INSTITUTE OF
BIOLOGY

NATIONAL CENTRE FOR SCIENTIFIC RESEARCH "DEMOKRITOS"

INSTITUTE OF BIOLOGY

2005
ANNUAL REPORT

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P E R S O N N E L

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Effie Tsilibary	MD, Cell Biologist

ACTING DIRECTOR

Almyrantis Ioannis	Chemist

SCIENTIFIC STAFF

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Sophianopoulou Vassiliki	Biologist
Tsilibary Effie	MD, Cell Biologist

Senior Researchers

Georgoussi Zafiroula-Iro	Biochemist
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Kletsas Dimitris	Biologist
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Prombona Anastasia	Biologist
Stamatakis Konstantinos	Biologist
Vlassi Metaxia	Physicist-Chrystallographer

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Labropoulou Vassiliki	Biochemist
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Tzinia Athina	Biochemist

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Ignatiadou Lydia (Dr. Hydrobiologist)- *Emeritus*	Iatrou K.
Machado Ednildo (Dr. Biologist)	Iatrou K.
Mazomenos Vassilios (Dr. Chemical Ecologist)- *Emeritus*	Lab of Chemical Ecology and Natural Products
Merkouris Emmanouel (Dr. Chemist)- *Collaborating*	Georgoussi I.
Papageorgiou George (Dr. Biochemist)- *Emeritus*	Stamatakis K.
Papageorgiou Spyros (Dr. Physicist)- *Emeritus*	Almirantis I.
Sideris Eleftherios (Dr. Geneticist)- *Emeritus*	Sophianopoulou V.
Stathakos Dimitrios (Dr. Biochemist)- *Emeritus*	Kletsas D.

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Efrose Rodica	Iatrou K.
Kenoutis Christos	Iatrou K.
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El Hamitie Abraam	Grammatikakis N.
Erpapazoglou Zoi	Sophianopoulou V.
Georganta Irene	Georgoussi I.
Georgomanolis Theodoros	Swevers L.
Giannouli Christina	Kletsas D.
Handris Panagiotis	Kletsas D.
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Mazarakou Georgia	Georgoussi I.
Mavrogonatou Eleni	Kletsas D.
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Tartas Athanassios	Vlassi M.
Tsagaraki Ioanna	Tzinia A.
Tzanopoulou |Stamatia	Pelekanou M.
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Fellow	Supervisor
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Magkrioti Christiana	Iatrou K.
Nikopoulos Kostas	Iatrou K.
Papaethimiou Ioannis *(MSc.)*	Iatrou K.
Tsikou Daniela *(MSc)*	Iatrou K.

COLLABORATING GRADUATE STUDENTS

Student (University)	Supervisor
Bombori Anna (Univ. of Athens)	Sophianopoulou V.
Fanourakis Galenos (Univ. of Athens)	Voutsinas G.
Fragopoulos Evaggelos (Univ. of Rome)	Tsilibary E.
Litsiou Eleni (University of Athens)	Voutsinas G.
Manta Maria (Univ. of Athens)	Tzinia A.
Ninios Ioannis (Univ.of Athens)	Sekeri K.
Papadopoulou Adamantia (Univ. of Athens, MSc)	Kletsas D.
Salpea Paraskevi (Univ.of Athens)	Sekeri K.

Sellis Diamantis (Univ.of Athens. MSc)	Almyrantis I.
Siskos Elias (Univ. of Cardiff)	Lab of Chemical Ecology and Natural Products – **PhD obtained in 2005**
Tsotakos Nikolaos (Univ. of Thessaloniki)	Tsilibary E.

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Student (University)	**Supervisor**
Bouchliou Eleni (Univ. of Thessaloniki)	Kitsiou P.
Galeou Aggeliki (Univ. of Athens)	Prombona A.
Gerolimatou Eleni (Univ. of Ioannina)	Voutsinas G.
Giarika Athina (Univ. of Thrace)	Voutsinas G.
Gika Eliana (Univ. of Athens)	Prombona A..
Glinou Vicky (Univ. of Athens)	Voutsinas G.
Haddad Rita (Univ. of McGill, Canada)	Iatrou K
Kapi Marianna (Univ. of Athens)	Prombona A.
Kotsogiannis Themistoklis (Univ. of Athens)	Georgoussi I.
Lamprakis Christos (Univ. of Thrace)	Vlassi M.
Lapa Stavriana (Univ. of Athens)	Voutsinas G.
Leptidis Stefanos (Univ. of Thrace)	Vlassi M.
Mela Ioanna (Athens Polytechnic School)	Kletsas D.
Noutsou Maria (Univ. of Thrace)	Iatrou K.
Papaioannou Danae (Univ. of Thrace)	Georgoussi I.
Pantazopoulou Marina (Univ. of Athens)	Sophianopoulou V.
Paparidou Olga (Univ. of Athens)	Voutsinas G.
Papaspiridakos Stefanos (Univ. of Athens)	Voutsinas G.
Pittis Alexandros (Univ. of Athens)	Sophianopoulou V.
Tsivola Lamprini (Univ. of Ioannina)	Voutsinas G.
Varouksi Niki (Univ. of Athens)	Sekeri K.

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
- Developing environmentally friendly pesticides and methods of protection of agricultural products, based on biotechnology

For the achievement of cutting-edge research, efforts have been made to extend the program of research programs/collaborations between different institute projects/researchers, with funds from recently obtained grants. Furthermore, new collaborations have started between researchers from the Inst. Biology and other institutes of NCSR Demokritos, with the aim to achieve excellence in multidisciplinary thematic areas, a unique strength of this Center.

Two years have elapsed since my appointment as Head of the Institute of Biology, during which non-stop efforts were dedicated to securing funds for the Institute of Biology which are necessary for a smoothly running operation. These hard efforts together with hard work have thus far been successful: The Institute of Biology together with the Institutes of Materials Science and Physical Chemistry have secured competitive funds for the amount of 1.300.000 € through the GSRT (General Secretariat for Research and Development) Program for Competitiveness/Infrastructure ("EPAN YPODOMON") for which the undersigned is the co-ordinator. 500.000 € from this amount will be dedicated to the purchase of new equipment for the IB. Another program was also funded, "AKMON", for the upgrading of the Laboratories of Human Tissues (human tissue bank) and Experimental Animals (Animal Colony). The total amount for this program is ~370.000 €. Another program listed "pending", "PEP for Attiki" has been funded, on the total mount of 1.000.000 €, and it will also support collaborative research between the insitutes of Biology, Material Science, and Physical Chemistry. I would like to express my gratitude to all researchers who devoted time and effort for the preparation of the above-mentioned grant applications which were funded: More specifically, Drs. Vlassi, Kletsas, Pelecanou and Prombona worked intensely with dedication for the submission of these proposals.

Finally, the evaluation process for which all the members on the EGS (Scientific Advisory Committee of the IB: Drs. Almyrantis, Vlassi, Kletsas, Pelecanou, Sophianopoulou) worked along with me around the clock, brought ~247.000 €. This evaluation, a process which left many unanswered questions in scientists inside and outside the center (including the undersigned) will not be elaborated upon. Suffice it to say that, according to my belief, the submitted report does not reflect the real situation, but an inaccurate report has no impact on the continuous, intense efforts for upgrading. If the IB constantly keeps up the commitment towards upgrading, we can certainly afford some contrary efforts trying to underscore the effectiveness of the endeavour. The fact which counts in the end is not impressions or opinions, but the final result.

In 2005 Dr. Piperakis transferred to the University of Thessaly as part of its faculty. Warm wishes are extended for a successful continuation of his career. Dr. Sekeri also retired, a researcher with invaluable contributions to the IB: Dr. Sekeri worked productively, with

dedication and self-sacrifice, and as vice Director and then acting Director faced many problems of the institute with wisdom, astuteness and effectiveness. I sincerely hope she will keep her connections and collaborations with the IB, as happened with many other retired researchers. In December 2005, Dr. Chroni, a new investigator started her appointment: Thus sincere, warm wishes are extended to her for a successful and ascending career. Furthermore, in December Dr. Swevers was promoted to the level B' (Associate Researcher), and obtained tenure. Congratulations Luc, keep up this successful performance!

The researchers overall have not relented their efforts for productivity, publications, participation in research programs, etc. Four PENED programs, 4 collaborations with other countries, 2 KESY, and 2 European programs were funded / approved for funding, among other. I sincerely hope that 2006 will bring more success to the IB and the Center, which is characterized by escalating efforts towards excellence. Moreover, the renovation of the biggest part of the Animal Colony facilities is continuing, and additional renovations have been programmed.

Once again the retired (honorary) researchers proved to be present and active participants with publications, book chapters, collaborations with research programs, and other contributions to the aim of upgrading of the IB.

The first two years of my appointment have been extremely demanding in time, effort and perseverance, but results which depended on my personal efforts have been thus far very positive, insofar as fund raising and extent of research collaborations were concerned. Problems arising along the way were unavoidable, but solutions were successfully found and applied. These copious, non-stop efforts were not "one-way" road: I was continuously and also received invaluable help from the members of the Scientific Advisory Committee of the IB (EGS), all of who worked equally hard as myself in solving problems, advising me in decision-making, and assisting me in the hard task of running this Institute towards the aim of upgrading. Other researchers contributed as well with dedication, whenever asked (Drs. Prombona, Stamatakis, etc). Dr. Iatrou was in charge of the external seminar series which have been extremely informative. I also wish to extend my gratitude to the IB researchers who participated in different committees performing various tasks, thus supporting the smooth operation of this Institute.

My task entailed innumerable difficulties and daily problems, however the trust and loyalty of the majority of researchers has been a delightful source of hope and optimism for achieving the endeavor of upgrading this institute. I firmly believe that, continuous efforts and harmonious collaborations will end up in projecting the IB as an internationally competitive research center. I am asking all of you to escalate your efforts, participate in national and European research programs and seek funds even form the private sector, so that you can adequately support a smooth operation of your research, which should become even more competitive. My trust lies in the research potential of all of you, and hope that you will keep proving your contributions and ascending success on a daily basis.

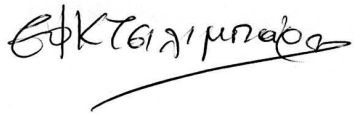
Additionally, I wish to extend warm thanks to Ms. Bazigou, accountant of the IB, who has been extremely supportive and efficient in facing all technical and other problems, and was also extremely efficient in providing a timely and informative update of the finances of the IB whenever asked. Ms. Bazigou departed several months ago, after accepting a tenured position in the Ministry of Economy and Economics. I extend to her my warmest wishes for a successful new career as a tenured employee and a mother. She has been replaced by Ms. Kostakou, a competent administrative member of the IB who returned after three years of absence, and contributes to an efficient support of researchers and a smooth operation of the IB with her knowledge, effectiveness and experience.

