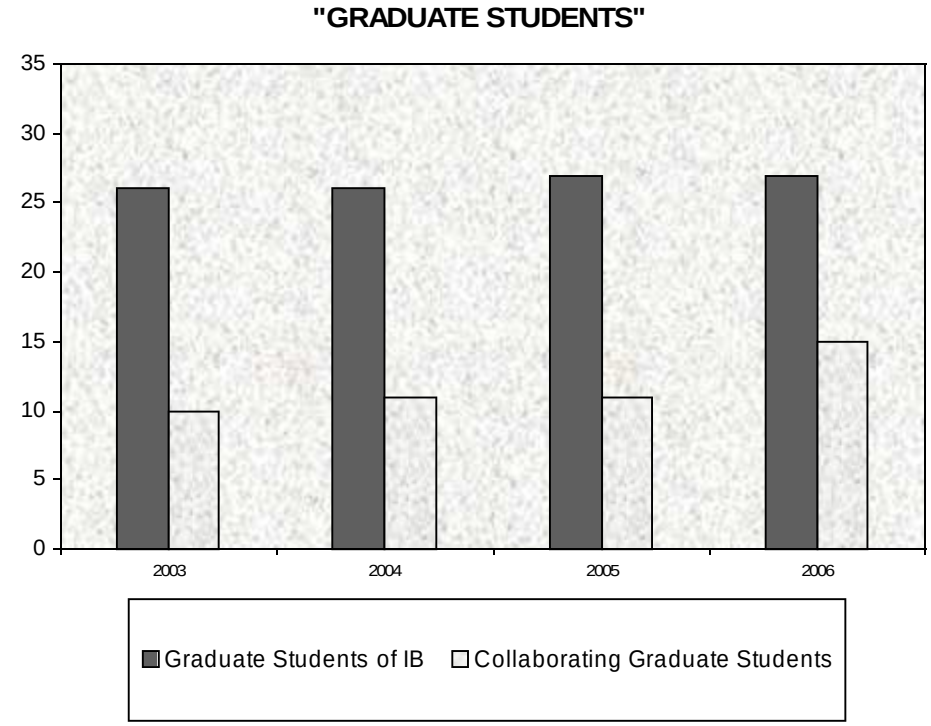
"TENURED EMPLOYEES"

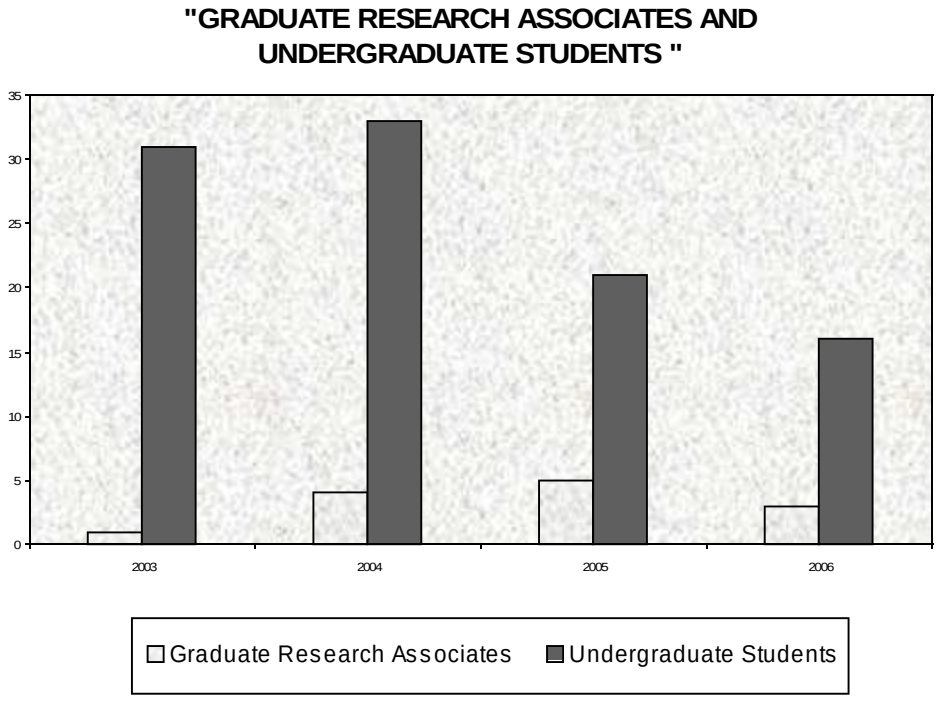


"POSTDOCTORAL FELLOWS & EMERITUS SCIENTISTS"