I could not possibly omit from the above list of "contributors" the heart and soul of the IB, Ms. Papadaki, the IB secretary who substantially and effectively participated in all activities of the IB

and the office of the Director. I extend warm thanks to Ms. Papadaki, who recently became a mother.

I wish progress and success for all!

Effie C. Tsilibary, MD, PhD



Director of IB
February 2006

PROGRAMME A:

REGULATION OF CELL FUNCTION

AGED-RELATED DISEASES

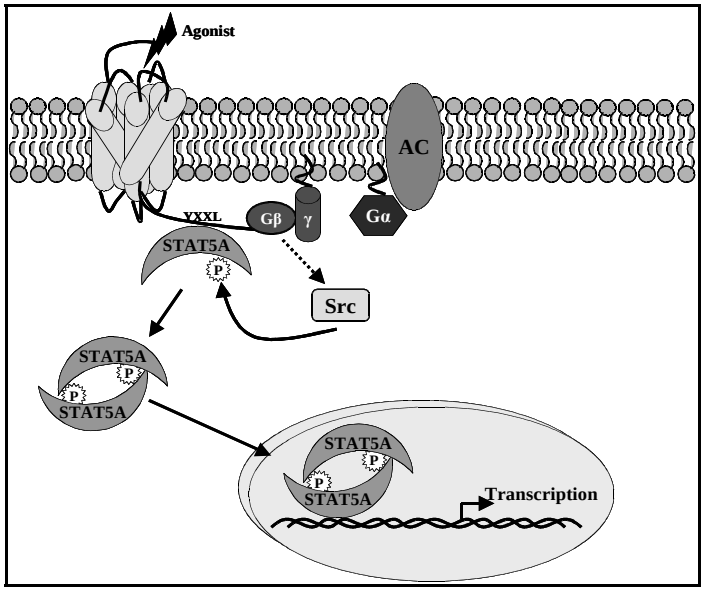
Research Group: Signal Transduction Mechanisms –Molecular Pharmacology

Research Staff

Iro Georgoussi, Senior Researcher
Emmanouel Merkouris, Collaborating Research Scientist
Thalia Teli, Postodoctoral Fellow
Georgia Mazarakou, Graduate Student
Evaghelia Morou, Graduate Student
Leonidas Leodiadis, Graduate Student
Irene Georganta, Graduate Student
Maria papakonstantinou, Graduate Student
Themistoklis Kotsogiannis, Undergraduate Student
Danae Papaioannou, Summer Undergraduate Student

Research Interests

Our research interests are focused on the elucidation of the molecular mechanism governing the G protein coupled receptor family using as a model system the opioid receptors. More specifically our aims are:



a) To define novel signaling pathways mediated by the three opioid receptor subtypes (μ, δ and κ)

b) to define novel interactive partners of GPCRs with the ultimate goal to identify new pharmacological targets

c) to identify transcription factors and specific genes that are activated upon opioid administration and contributes to tolerance and dependence on narcotic substances

d) to develop High Throughput Screens (HTP) for mammalian GPCRs, and for antennal mosquito odorant receptors that will lead in the identification of new mosquito disruptors host seek response using functional reference systems in insect cell lines (*collaboration Prof. K. Iatrou group*).

2005 Findings

***Mapping the structural and functional domains of the opioid receptors*:** In an attempt to develop specific activators, or inhibitors of the cellular signaling of heptahelical receptors we have constructed a minigene encoding the third intracellular loop of the δ-opioid receptor (δ-i3L). Expresion of this δ-i3L minigene inhibited not only δ and μ-opioid receptor interaction with Gi/Go proteins but also of the α2-adrenergic receptor that couples with the same G protein pool. In contrast, the δ-i3L minigene did not affect the signal transduction pathway mediated by the Gq and Gs activated-M1-muscarinic and β2 adrenergic receptors respecively. Mutation of arginine254 of the i3L minigene to alanine in the conserved RXXRR motif did not alter its inhibitory effects. These data indicate that the δ-i3L minigene can be considered as a "generic" inhibitor of heptahelical receptor signaling and a useful tool for determining the contribution of a given G protein population to signaling by a receptor that couples to multiple G proteins.

Identification of novel interactive partners of the opioid receptors. Experimental results from our laboratory have demonstrated for the first time the ability of RGS4, but not RGS9 protein, to interact specifically with the mu and delta opioid receptors. The interaction of RGS4 protein occurs within the third intracellular loops and the carboxyl-tails of these receptors. Mutanted and truncated versions of the GST-fusion peptides encompassing the C-terminal tails of the mu and delta opioid receptors have shown that RGS4 interacts within the designated "helix 8", because it is distal to the seventh transmembrane helices of both opioid receptors. Overxpression of RGS4 in HEK293 cells alters the signaling of the mu opioid receptor, whereas recruitment of RGS4 to the mu-opioid receptor occurs upon activation of the receptor. Formation of a signal transduction complex employing RGS4, monomeric Gα and Gβγ is under further investigation (*Collaboration: Prof. H. Hamm Vanderbilt University, Nashville, TN, USA*).

Interactions between opioid receptors with other transmembrane proteins. To investigate the possibility of interactions between the mu opioid receptors with membrane bound proteins, we developed a HEK293 cell line stably expressing a myc-tagged version of the mu opioid receptor. Subsequently, the ability of myc-tagged mu-opioid receptor to interact with EAAT1 and EAAT2 glutamate transporters was tested by immunoprecipitation experiments. Additionally, we examined whether the neurotensin receptor1 can hetero/ oligomerize with the mu-opioid receptor.

Alterations of cellular signaling and synaptosomal plasticity: Phosphorylation of transcriptional factors. In an attempt to define the molecular mechanisms that lead to alteration of transcription upon morphine exposure we demonstrated that activation of the mu-opioid receptor leads to STAT5A and STAT5B phosphorylation of Signal Transducers and Activators of Transcription, via a Src tyrosine kinase in a Gi/Go independent manner in COS-7. Furthermore, we identified for the first time that the STAT5 transcriptional factors interact with the carboxyl-tail of mu- opioid receptor and propose a new signaling pathway mediated by the mu opioid receptor (figure). Based on this signaling pathway we investigated whether chronic administration of cells to morphine causes the same levels of STAT5 phosphorylation. Our results indicate that prolonged morphine exposure of SH-SY5Y cells, that endogenously express the mu- opioid receptor, resulted in a significant phosphorylation of these proteins. Our goal is to elucidate the differences in STAT phosphorylation and the specific genes that are activated upon opioid administration.

High Troughput Screens (HTP) for mammalian and insect GPCRs in functional cellular systems. In collaboration with Professor K. Iatrou and Dr L. Swevers *(Laboratory of Insect Molecular Genetics and Biotechnology)* we have successfully developed a HTP screening system for opioid analogs. Similar studies are employed for the serotonin 5-HT4 in the presence of the Gα16 protein in lepidopteran Bm5 cells. At the same time, the pharmacological characterization and identification of the signaling pathways mediated upon activation of two cloned mosquito antennal odorant receptors (OR1 and OR2) is in progress.

2005 Publications

Mazarakou G**.** and Georgoussi Z. (2005) STAT5A interacts with and is phosphorylated upon activation of the μ-opioid receptor J. Neurochem.93, 918-931.

Swevers L., Morou E*., Balatsos N., Iatrou K. and Georgoussi Z. (2005) Functional expression of the mouse δ-opioid receptor in insect cells: development of a cell-based high throughput screening system for detection of opioid receptor ligand mimetics. Cell Mol. Life Sciences. 62, 919-930 (* equal contribution).

Georgoussi Z., Leontiadis,L., Mazarakou G., Merkouris M., Karren H. and Hamm H. (2005) 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 Cellular Signaling, 22 August [Epub ahead of print], *in press*.

Morou E and Georgoussi Z. (2005) Expression of the third intracellular loop of the delta opioid receptor inhibits signalling by opioid receptors and other GPCRs. J. Pharmacol. Exp. Ther.315, 1368-1379.

Douris V. Swevers, L., Labropoulou V., Andronopoulou E, Georgoussi Z. and Iatrou K. (2005) Stably transformed insect cell lines: tools for expression of secreted and membrane-anchored proteins and high throughput screening platforms for drug and insecticide discovery. Advances in Virus Research *in press*.

2005 Presentations at International Scientific Conferences

Georgoussi Z, Swevers L., Douris V., Stefanou D, Morou E. Balatsos N. and Iatrou K. A modular expression system derived from insect and baculovirus regulatory elements as the basis for the development of versatile cell-based HTS platforms for lead discovery for known targets: the insect ecdysteroid and mammalian opioid receptor HTS paradigms. Screening Europe 2005, February 14-16 2005, Geneva, Switzerland

Impact Factors (for 5 publications): 21,735

Citations 2005 (without self- citations): 15

Total Citations 2003-2005 (without self- citations): 34

h-factor: 9

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
Aliki Siganou, Research Technician

Research Interests

The general area of our research is Cell Signaling. More specifically and upon transferring our program from the US (Harvard University, BIDMC) to Greece (Institute of Biology, NCSR Demokritos) last July we have continued studying two groups of proteins which play a pivotal role in the above process. These two groups involve the Oncogenic Kinases and the Molecular Chaperones/ Heat Shock Proteins (HSPs). Our aim is to learn how those Signaling Pathways (MAPK, NF-κB, ErbB) known to play a "key-role" in the process of Oncogenesis and Apoptosis are regulated and to delineate the mode of modulation of the kinases involved. In molecular terms, our interest currently focuses on how Cdc37, Hsp90 and Hsp90N as and their cofactors affect the function of Cdk4, ErbB2, Raf, Akt and I-kappaB kinases (IKK) during the cell cycle and division both during physiological and non-physiological conditions (various forms of cancer and immune system diseases). A desired outcome is, first, to produce animal and cell culture models based on *RNAi/gene knock-in* for the kinase-modulatory activity of the above Molecular Chaperones. As a further aim we envision the development of chemotherapeutical drugs based on the structure of members of this novel group of Signaling Regulators.

2005 Findings

Upon transferring our work from the US (Harvard University/ BIDMC) to the Institute of Biology at the NCSR Demokritos we have encountered a major hardship and delay in establishing a working environment, especially in regards to finding a proper laboratory space and the necessary instrumentation and equipment. We remain hopeful that the problems will be solved soon. In general lines we have continued testing experimentaly the model we had proposed 9 years ago, namely that "the activity of a select group of kinases might -in addition to the classic mechanisms- be possibly conformationally modulated by Cdc37 and the chaperone machinery". To this end, our recent data indicate that p50Cdc37 is the same molecule suspected since the 1980s to, together with Hsp90, bind to and possibly regulate Src (reviewed in: Brugge J., 1986: Interaction of the Rous sarcoma virus protein pp60src with the cellular proteins pp50 and pp90. Curr Top Microbiol Immunol 123:1-22). As our current data also show, p50Cdc37 is a crucial regulatory factor for a group of G1-specific kinases which is functionally dependent on interactions with the Chaperone Machinery (HSPs). This group of kinases includes Raf and Src, ErbB2, Akt, the I-kappaB kinases (IKKs), MLK3, the heme-regulated eIF-2alpha kinase (HRI) and, among the cyclin-dependent-kinases, Cdk4, Cdk6 and Cdk9. Further, as our overall data show, the entire assembly and coordinate regulation of at least three crucial signaling kinase modules (Raf>Mek>Erk, IKK>IkappaB>NFkappaB and Cdk4>Cdk4/CyclinD>pRb) might be mediated by the chaperone machinery under physiological conditions. In the above process, the ATPase/folding activity of the participating Hsp90 and Hsp70 plays a central role. Finally, we have reported a novel tripartite connection, that of the MAPK kinase module and of the 14-3-3 family of adaptors with the Heat Shock Factor-1 (HSF-1) and formulated a hypothesis of how the nucleocytoplasmic localization, and as result the transcriptional activity of the later might be affected by upstream signaling.

Impact Factors: 0

Research Group: Mechanisms of Cell Proliferation and Ageing

Research Staff
Dimitris Kletsas, Senior Researcher
Dimitrios Stathakos, Emeritus Scientist Haris Pratsinis, Postdoctoral Fellow
Panagiota Malakassi, Postdoctoral Fellow
Panagiotis Handris, Graduate Student
Christina Giannouli, Graduate Student
Ioannis Karakatsanis, Graduate Student
Eleni Mavrogonatou, Graduate student
Adamantia Papadopoulou, Collaborating Graduate Student (MSc)
Eleni Gkioni, Graduate Research Associate
Ioanna Mela, Training Student
Eleni Sevaslidou, Research Technician

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, whose the degeneration provokes severe dysfunctions during ageing, e.g. the intervertebral disc. Finally, the characteristics of centenarians, as an example of successful ageing, are also studied.

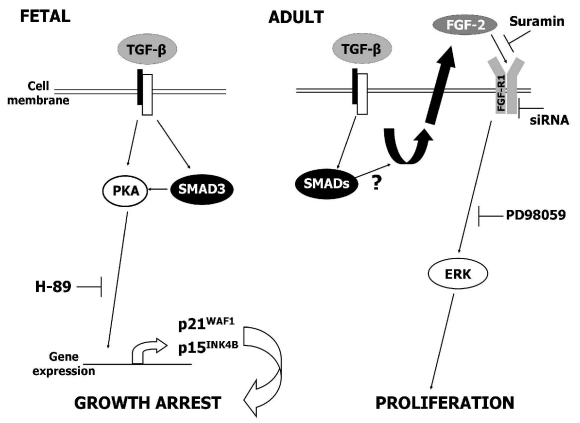
Aim of these studies is the elucidation of the mechanisms underlying the regulation of tissue homeostasis, especially during ageing, and further, through research networks the contribution in the development of cell replacement therapies. Finally, we study natural products and new synthetic compounds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

2005 Findings

We have continued our studies on the action of the multifunctional growth factor TGF-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 the formation of an autocrine loop: it induces the synthesis and release of FGF-2 which, through its receptor FGFR-1, stimulates cell proliferation 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 $p15^{INK4}$, probably through the overactivation of Smad3 (Fig. 1).

Main goal of our studies is the investigation on the role of the senescent cell in the development of age-related diseases, including cancer. Telomere shortening or exogenous stresses can lead to a DNA damage response (DDR), and the activation of the tumor-suppressor protein p53. We have shown that p53 overactivation in senescent cells is responsible for the induction of the pro-inflammatory protein ICAM-1. This was

demonstrated in cellular systems in vitro, as well as in a classical inflammatory and age-related disease, i.e. atherosclerosis, indicating the involvement of cellular senescence in this disorder. In addition, in cells of intervertebral disc, a tissue whose degeneration is involved in major age-related disfunctions, DDR induced by exogenous stresses leads to activation of p53, growth arrest and premature senescence. Besides, we have found signs of DDR in human precancerous lesions as a result of aberrant division, while progression to carcinoma was associated with p53 inactivation.



Finally, we have continued our studies on a. the structural changes of the nucleus of the senescent cell, b. the role of senescent stromal cells in the progression of carcinogenesis, c. the characteristics of Greek centenarians and d..the cytostatic/cytotoxic, anti-ageing and the would healing activity of natural products and new synthetic compounds.

2005 Publications

Gorgoulis, V.G., Vassiliou, L.V., Karakaidos, P., Zacharatos, P., Kotsinas, A., Liloglou, T., Venere, M., Ditullio, R.A. Jr, Kastrinakis, N.G., Levy, B., Kletsas, D., Yoneta, A., Herlyn, M., Kittas, C., Halazonetis, T.D. (2005). Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 434, 907-913

Gorgoulis, V.G., Pratsinis, H., Zacharatos, P., Demoliou, C., Sigala, F., Asimacopoulos, P.J., Papavassiliou, A.G., Kletsas, D. (2005). p53-dependent ICAM-1 overexpression in senescent human cells identified in atherosclerotic lesions. Lab. Invest. 85, 502-511

Trougakos, I.P., Lourda, M., Agiostratidou, G., Kletsas, D., Gonos, E.S. (2005). Differential effects of clusterin/apolipoprotein J on cellular growth and survival. Free Radic. Biol. Med. 38, 436-449

Gioka, C., Bourauel, C., Hiskia, A., Kletsas, D., Eliades, T., Eliades, G. (2005). Light-cured or chemically cured orthodontic adhesive resins? A selection based on the degree of cure, monomer leaching, and cytotoxicity. Am. J. Orthod. Dentofacial Orthop. 127, 413-419 (quiz 516)

Kousidou, O.C., Mitropoulou, T.N., Roussidis, A.E., Kletsas, D., Theocharis, A.D., Karamanos, N.K. (2005). Genistein suppresses the invasive potential of human breast cancer cells through transcriptional regulation of metalloproteinases and their tissue inhibitors. Int. J. Oncol. 26, 1101-1109

Kostakis, I.K., Tenta, R., Pouli, N., Marakos, P., Skaltsounis, A.L., Pratsinis, H., Kletsas D. (2005). Design, synthesis, and antiproliferative activity of some novel aminosubstituted xanthenones, able to overcome multidrug resistance toward MES-SA/Dx5 cells. Bioorg. Med. Chem. Lett. 15, 5057-5060

Zervolea, I., Pratsinis, H., Tsagarakis, S., Karavitaki, N., Stathakos, D., Thalassinos, N., Kletsas, D. (2005) The impact of chronic in vivo glucocorticoid excess on the functional characteristics of human skin fibroblasts obtained from patients with endogenous Cushing's syndrome. Eur. J. Endocrinol. 152, 895-902

Stathakos, D., Pratsinis, H., Zachos, I., Vlahaki, I., Gianakopoulou, A., Zianni, D., Kletsas, D. (2005) Greek centenarians: assessment of functional health status and life-style characteristics. Exp. Gerontol. 40, 512-518

Trougakos I.P., Lourda M., Agiostratidou G., Kletsas D., Gonos E.S. (2005) Differential effects of clusterin/apolipoprotein J on cellular growth and survival. Free Rad. Biol. Med. (in press).

Kundakovic, T., Fokialakis, N., Dobric, S., Pratsinis, H., Kletsas, D., Kovacevic, N., Chinou, I. (2005). Evaluation of the Anti-inflammatory and Cytotoxic Activities of Naphthazarine Derivatives from Onosma leptantha. Phytomedicine (in press).

Gorgoulis, V.G., Pratsinis, H., Zacharatos, P., Demoliou, C., Sigala, F., Papavassiliou, A.G., Kletsas, D. (2005). p53-Dependent ICAM-1 Over-Expression In Senescent Human Cells. Their Identification In Atherosclerotic Lesions. Lab. Investigation (in press).

2005 Presentations at International Scientific Conferences

H. Pratsinis, C.C. Giannouli, I. Karakatsanis, D. Kletsas (2005). Differential Proliferative Response of Fetal and Adult Human Skin Fibroblasts to Transforming Growth Factor-β. Institut Pasteur Euroconferences, Tissue Repair and Ulcer/Wound Healing: Molecular mechanisms, therapeutic targets and future directions, March 17-18, 2005, Paris, France.

H. Pratsinis, K. Würtz, C. Neidlinger-Wilke, D. Kletsas (2005). Autocrine growth stimulation of intervertebral disc cells subjected to cyclic strain. 32nd Annual Meeting of the International Society for the Study of the Lumbar Spine (ISSLS), May 10-14, 2005, New York, USA.

S. Roberts, E.H. Evans, J.P.G. Urban, D. Kletsas, N. Craig, S.M. Eisenstein (2005). Cells in herniated intervertebral discs demonstrate premature senescence: A cautionary note for disc repair. 32nd Annual Meeting of the International Society for the Study of the Lumbar Spine (ISSLS), May 10-14, 2005, New York, USA.

H. Pratsinis, S. Tsagarakis, I. Zervolea, D. Stathakos, N. Thalassinos, D. Kletsas (2005). The Unexpected Anabolic Phenotype and Extended Longevity of Skin Fibroblasts after Chronic Glucocorticoid Excess. 4th Annual International Conference on Hormesis: Implications for Toxicology, Medicine and Risk Assessment, June 6-8, 2005, University of Massachusetts Amherst, USA.

Impact Factors (for 8 publications): 53,799

Citations 2005 (without self- citations): 113

Total Citations 2003-2005 (without self- citations): 253

h-factor: 13

Research Group: The Role of Nuclear proteins and Chromatin Function

Research Staff
Kalliopi Sekeri, Research Director
Thomais Sourlingas, Researcher
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 variants and more specifically, linker histone variants, 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.

2005 Findings

The effects of the histone deacetylase inhibitor, trichostatin A (TSA), on linker histone H1o expression, histone H4 acetylation and apoptosis were studied in six leukemic cell lines (MOLT-4, U937, NB-4, K562, HL60, Jurkat). It was found that there is a differential response to TSA depending on the cell line with respect to the level of histone H4 acetylation and the degree of H1o induction. Moreover these two molecular parameters were found to be directly associated with the differential degree of apoptosis induced by TSA in these leukemic cell lines.

Moreover we studied the somatic subtypes of the H1 linker histone family in human peripheral blood lymphocytes as a function of increasing donor age using capillary zone electrophoresis. The results showed that the expression of one subtype decreased substantially in the senior (60-70 yrs.) and even more so in the elderly (80-90 yrs.) age groups. These H1 subtypes have not previously been studied with respect to the ageing process. This study has been completed by the identification of all the H1 somatic subtypes in human peripheral blood lymphocytes and the identification of the H1 subtype that decreases during ageing. This study was accomplished in collaboration with Prof. D. Doenecke, Institut für Biochemie und Molekulare Zellbiologie, Georg-August-Universität Göttingen, and a manuscript is now in preparation.