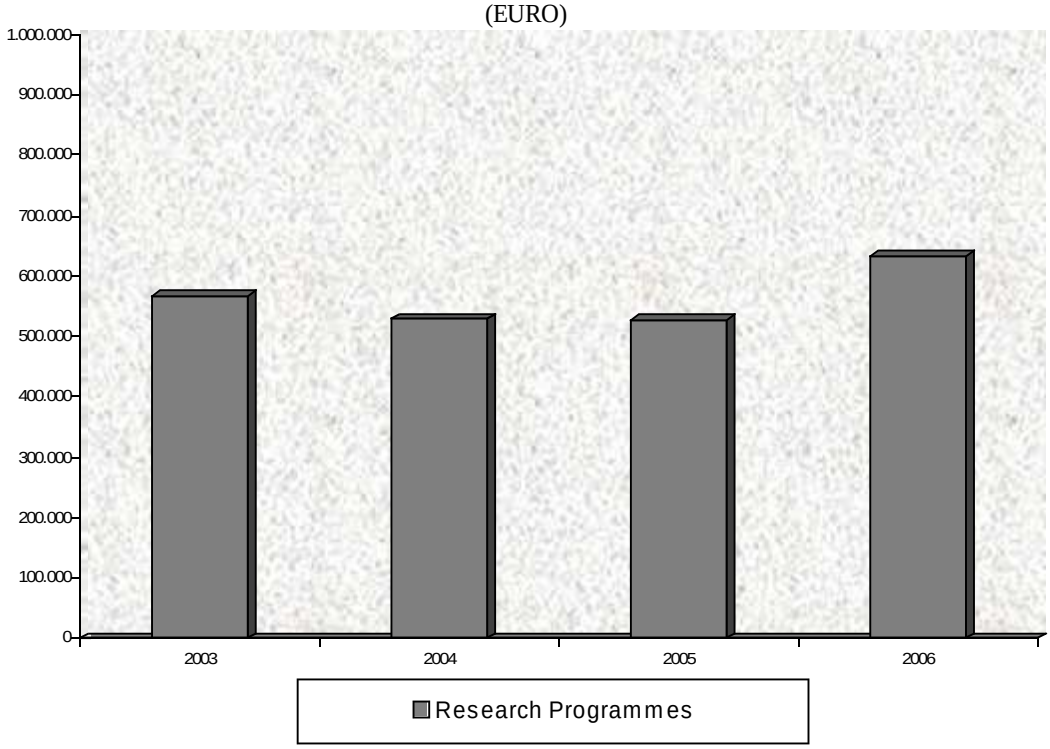
CHANGES OF IB STAFF
DURING 2003-2006





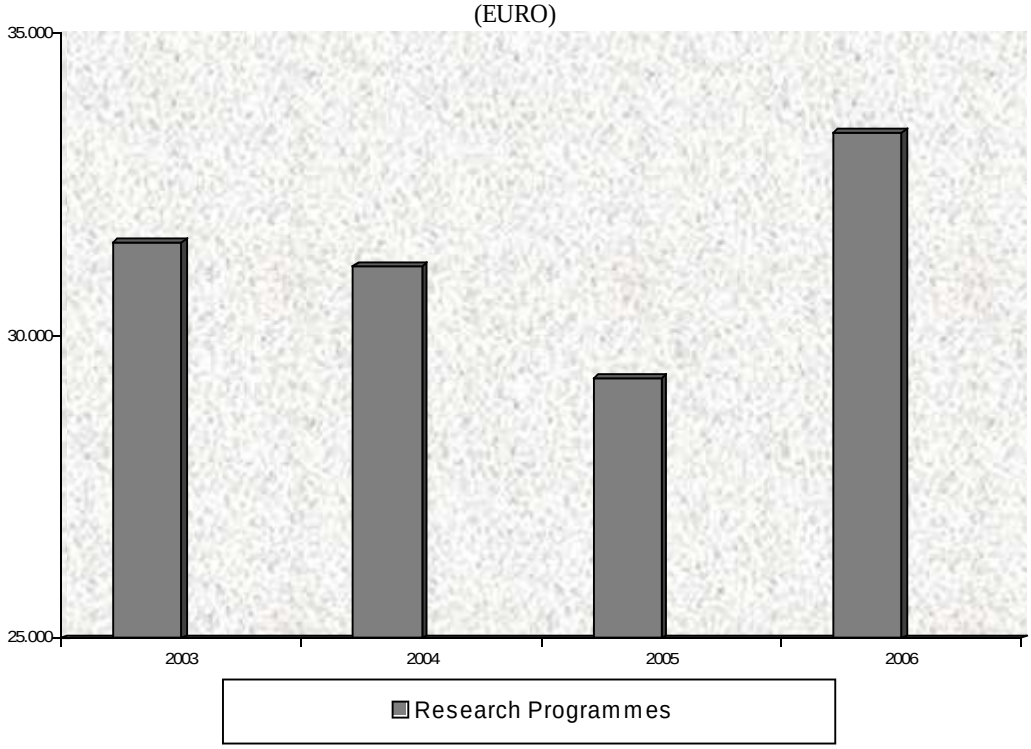
CUMULATIVE EXTERNAL FUNDING OF THE INSTITUTE DURING 2003-2006

(EURO)



EXTERNAL FUNDING OF THE INSTITUTE PER SCIENTIST DURING 2003 - 2006

(EURO)



PUBLICATIONS IN PEER-REVIEWED JOURNALS
AND CUMULATIVE IMPACT FACTOR DURING 2003-2006