Also under investigation is the acetylation of cytoplasmic proteins as potential signaling factors in physiological peripheral blood lymphocytes in comparison to leukemic cells. Results have shown that tubulin is acetylated after treatment with trichostatin A. However, whereas the levels of tubulin acetylation remain constant in physiological lymphocytes, irrespective of the age of the donor, in the six leukemic cell lines that were studied,

differential responses to treatment with the inhibitor were found. In light of the fact that histone deacetylase inhibitors are used as anticancer agents that more often than not lead the cells to apoptosis, the degree of apoptosis in relation to the levels of acetylation of tubulin after treatment with TSA were also studied in these leukemic cell lines. The results showed that in the cell lines where tubulin acetylation was highest (MOLT-4, NB-4, HL60) after TSA treatment, the degree of apoptosis was also highest. These results indicate that tubulin acetylation may be a cytoplasmic signal or marker of apoptosis. This study has been undertaken in collaboration with the laboratory of «Chromatine et Expression des Genes», INSERM U309, Institut Albert Bonniot, Faculte de Medecine, Domaine de la Merci (Dr. Saadi Khochbin, Research Director)

Moreover, in collaboration with the laboratory of Dr. A. Prombona ("Regulation of Transcription of Plants by the Biological Clock") a research project has been initiated whose purpose is to study 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. Preliminary results showed that c-myc and wee1 gene expression levels may also be rhythmically expressed. The effect of curcumin, an inhibitor of p300 acetyltransferase activity, on the level of histone H4 acetylation was also studied by 2-D electrophoretic analysis so as to ascertain its ability in future experiments to change histone acetylation levels in the promoter regions of circadian clock genes. These induced, by curcumin, changes in histone acetylation levels may in turn effect changes in the expression levels of these genes. Results showed that this substance, which has till now not been studied to a great extent, reduced discernibly the mass histone H4 acetylation levels.

Citations 2005 (without self- citations): 13
Sekeri K.E (in common publications with T. Sourlingas): 13
Sourlingas T. G.: 3

Total Citations 2003-2005 (without self- citations): 31
Sekeri K.E (in common publications with T. Sourlingas): 31
Sourlingas T. G.: 11

h-factor:
Sekeri K.E : 9
Sourlingas T. G.: 5

Research Group: Pathobiology of the Cell and Exracellular Matrix

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

Research Interests

The laboratory focuses on the regulation of cell function which is mediated by matrix and other cell surface receptors and matrix-ignited signalling. Receptors examined include integrins, growth factor receptors (insulin receptor), cell surface sialoproteins, etc. We are mainly interested in understanding pathological conditions involving matric and matrix receptors (in vitro models of diabetes, Alzheimer disease, osteoporosis/osteoarthritis, etc). The research topics are briefly described below:

- Regulation of gene expression of the transmembrane sialoprotein podocalyxin in cultured human glomerular epithelial cells (podocytes), and additionally chick development. The aim is to understand molecular mechanisms involved in the up- or down-regulation of the expression of podocalyxin. This will contribute to a better understanding of changes of expression of podocalyxin in renal diseases including diabetes, minimal change disease, etc. IN these conditions reduced expression of podocalyxin results in altered structural and functional properties of renal podocytes, and the aim is to be able to control podocalyxin expression.
- Neuroblastoma cell properties in conditions of Alzheimer's disease (culture in the presence of Aβ amyloid), with the aim to reduce amyloid aggregates which have been correlated with neuronal degeneration of Alzheimer.
- Functional properties of Glutamate transporters in physiological and neuro-degenerative conditions, with the aim to understand the role of these transporters in neuronal degeneration, and the control of these transporters in the pathological conditions described above.
- Regulation of functional properties of osteoblastic cell lines in inflammation (arthritis) and osteoporosis, with the aim to intervene and prevent alterations of structural and functional properties of the connective tissue in these pathological situations.

2005 Findings

- ***Regulation of gene expression of the transmembranous sialoprotein podocalyxin in human glomerular epithelial cells***. We examined the role of the transcription factor WT1 which has been suggested to regulate podocalyxin expression, however in conditions in which podocalyxin expression is dramatically downregulated (in the presence of increased glucose concentrations), WT1 expression remained unaltered. Thus additional

transcriptional factors should be involved in controlling the expression of podocalyxin. In addition to p53 which was observed to down-regulate podocalyxin expression, we observed reduced binding of the transcription factor CBP to WT1, and binding of this complex to the podocalyxin promoter becomes less effective, thus resulting in reduced podocalyxin expression.

- ***Regulation of β-pancreatic cell function in diabetic conditions (in the presence of increased glucose concentrations)***: Previous studies of the insulin receptor (IR)-mediated signalling indicated that high glucose resulted in decreased phosphorylation of IRS-2, PI3K and Akt but the expression of these kinases was not altered. Furthermore, insulin-dependent phosphorylation of mTOR, which is controlled by Akt and regulates protein synthesis was decreased, as was phosphorylation of BAD, a pro-apoptotic gene. It is possible therefore that glucose-induced changes of the insulin-mediated signalling pathway play a role in β-pancreatic cell dysfunction and apoptosis which is observed in type 2 diabetes.
- ***Functional properties of neuroblastic cells in Alzheimer conditions (in the presence of Aβ amyloid):*** Previous data from our laboratory indicate that collagenase MMP-9 expression is induced in SK-N-SH neuroblastic cells in the presence of amyloid Aβ and probably acts as α-secretase, i.e. protective against amyloid aggregation. In HEK293 cells stably overexpressing APP, additionally induced overexpression of MMP-9 (by transfections with the cDNA of MMP-9) resulted in the release of soluble sAPPa, which is derived from transmembranous APP by the action of α-secretase, and parallel decrease of Aβ levels. In the presence of phorbol esters (PMA: enhacners of PKC which are known to regulate the activity of α-secretases) the levels of sAPPa were increased by a factor of 2.5. The activity of MMP-9 was inhibited by EDTA and MMP-9 specific inhibitor SB-3-CT. Finally, immunoprecipitation and confocal microscopy experiments indicated interaction between APP and MMP-9 on the cell surface.
- ***Functional regulation of osteoblastic cell lines in inflammation:*** Cultured MG-63 osteosarcoma cells have increased expression of TIMP-1 in the presence of TNF-α. This protein which is a collagenase inhibitor has anti-apoptotic properties and the molecular mechanisms of this function are being examined.

2005 Publications

Charonis AS, Sideraki V, Kaltezioti V, Alberti A, Vlahakos D, Wu K, Tsilibary EC Basement membrane peptides: Functional Considerations and Biomedical Applications in Autoimmunity. *Current Medicinal Chemistry* 12: 1495-1502, 2005

Impact Factors (for 1 publication): 4,38

Citations 2005 (without self- citations): 89
(Tsilibary EC, Tsilibary E, Tsilibary PC): 72
(Tzinia A, Tzinia AK): 9
(Kitsiou P): 8

Total Citations 2003-2005 (without self- citations): 271
(Tsilibary, EC. Tsilibary E, Tsilibary PC): 221
(Tzinia A, Tzinia AK): 32
(Kitsiou P): 18

h-factor: Tsilibary:26, Tzinia: 5, 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
Elias Siskos, Collaborating Graduate Student
Anastassia Pantazi-Mazomenou, Research Technician

2005 Publications

Schwartz, B., McErlean, C., Fletcher, M., Mazomenos, B., Konstantopoulou, M., Kitching, W., De Voss, J. (2005). Spiroacetal Biosynthesis: (±)-1,7-Dioxaspiro[5.5]undecane in *Bactrocera cacuminata* and *Bactrocera oleae* (Olive Fruit Fly). Organic Letters 7(6), 1173-1176.

Konstantopoulou, M., Mazomenos, B. 2005. Evaluation of *Beauveria bassiana* and *B. brongniartii* strains and four wild-type fungal species against adults of *Bactrocera oleae* and *Ceratitis capitata.* Biocontrol 50, 293-305.

Herz, A., Hassan, S., Hegazi, E., Nasr, F., Youssef, A., Khafagi, W., Agamy, E., Ksantini, M., Jardak, T., Mazomenos, B., Konstantopoulou, M., Torres, L., Gonçalves, F., Bento, A., Pereira, J. (2005). Towards sustainable control of lepidopterous pests in olive cultivation. Gesunde Pflanzen 57, 117-128.

Kavallieratos, N., Athanassiou, C., Balotis, G., Tatsi, G., Mazomenos, B. (2005). Factors affecting male Prays oleae (Lepidoptera: Yponomeutidae) captures in pheromone-baited traps in olive orchards. J. Econ. Entomol. 98(5), 1499-1505.

Konstantopoulou, M., Raptopoulos, D., Stavrakis, N., Mazomenos, B. (2005). Microflora Species and Their Volatile Compounds Affecting the Development of an Alcohol Dehydrogenase Homozygous Strain (*Adh*-I) of *Bactrocera* (*Dacus*) *oleae* (Diptera:Tephritidae). J. Econ. Entomol. 98(6), 1943-1949.

2005 Presentations at International Scientific Conferences

Ksantini, M. Konstantopoulou, B. Mazomenos, T. Broumas, P. Milonas, T. Moschos, C. Souliotis, L. Torres, J. Pereira, A. Bento (2005). Prospects to use releases of the egg parasitoid Trichogramma (Hymenoptera, Trichogrammatidae) for biological control in olive cultivation-lessons from the EU-project "Triphelio". Dresdener Entomologentagung, 21-24 March 2005, Dresden, Germany.

A.A. Bento, J.A. Pereira, J.E. Cabanas, M. Konstantopoulou, L. Torres, B. Mazomenos (2005). Mating Disruption Trials for the Olive Moth, Prays oleae (Bern.), (Lep.:Yponomeutidae in Trás-os-Montes Olive Groves (Northeast of Portugal). 2nd European meeting of the IOBC/WPRS study group «Integrated protection of olive crops», Florence, Italy 26-28 October 2005.

E.M. Hegazi, B.E. Mazomenos, W.E. Khafagi, A. Zaitun, S. Mostafa, S. El-Kemny (2005). Long term monitoring of Palpita unionalis (HÜBNER) (Lepidoptera: Pyralidae) in olive groves in Egypt. 2nd European meeting of the IOBC/WPRS study group «Integrated protection of olive crops», Florence, Italy 26-28 October 2005.

E.M. Hegazi, M.B. Hafez, B.E. Mazomenos, W.E. Khafagi, A. Zaitun, S. Showel, S. Mostafa, S. El-Kemny (2005). Efficancy of mating disruption for controlling the jasmine moth, Palpita unionalis: A case study over three consecutive olive growing seasons. 2nd European meeting of the IOBC/WPRS study group «Integrated protection of olive crops», Florence, Italy 26-28 October 2005.

N.G. Kavallieratos, C.G. Athanassiou, George N. Balotis, G.T. Tatsi, B.E. Mazomenos (2005). Factors affecting male *Prays oleae* (Lepidoptera: Yponomeutidae)- Captures in pheromone-baited traps in olive orchards. 2nd European meeting of the IOBC/WPRS study group «Integrated protection of olive crops», Florence, Italy 26-28 October 2005.

Impact Factors (for 5 publications): 7,551

Citations 2005 (without self- citations): 22

Total Citations 2003-2005 (without self- citations): 76

h-factor: 9

Research Group: Environmental Mutagenesis-Carcinogenesis

Research Staff

Gerassimos Voutsinas, Researcher
Vassilis Nikas, Graduate Student
Dimitra Anastasiou, Graduate Student
Stefanos Kachrilas, Graduate Student
Eleni Litsiou, Collaborating Graduate Student
Galenos Fanourakis, Collaborating Graduate Student
Eleni Gerolimatou, Undergraduate Student
Athina Giarika, Undergraduate Student
Olga Paparidou, Undergraduate Student
Stefanos Papaspiridakos, Undergraduate Student
Lamprini Tsivola, Undergraduate Student
Vicky Glinou, Training Student
Stavrianna Liapa, Training Student
Sokratis Avgeris, Research Technician

Research Interests

1. Genetic and epigenetic alterations in genes involved in metabolism, cell cycle control and apoptosis and their relation to human disease
2. Apoptotic signal transduction in carcinogenesis and drug resistance

2005 Findings

Human retinoblastoma cells are resistant to apoptosis induced by death receptors: Role of caspase-8 gene silencing

Rb cells express Fas, DR4, and DR5 on their surfaces, yet were resistant to DR-mediated apoptosis. This was not due to DR mutations or secretion of the soluble decoy Fas, antiapoptotic NF-kappaB activity, or FLIP overexpression, but to the absence of caspase-8 expression. The demethylating agent 5-aza-2'-deoxycytidine restored caspase-8 expression and sensitivity to DR-mediated apoptosis. Rb cells are resistant to DR-mediated apoptosis because of a deficiency in caspase-8 expression secondary to epigenetic gene silencing by overmethylation.

2005 Publications

Poulaki, V., Mitsiades, C.S., McMullan, C., Fanourakis, G., Negri, J., Goudopoulou, A., Halikias, I.X., Voutsinas, G., Tseleni-Balafouta, S., Miller, J.W., Mitsiades N. (2005) Human retinoblastoma cells are resistant to apoptosis induced by death receptors: Role of caspase-8 gene silencing. Investigative Ophthalmology and Visual Science 46, 358-366.

Impact Factors (for 1 publication): 4,148

Citations 2005 (without self- citations): 16

Total Citations 2032-2005 (without self- citations): 40

h-factor: 4

PROGRAMME B:

MODEL SYSTEMS FOR THE STUDY OF CELL FUNCTION

Research Group: Molecular Genetics of Insects and Biotechnology

Research Staff
Kostas Iatrou, Research Director
Vassiliki Lampropoulou, Researcher
Luc Swevers, Researcher
Lydia Ignatiadou, Emeritus Scientist
Ednidlo Machado, Collaborating Research 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
Ioannis Papaethimiou, Graduate Research Assosiate (MSc)
Daniela Tsikou, Graduate Research Assosiate (MSc)
Christiana Magrioti, Graduate Student
Kostas Nikopoulos, Graduate Research Assosiate
Rita Haddad, Training Student
Maria Noutsou, Training Student
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

2005 Findings

a) Silkmoth Oogenesis
Expression analysis and further functional characterization was carried out of the regulatory factor BmSH3, a putative modulator of the function of the nuclear receptor BmE75C. Antibodies were raised against specific domains of BmSH3 and used in Western blot analysis and immunocytochemistry for developmental analysis. Furthermore, fusion proteins of GFP with BmSH3 were expressed in silkmoth-derived tissue culture cells to study the subcellular localization of BmSH3.

In addition, the role of the prostaglandin signaling pathway in the regulation of the transition from vitellogenesis to choriogenesis was investigated. Inhibitors of prostaglandin synthesis blocked the transition to choriogenesis while the effect could be reversed by the addition of exogenous prostaglandins and cAMP. Current experiments aim at the determination of the exact stages of oogenesis during which prostaglandin signaling takes place.

b) Cell-Based High-Throughput Screening Systems
Data obtained from the Bm5 cell-based high-throughput screening system for ecdysone mimetics were used to obtain a quantitative structure-activity relationship (QSAR) model of the moulting hormone activity that is specific to lepidopteran insects (collaboration with Dr.

Nakagawa, University of Kyoto, Japan). Furthermore, the QSAR model was validated through docking studies of the QSAR model in the ligand-binding pocket of the silkmoth ecdysone receptor (collaboration with Dr. Nakagawa).

Progress was also made regarding the development of Bm5 cell-based high-throughput screening systems that are specific for the human serotonin receptor (collaboration with Dr. Z. Georgoussi, Laboratory of Cell signaling and Molecular Pharmacology). Co-expression of the serotonin receptor and the $G_{\alpha 16}$ protein in Bm5 cells results in efficient transduction to the calcium mobilization pathway. Because calcium release can be easily monitored by fluorescent or luminescent methods, Bm5 cell lines that are transformed for the serotonin receptor and the $G_{\alpha 16}$ protein have the potential to be employed as high-throughput screening systems for serotonin agonists and antagonists.

c) Baculovirus-based Mammalian Gene Transduction Vectors

In the framework of the EPAN project "Baculovirus Artificial Chromosomes (BVACs) and technologies for gene therapy and continuous high-level expression of therapeutic proteins in insect production systems" (coordinator: Dr. K. Iatrou), several baculovirus transfer vectors were constructed that allow the introduction of (mammalian and insect) gene expression and transposition constructs into the baculovirus (Bombyx mori nuclear polyhedrosis virus or BmNPV) genome. The transfer vectors are currently being used to generate recombinant baculoviruses that allow recombinant gene expression as well as transposition of the gene expression cassette into the host cell genomes. Expression and genome transposition cassettes were generated for the green fluorescent protein (GFP) and two neural cell adhesion molecules fused to the Fc domain of human IgG (L1-Fc and F3-Fc). In addition, transfer vectors for expression of Mariner transposase protein were also generated.

In the framework of the same project (coordinator: Dr. K. Iatrou), the lipid profile was characterized of the Bm5(SF)-PP cell line, a cell line that grows in serum-free (SF) media and constitutively produces the promoting protein (PP), a small secreted protein characterized by a lipid-binding ML-domain (collaboration with Dr. Jens Coorssen, University of Calgary, Canada). In comparison with the parental cell line, the lipid profile of the PP-producing cell line shows dramatically decreased levels of cholesterol and sphingomyelin which is consistent with a disappearance of rigid "lipid rafts" in the cellular membrane. The resulting increased fluidity of the membrane is proposed to be responsible for the increased levels of baculovirus infection and production observed in this cell line. The transformed PP-producing cell line is predicted to have important biotechnological applications since it will allow the production of high levels of baculovirus-based mammalian transduction vectors in media that are devoid of human pathogens, viruses or prions.

2005 Publications

Swevers, L., Morou, E., Balatsos, N., Iatrou, K. and Georgoussi, Z. (2005). Functional expression of mammalian opioid receptors in insect cells and high throughput screening platforms for receptor ligand mimetics. Cell. Mol. Life Sci. 62, 919-930.

Lapointe, R, Wilson, R., Vilaplana, L., O'Reilly, D.R., Falabella, P., Douris, V., Bernier-Cardou, M., Pennacchio, F., Iatrou, K., Malva, C., and Olszewski J.A. (2005). Expression of a Toxoneuron nigriceps polydnavirus (TnBV) encoded protein, TnBV1, causes apoptosis-like programmed cell death in lepidopteran insect cells. J. Gen. Vir. 86, 963-971.

Espagne, E., Douris, V., Lalmanach, G., Provost, B., Cattolico, L., Iatrou, K., Drezen, J-M., and Huguet, E. (2005). A virus required for hymenopteran parasite survival into lepidopteran host expresses genes encoding cystatins. J. Vir. 79, 9765-9776.

Iatrou, K., and Swevers, L. (2005) Lepidopteran Cells Expressing the Silkmoth Promoting Protein Display Enhanced Susceptibility to Baculovirus Infection and Yield High Titers of Budded Virus in Serum-Free Culture Media. J. Biotech. 120, 237-250.

Wheelock, C.E., Nakagawa, Y., Harada, T. Oikawa, N., Akamatsu, M., Smagghe, G., Stefanou, D., Iatrou, K., and Swevers, L. (2005). 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. 2005 Oct 21; [Epub ahead of print].

Ignatiades, L. (2005). Scaling the eutrophic status of the Aegean Sea, eastern Mediterranean J. Sea Res., 54, 51-57.

2005 Presentations at International Scientific Conferences

Z. Georgoussi, L. Swevers, V. Douris, D. Stefanou, E. Morou, N. Balatsos and K. Iatrou. A modular expression system derived from insect and baculovirus regulatory elements as the basis for the development of versatile cell-based HTS platforms for lead discovery for known targets: the insect ecdysteroid and mammalian opioid receptor HTS paradigms. Screening Europe 2005 Conference, Geneva, Switzerland, February 14-16, 2005.

L. Swevers, D. Stefanou, T. Soin, G. Smagghe, Y. Nakagawa, and K. Iatrou. A widely applicable ecdysone-responsive reporter cassette for the generation of transformed insect cell line-based high-throughput screening systems for ecdysone mimics. First International Symposium of Entomological Science: Development and Metamorphosis, Kyoto University, Japan, March 2-3, 2005.

C. Kenoutis, R. Efrose, L. Swevers, A. Lavdas, M. Gaitanou, R. Matsas, P.J. Farrell and K. Iatrou. Baculovirus artificial chromosomes and gene delivery and expression in insect and mammalian cells: development, use and safety considerations. 7th Conference on Protein Expression in Animal Cells, September 18-22, 2005, Heraklion, Crete, Greece.

T. Soin, Swevers, L., Iatrou, K., Ghekire, A., Janssen, C., and Smagghe, G. (2005). Presence of ecdysteroids and EcR and RXR homolog receptors in Neomysis integer (Arthropoda: Mysidae) to explain endocrine disruptive activities. Ivth International Conference on Arthropods. Chemical, Physiological and Environmental Aspects. September 18-23, Zakopane, Poland.

C.E Wheelock, Harada, T., Smagghe, G., Iatrou, K., Swevers, L., Akamatsu, M., and Nakagawa, Y. (2005). 3-D QSAR analysis of non-steroidal ecdysone agonists and homology modeling of the ligand-receptor binding. 33rd Symposium on Structure-Activity Relationships, November 16-17, Osaka University Convention Center, Osaka, Japan.

L. Ignatiades, (2005). Eutrophication trends and consequences in the inshore-offshore system of the Aegean Sea, Eastern Mediterranean. (Abstracts, pp.20) XVII International Botanical Congress, July 17-23 (Invited Speaker).

Impact Factors (for 6 publications):27,429

Citations 2005 (without self- citations): 128
Iatrou K. (Swevers' publications are included): 49
Swevers L.: 3
Lampropoulou V.: 31
Ignatiades L.: 45

Total Citations 2003-2005 (without self- citations): 354
Iatrou K. (Swevers' publications are included): 134
Swevers L.: 10
Lampropoulou V.: 93
Ignatiades L.: 117

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, Researcher
Anastasia Repouskou, Graduate Student
Marianna Kapi, Undergraduate Student
Eliana Gika, 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 transcription. Investigation of PvLHY and PvTOC1 roles in the function of the central oscillator of bean. Study of the gating of light input signals by the biological clock.

- ***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 and study of its effects on the biological clock function and the cell cycle. Overexpression of clock components and study of the effects on cell cycle and cell proliferation. Our goal is the design of drugs that control the proliferation of cancer cells.

2005 Findings

I. Our progress in the study of the plant biological clock function regards the cloning of the missing 5' fragment of the central clock component of Phaseolus vulgaris *PvTOC1* cDNA. The 5' end of the cDNA was cloned by using heterologous primers in RT-PCR from bean total RNA. The available *PvTOC1* cDNA is thus full-length. In addition, we isolated the partial cDNA of an additional clock component of *Phaseolus vulgaris* named *PvELF3*. The orthologous factor from *Arabidopsis thaliana* is involved in the gating mechanism of the light input signals by the biological clock. Preliminary experiments indicate a diverse role of *PvELF3* in the *Phaseolus* light signaling to the clock.

II. Regarding the study of the involvement of the biological clock in cell amplification and carcinogenesis, we were able to induce the biological clock function in the mouse malignant cell line neuroblastoma N2A. Induction was checked by studying with semi-quantitative RT-PCR the rhythmic expression levels of the clock genes *per2*, *cry1* and of the proto-oncogene *c-myc*, which was found to be rhythmic. In addition, we treated N2A cells with 50 μM curcumin, an inhibitor of the histone acetyl transferase p300. Modification in the global acetylation level of histone H4 was detected after a 3-hour treatment. This condition will be used in future experiments to study the involvement of acetylation in the regulation of expression of clock and cell cycle genes. This work is in collaboration with the laboratory of Histone Biochemistry (Dr. T. Sourlingas).

2005 Publications

A. Prombona and J. Argyroudi-Akoyunoglou: Diverse signals synchronize the circadian clock controlling the oscillations in chlorophyll content of etiolated *Phaseolus vulgaris* leaves, Plant Science 167 (2004), 117-127.

Impact Factors: 0

Citations 2005 (without self- citations): 7

Total Citations 2003-2005 (without self- citations): 22

h-factor: 5

Research Group: Microbial Molecular Genetics and Radiation Genetics

Research Staff
Vassiliki Sophianopoulou, Researcher Director
Eleftherios Sideris, Emeritus Scientist
Zoi Erpapazoglou, Graduate Student
Dimitra Bouzarelou, Graduate Student
Anna Bombori, Collaborating Graduate Student
Marina Pantazopoulou, Undergraduate Student
Alexandros pittis, Undergraduate 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) functional / physiological characterization and subcellular 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
  a) functional expression and characterization of genes encoding putative NATs from higher plants b) functional expression and structure-function relationships of human NAT homologues, specific for L-αscorbate transport

2005 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: ShrA protein of Aspergillus nidulans has a restricted range of amino acid transporter targets

  ii) Studies of the role of PrnB native Cys residues on its structure/function: Position 352 of PrnB is involved in a hydrophobic interaction that is essential for proline transport

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

Studies on ExpA, a non-plant Expansin-like protein in *A. nidulans*: ExpA is a cell wall-enzyme, acting probably as an endoglucanase. Strong indications suggest that *expA* transcription and translation occur in different developmental stages.

- ***Functional genomics:*** Functional expression of human Ascorbate transporters SVCT1 and SVCT2 in *A. nidulans*

2005 Publications

E. Tsoulou, C. A. Kalfas and E. G. Sideris (2005). Conformational properties of DNA after exposure to γ-rays and neutrons Radiat. Res. 163 (1): 90-98

2005 Presentations at International Scientific Conferences

M. Billini, V. Sophianopoulou, K. Stamatakis (2005). Molecular and functional characterization of a Na+/H+ antiporter of the cyanobacterium *Synechococcus* sp. PCC 7942. Decoding Nature: Hierarchy of Interactions, Abstract of the 2nd International PhD Student Symposium, March 17-19, Gottingen, Germany, page 39.

Impact Factors (for 1 publication): 3,2

Citations 2005 (without self- citations): 25
V. Sophianopoulou: 17
E. Sideris: 8

Total Citations 2003-2005 (without self- citations): 78
V. Sophianopoulou:54
E. Sideris: 24

h-factor: 8 (V. Sophianopoulou)

Research Group: Biophysics and Biotechnology of Membranes

Research Staff
Kostas Stamatakis, 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 the photosynthetic Hydrogen production.

2005 Findings

Cells of fresh water cyanobacterium *Synechococcus* sp. PCC 7942 import NaCl passively and export Na^+ actively, primarily *via* Na^+/H^+ antiporter. During 2005 genes encoding Na^+/H^+ antiporters of freshwater cyanobacterium *Synechococcus* sp. PCC 7942 was characterized at the molecular level, and functional characterized in an appropriate E. coli strain, with inactive all its endogenous Na^+/H^+ antiporters, complemented with the Na^+/H^+ antiporter genes.

In 2005 we study the water transport across plant cell membranes. We present a model assay, based of chlorophyll (Chl) *a* fluorometry, with which net water transport across the cell membrane of freshwater cyanobacterium *Synechococcus* sp PCC7942 (S7942) can be followed kinetically with millisecond time resolution. It is the fastest assay of water transport across a cell membrane ever reported. Arrhenius plots of the water uptake rates gave activation energies of $E\alpha = 4.9$ kcal mol $^{-1}$, in the absence of inhibitors of water membrane transporters, and $E\alpha = 11.9$ kcal mol $^{-1}$ in its presence. These results satisfy the usual criteria for facilitated water transport through protein water pores of plasma membranes *(aquaporin*s), namely sensitivity to Hg^{2+} ions and low activation energy.

2005 Publications

Stamatakis, K., Ladas, N., Papageorgiou, G. C (2005). Facilitated water transport in cyanobacterium Synechococcus sp. PCC 7942 studied by phycobilisome-sensitized chlorophyll a fluorescence. Photos. Research 84,181–185

2005 Presentations at International Scientific Conferences

Billini M., Sophianopoulou V., and Stamatakis K. (2005). Molecular and functional characterization of a Na^+/H^+ antiporter of the cyanobacterium *Synechococcus* sp. PCC 7942. 'Decoding Nature: Hierarchy of Interactions' 2nd International PhD Student Symposium, 17-19 March 2005, Gottingen, Germany.

Impact Factors (for 1 publication): 2,239

Citations 2005 (without self- citations): 5

Total Citations 2003-2005 (without self- citations): 17

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
Christoforos Nikolaou, Graduate Student
Diamantis Sellis, Collaborating Graduate Student (MSc)

Research Interests

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

- Deviations from randomness at the level of nucleotide n-tuplets. Patterns related to the functionality of genomic regions and to the global genome structure.
- Deviations from randomness at the "middle" length scale (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.

2005 Findings

The Chargaff's 2nd parity rule in bacterial genomes has been studied. The impact of transcription and replication on the observed deviations from this rule has been determined. Two types of skews pattern, related to these deviations, are met in prokaryotes: the one is of a simpler form and helps the determination of the replication origin while the other is a rather fuzzy one, without apparent correlation to any functional localization. They are typical for eubacteria and archaea respectively, but not without some notable exceptions. We have formulated the hypothesis that these patterns are related to two distinct modes of replication in bacterial species, the one characterized by a unique origin of replication and the other by multiple origins. Computer simulations of bacterial evolution corroborate the above hypothesis.

The word (nucleotide n-tuplet) preference in the genomic text has been systematically correlated to the functionality of heterogeneous genomic segments and to genomic evolution. Simple cumulative measures of n-tuplet occurrence have been introduced in the genome study, in combination with modifications of the standard "genomic portrait" and "rank diagram" n-tuplet occurrence visualization tools. A minimal evolutionary scenario has been

proposed which may account for the observed genomic features, which is corroborated by computer simulations.

2005 Publications

Almirantis, Y., Nikolaou, C. (2005). Multi-criterial coding sequence prediction. Combination of GeneMark with two novel, coding-character specific quantities. *Computers in Biology and Medicine.* 35, 627-643.

Nikolaou, C., Almirantis, Y. (2005). "Word" preference in the genomic text and genome evolution. Different modes of n-tuplet usage in coding and noncoding sequences" *Journal of Molecular Evolution* 61, 23-35.

Nikolaou, C., Almirantis, Y. (2005). A study on the correlation of nucleotide skews and the positioning of the origin of replication: different modes of replication in bacterial species. *Nucleic Acids Research* 33, 6816-6822.

Almirantis, Y. (2005). The Paradox of the Planetary Metals. *Journal of Scientific Exploration* 19, no.1, 31-42

2005 Presentations at International Scientific Conferences

Nikolaou C. and Almirantis Y. Scale-dependent deviation from Chargaff's second parity rule in relation with multiple replication origins in bacteria. 4th European Conference on Computational Biology, September 27- October 1 2005, Madrid, Spain.

Papageorgiou, S. Physical forces may cause *Hox* gene collinearity. British Societies for Cell and Developmental Biology Joint Spring Meeting. 6th-9th April 2005, University of Warwick, UK.

Nikolaou C. and Almirantis Y. Different modes of replication in bacterial species. A study on the correlation of nucleotide skews and the positioning of the origin of replication. 57th Meeting of the Hellenic Society of Biochemistry and Molecular Biology, December, 9-11 2005, Athens, Greece.

Impact Factors (for 3 publications): 12,055

Citations 2005 (without self- citations): 10

Total Citations 2003-2005 (without self- citations): 39

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, dynamics and interactions of compounds with biological and pharmacological interest. Two types of compounds are mainly considered:

I. Peptides and small proteins

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

2005 Findings

In 2005 the NMR structural study of humanin (HN), a peptide with novel neuroprotective activity against the insults of Alzheimer's disease, was completed and published in *Biochemical and Biophysical Research Communications*. In aqueous solution HN exists predominantly in an unstructured conformation in equilibrium with turn-like structures, while in the less polar environment of 30% TFE, the peptide readily adopts helical structure with long-range order spanning residues 5 to 18. The structural study of the 1000-fold more active HN derivative (HNG) bearing a Gly in the place of Ser14, is also almost completed and comparisons of the structural characteristics of the two peptides will hopefully identify the structural elements that can be related to their mechanism of neuroprotective action. Within the framework of NMR structural studies of bioactive peptides, the interaction of β-amyloid peptide of Alzheimer's disease with potential aggregation inhibitors, as e.g., thioflavin and oleuropein, was approached with NMR methods and a publication is in preparation.

In the area of radiopharmaceuticals, our work on the synthesis and complete characterization of a new class of ReO(V) and TcO(V) complexes carrying a derivative of the bioactive 4-aminobenzothiazole as ligand and thiophenols as coligands appeared in *Inorganic Chemistry* as part of our effort in developing imaging agents for Alzheimer's disease and cancer. The active 4-aminobenzothiazole moiety has also reacted with the Re(I) and Tc(I) metal cores and carbonyl coligands and the complexes obtained were tested for uptake in cancer cell lines with positive results. In 2005 the synthesis of Re(I) and Tc(I) complexes with S-derivatized cysteine was published in *Inorganic Chemistry* with the collaboration of the distinguished radiochemist, Roger Alberto, introducing a new way for attaching a biomolecule to the complexes. Furthermore, a series of new ligands was tested with the rhenium and technetium cores in the effort for optimisation of the pharmacological properties of the synthesized complexes; a publication has been submitted to *Inorganic Chemistry* on the subject.

2005 Publications

Benaki, D., Zikos, C., Evangelou, A., Livaniou, E., Vlassi, M., Mikros, E., Pelecanou, M. (2005). Solution structure of humanin, a peptide against Alzheimer's disease-related neurotoxicity. Biochem. Biophys. Res. Commun. 329, 152-160

Karagiorgou, O, Patsis, G., Pelecanou, M., C. P. Raptopoulou, C. P., Terzis, A., Siatra-Papastaikoudi, T., Alberto, R., Pirmettis, I., Papadopoulos M. (2005). S-(2-(2-

pyridyl)ethyl)cysteamine and S-(2-(2-pyridyl)ethyl)-D,L-homocysteine as ligands for the "*fac*-$[M(CO)_3]^+$" (M = Re, ^{99m}Tc) core. Inorg. Chem. 44, 4118-4120.

2006 Presentations at International Scientific Conferences

D. Benaki, M. Pelecanou, E. Mikros."NMR study of the interaction of the β-amyloid peptide (1-28) of Alzheimer's disease with potential aggregation inhibitors" XXI International Conference on Magnetic Resonance in Biological Systems (XXI ICMRBS), Hyderabad, India, January 2005

Ch. Zikos, A. Evangelou, D. Benaki, E. Mikros, M. Pelecanou, E. Livaniou, G. P. Evangelatos, E. Livaniou "High-Yield solid-phase synthesis of humanin, an Alzheimer's disease associated 24-mer peptide, and humanin analogues" Czech and Slovak National Conference on Biologically Active Peptides, Prague, May 2005

S. Tzanopoulou, G. Patsis, M. Sagnou, A. Panagiotopoulou, A. Papadopoulos, I. Pirmettis, M. Papadopoulos, M. Pelecanou "Complexes of 4-aminophenyl)benzothiazole derivatives with technetium and rhenium, and assessment of their properties as diagnostic and/or therapeutic radiopharmaceuticals" International Symposium on Trends in Radiopharmaceuticals, Vienna, Austria, 14-18 November 2005

O. Karagiorgou, G. Patsis, M. Pelecanou, C. P. Raptopoulou, A. Terzis, T. Siatra-Papastaikoudi, I. Pirmettis, M. Papadopoulos, S-(2-(2'-pyridyl)ethyl)mercaptoacetic acid and S-(2-(2'-pyridyl)ethyl)mercaptopropionic as ligands for the "fac-$[M(CO)_3]^+$ core (M=Re, ^{99m}Tc), International Symposium on Trends in Radiopharmaceuticals, Vienna, Austria, 14-18 November 2005

A. E. Chiotellis, Ch. Tsoukalas, M. Pelecanou, A. Papadopoulos, I. Pirmettis, M. Papadopoulos, E. Chiotellis "A convenient synthetic procedure yielding 2-picolinamino-N,N-diacetic acid monoamide derivatives, for labelling with the fac-$[M(CO)_3]^+$ core", International Symposium on Trends in Radiopharmaceuticals, Vienna, Austria, 14-18 November 2005

Impact Factors (for 2 publications): 6,225

Citations 2005 (without self- citations): 18

Total Citations 2003-2005 (without self- citations): 49

h-factor:11

Research Group: Protein Structure by Crystallography and Theoretical Modeling

Research Staff
Metaxia Vlassi, Senior Researcher
Maria Palaiomilitou, Postodoctoral Fellow
Athanassios Tartas, Graduate Student
Christos Labrakis, Undergraduate Student
Stefanos Leptidis, Undergraduate Student

Research Interests

Our current research activities focus on structural studies of protein-protein interaction modules involved in many important biological functions and of enzymes and peptides of medical interest with the aim to elucidate their structure/stability/function relationships. 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).

2005 Findings

1) In the framework of structural studies on protein-protein interaction modules:
- ✓ Using bioinformatics techniques combined with biochemical and circular dichroism data, we predicted the structural determinants of the TPR-mediated interaction of the Ssn6 and Tup1 proteins (see previous annual reports) and propose a recognition mechanism for this particular interaction. A manuscript describing this work is in preparation.
- ✓ Our work on the elucidation of structure/thermostability/function relationships of the tumor suppressor protein, BRCA1, using a 3D-model of its BRCT region we produced (see previous annual reports), is published in Biophysical Chemistry (see publications).
- ✓ We have modeled, *in silico,* the 3D-structures of two proteins potential members of a two-component system (by Prof. Kyriakides & co-workers). Applying docking techniques, the two independent models were subsequently used for the modeling of the 3D-structure of their complex. A manuscript describing this work is submitted for publication in BMC Bioinformatics.

2) In the framework of studies on enzymes of medical interest
- ✓ A number of new molecules predicted to function as inhibitors of the enzyme GAPN from pathogenic bacteria (see previous annual reports), have been designed *in silico,* using the structure-based design approach. This work is funded by GSRT (02-PRAXE-197).
- ✓ We have expressed in *E.coli* cells and purified an enzyme involved in cell division (in collaboration with W.Warren, James Cook Univ. Australia) as a starting point for crystallographic analysis.

3) Our work on the peptide humanin in collaboration with M. Pelecanou and the NMR group (see previous annual reports), is published in Biochem. Biophys. Res. Comm (see publications below).

2005 Publications

Pyrpassopoulos, S, Ladopoulou, A., Vlassi, M., Papanikolau, Y., Vorgias, C.E., Yannoukakos, D. and Nounesis, G (2005). Thermal denaturation of the BRCT tandem repeat region of human tumour suppressor gene product BRCA1. *Biophys Chem*. 114, 1-12.

Benaki, D., Zikos, C., Evangelou, A., Livaniou, E., Vlassi, M., Mikros, E., Pelecanou, M.

(2005) Solution Structure of Humanin, a Peptide against Alzheimer's Disease-Related Neurotoxicity. *Biochem Biophys Res Comm* 329, 152-160.

2005 Presentations at International Scientific Conferences

A. Iddar, L. Fourat, A. Serrano, M. Vlassi, A. Soukri (2005). Biochemical and modeling studies on the non phosphorylating glyceraldehyde-3-phosphate dehydrogenase from pathogenic bacteria as potential target for *in silico* drug design against bacterial infection. 1er Congres International de Modélisation Moléculaire, November 23-25, 2005, Casablanca, Morocco.

Impact Factors (for 2 publications): 4,57

Citations 2005 (without self- citations): 23

Total Citations 2003-2005 (without self- citations): 57

h-factor: 9

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.

Concerning the development point of view, we continued to produce demineralised freeze-dried cancellous bone in order to be studied in vitro for its osteogenesis , osteoinduction and osteoconduction capacities and further to be used in dental surgery.

Service Unit Activities during 2005

The numbers of the various types of grafts, which were produced and delivered to Hospitals, during 2005, are listed in the following table.

GRAFTS	DELIVERY
Cancellous Bone	603
Cortical bone	13
Mixed bone	7
Dura mater	130
Cranium bone	15
Collaborations	17

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

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.

During 2005, the Animal Facility made available the following animals:

Users	***Rats***	***Mice***	***Rabbits***
Institute of Biology	20	21	10
Institute of Radioisotopes & Radiodiagnostics	104	335	7
External Users	1056	15	
Total of animals provided	**1180**	**371**	**17**

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
Maria Konstantopoulou, Technical Specialist (she replaces temporarily Dr. Sagnou)

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.

2005 Findings

During the year 2005, 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.

2005 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

During the year 2005, 11 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 2005, three of our graduate students finished their thesis work and became PhDs.

Moreover, 21 students from the University are carrying out their pre-graduate project thesis work at the Institute. 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 2005 the Biology Institute organized one course in which had as participants graduate students of the IB and of other Institutes of N.C.S.R. "Demokritos". The following course was given by scientists of the Biology Institute:

- *Cell Signaling* [course lecturers: I. Georgoussi (course coordinator), and D. Kletsas].

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

- *Teaching in the framework of the postgraduate course: "Biochemistry"* (**Dr. Iro Georgoussi,** Department of Biology, University of Athens)
- *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).
- *Lecture with title "Signal Transduction from Membrane Receptors" in the framework of the seminar program of "Molecular Endocrinology"* (**Dr. D. Kletsas**, Medical School, University of Athens).
- *Lecture with title "Ageing and Carcinogenesis" in the framework of the postgraduate course "Developmental Biology"* (**Dr. D. Kletsas**, Medical School, University of Athens).
- *Cell Cycle: Checkpoints and Consequences for Physiological Cell Function* (**Dr. Th. Sourlinga** Department of Biology, University of Athens).
- *Lecture with title "Renal Development and Related Diseases" in the framework of the postgraduate course "Developmental Biology"* (**Dr. E. Tsilibari**, Department of Biology, University of Athens)
- *Pancreatitis, Pathobiochemistry and Complications of Diabetes (***Dr. E. Tsilibari**, Department of Biology, University of Athens)
- *Lecture with title "Insect molecular biology and insect pest control"* (**Prof. K. Iatrou**, Department of Biology, University of Athens)
- *Lecture with title Functional Expression and Study of Transmebrane Transporters of Higher Organisms" in the framework of the course "Model Systems of Molecular*

Microbiology" of the postgraduate programme Microbial Biotechnology (**Dr. V. Sophianopoulou**, Department of Biology, University of Athens)

- *Teaching in the framework of the postgraduate program "Bioinformatics", the course "Introduction to Computational Biology"* **(Dr. I. Almyrantis,** Department of Biology, University of Athens)
- *Teaching in the framework of the postgraduate courses: "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 2005, 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 (2005) are presented analytically in the following pages.

Finally, the educational endeavours of the Biology Institute also include those accomplished by the Human Tissue Bank (**E. Vavouraki**) who, on a weekly basis, gives tours of their facilities and informative seminars to High School, University and Military School students.

COMPLETION/AWARD OF DOCTORAL THESES IN 2005

GRADUATE STUDENT	TITLE OF DOCTORAL THESIS	ADVISOR (in Institute of Biology)	UNIVERSITY
Christoforos Nikolaou	Study and quantification of non-randomness at several length scales and correlation with their functional role	Ioannis Almyrantis	Biology Dpt., University of Athens
Georgios Megaritis	Molecular mechanisms regulating delta and mu-opioid receptor expressed in Rat-1 fibroblasts	Iro Georgoussi	Biology Dpt., University of Athens
Elias Siskos	*Insecticidal actions of Citrus aurantium*	Vassilios Mazomenos	Univ. of Cardiff, UK

LECTURE CONTRIBUTIONS TO
THE 2005 SUMMER SCHOOL
OF THE NCSR "DEMOKRITOS"

(July 2005)

DATE	SPEAKER	TITLE
11/7/05	**Dr. G. Voutsinas** Institute of Biology, NCSR "Demokritos"	Introduction to cancer genetics
13/7/05	**Dr. M. Vlassi** Institute of Biology, NCSR "Demokritos"	Elucidation of structure-function relationships using X-ray Crystallography: The example of a tumor suppressor protein
14/7/05	**Dr. I. Georgoussi** Institute of Biology, NCSR "Demokritos"	Heptahelical receptors that couple to G proteins: Molecular targets in the development of new therapeutic drugs
14/7/05	**Dr. E. Tsilibary** Institute of Biology, NCSR "Demokritos"	Cell-matrix interactions in physiological and pathological conditions
15/7/05	**Dr. D. Kletsas** Institute of Biology, NCSR "Demokritos"	Cell sencescense and tissue homeostasis
15/7/05	**Dr. T. Sourlingas** Institute of Biology, NCSR "Demokritos"	Histone Variants and Histone Posttranslational Modifications: Fundamental Factors for Chromatin Remodelling during Ageing and Apoptosis
15/7/05	**Dr. A. Prombona** Institute of Biology, NCSR "Demokritos"	The biological clock: fundamental regulator of the organisms' physiology
18/7/05	**Dr. D. Benaki** Institute of Biology, NCSR "Demokritos"	Structural studies with NMR
18/7/05	**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?
19/7/05	**Dr. L. Swevers** Institute of Biology, NCSR "Demokritos"	Long term developmental programs of cellular differentiation: the paradigm of insect oogenesis
19/7/05	**Dr. K. Iatrou** Institute of Biology, NCSR "Demokritos"	Functional genomics and insect recombinant protein expression systems for improvement of health, agriculture and environment
19/7/05	**Dr. V. Lampropoulou** Institute of Biology, NCSR "Demokritos"	Parasitism of Lepidoptera insects by hymenopteram: The symbiotic polydnaviruses and suppression of host immunity
20/7/05	**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".
21/7/05	**Dr. V. Sophianopoulou** Institute of Biology, NCSR "Demokritos"	Model systems of molecular microbiology: the example of using fungal systems for cloning and functional characterization of plant and human genes

SEMINAR PROGRAMME 2005
INSTITUTE OF BIOLOGY

ΗΜΕΡ.	ΟΜΙΛΗΤΗΣ	ΤΙΤΛΟΣ
13/1/05	**Z. Erpapazoglou** Institute of Biology, NCSR "Demokritos"	Homing of a DNA endonuclease gene by meiotic gene conversion in Saccharomyces cerevisiae.
13/1/05	**D. Bouzarelou** Institute of Biology, NCSR "Demokritos"	Maintaining the ribosomal reading frame: the influence of the E site during translational regulation of release factor 2
20/1/05	**M. Billini** Institute of Biology, NCSR "Demokritos"	A Role for Initiation Codon Contex in Chloroplast Translation
20/1/05	**P. Venieratos** Institute of Biology, NCSR "Demokritos"	Tissue-specific microRNA mir-375 regulates insulin secretion from pancreatic β-cells
27/1/05	**L. Leontiadis** Institute of Biology, NCSR "Demokritos"	Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate ERK in the striatum
27/1/05	**A. Talamagas** Institute of Biology, NCSR "Demokritos"	Aβ peptide modulates NO production and mitochondrial function and induces apoptosis
3/2/05	**T. Tzanopoulou** Institute of Biology, NCSR "Demokritos"	Crossover Study of ^{99m}Tc-TRODAT-1 SPECT and ^{18}F-FDOPA PET in Parkinson's Disease Patients
3/2/05	**I. Karakatsanis** Institute of Biology, NCSR "Demokritos"	The upregulation of urocinase synthesis by TGF-β and the induction of metastasis are linked with the src-mapk pathway in human cancer ovarian cells
10/2/05	**D Anastasiou** Institute of Biology, NCSR "Demokritos"	Regulation of Raf - Akt cross-talk
23/3/05	**Ch. Nicolaou** Institute of Biology, NCSR "Demokritos"	Quantification and scale-dependence of non-randomness in genomic DNA sequences in relation to their functional role
30/3/05	**V. Nikas** Institute of Biology, NCSR "Demokritos"	Targeted chemotherapy in epithelial ovarian carcinoma
6/4/05	**M. Xydous** Institute of Biology, NCSR "Demokritos"	The effect of histone acetylation levels in the regulation of the biological clock: Consequences for cellular function
13/4/05	**D. Anastasiou** Institute of Biology, NCSR "Demokritos"	Inhibition of HSP90 by geldanamycin in bladder cancer
20/4/05	**E. Mavrogonatou** Institute of Biology, NCSR "Demokritos"	The effect of hyperosmotic stress on the proliferation and senescence of intervertebral disc cells
18/5/05	**D. Bouzarelou** Institute of Biology, NCSR "Demokritos"	Cloning, molecular and functional characterization of an expansin-like protein in *aspergillus nidulans*
25/5/05	**P. Veniaratos** Institute of Biology, NCSR "Demokritos"	A study of the effects of high glucose on insulin-mediated signaling in cultured pancreatic b-cells
25/5/05	**I. Tsagaraki**	A study of pro- and anti- apoptotic mechanisms of

	Institute of Biology, NCSR "Demokritos"	osteoblasts, in the presence of cytokines
1/6/05	**T. Tzanopoulou** Institute of Biology, NCSR "Demokritos"	Synthesis and characterization of rhenium and technetium complexes as potential radiopharmaceuticals
8/6/05	**M. Billini** Institute of Biology, NCSR "Demokritos"	Study of Na+/H+ antiporters in the freshwater cyanobacterium *Synechococcus* sp. PCC 7942
15/6/05	**L. Leontiadis** Institute of Biology, NCSR "Demokritos"	Functional interactions of the opioid receptors with various proteins
26/5/04	**A. Talamagas** Institute of Biology, NCSR "Demokritos"	MMP-9 acts as a secretase and co-localizes with APP on the cell surface
6/7/05	**T. Georgomanolis** Institute of Biology, NCSR "Demokritos"	Characterisation of the gene BmSH3 of the silkmoth Bombyx mori
13/7/06	**Z. Erpapazoglou** Institute of Biology, NCSR "Demokritos"	Study of the topogenesis mechanisms of transmembrane transporters in *Aspergillus nidulans*
25/8/05	**Dr. E. Tzima** Scripps Research Institute, La Jolla, California	How endophelial cells sense stress
28/9/05	**Dr. A. Reboutsika** BSRC A. Fleming	Internal control of the identity of nerve stem cells
7/10/05	**Dr. Ch. Louis** IMBB - ITE	Malaria tomorrow: can molecular entology replace DDT?
14/10/05	**Dr. D. Karagogeos** IMBB - ITE	Functional significance of TAG-1 affinity molecule in the developing mature nervous system
20/10/05	**M. Xydous** Institute of Biology, NCSR "Demokritos"	Survey of Australians using cannabis for medical purposes
20/10/05	**P. Venieratos** Institute of Biology, NCSR "Demokritos"	Synergistic activation of JNK/SAPK induced by TNF-a and IFN-γ: Apoptosis of pancreatic b-cells via the p53 and ROS pathway
27/10/05	**T. Tzanopoulou** Institute of Biology, NCSR "Demokritos"	Structural characterization of interaction between δ- and α- subunits of F_0F_1-ATPase in E.coli with NMR
27/10/05	**I. Tsagaraki** Institute of Biology, NCSR "Demokritos"	TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism
3/11/05	**D. Bouzarelou** Institute of Biology, NCSR "Demokritos"	Degradation-mediated protein quality control in the nucleus
3/11/05	**A. Repouskou** Institute of Biology, NCSR "Demokritos"	Rhythmic histone acetylation underlies transcription in the mammalian circadian clock
4/11/05	**Prof. S. Efthimiopoulos** Univ. of Athens, Dpt. of Biology	Protein interactions of APP precursor protein
11/11/05	**Dr. E. Gizeli** IMBB, ITE	Applications of biosensors in biology and biotechnology

18/11/05	**Dr. N. Economakos** EIE	Acylureas: a new class of chemicals which inhibit glycogenolysis, thus are potential ypoglycemic drugs
24/11/05	**V. Nikas** Institute of Biology, NCSR "Demokritos"	p53 induced cell sencescence is a significant inhibition mechanism of carcinogenesis in tumor deficient in p53
25/11/05	**Dr. S. Gonos** EIE	Molecular mechanisms of ageing and longevity
1/12/05	**M. Billini** Institute of Biology, NCSR "Demokritos"	The Zipper Model of translational control: a small upstream ORF is the switch that controls structural remodelling of an mRNA leader
1/12/05	**S. Alimperti** Institute of Biology, NCSR "Demokritos"	Studying the molecular base of evolution. Hsp90: A capacitor of evolution
2/12/05	**Dr. N. Tevernarakis** IMBB,ITE	Molecular mechanisms of neurodegeneration
7/12/05	**Dr. L. Stefanis** Academy of Athens	The role of α-synuclein in Parkinson pathogenesis
8/12/05	**E. Salpea** Institute of Biology, NCSR "Demokritos"	Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases
26/12/05	**Dr. S. Taraviras** Medical School, Univ. of patras	Corelations between cell replication and cell differentiation
22/12/05	**A. El Hamitie** Institute of Biology, NCSR "Demokritos"	The Oncoprotein Kinase Chaperone CDC37 functions as an Oncogene in Mice and Collaborates with Both c-myc and Cyclin D1 in Transformation of Multiple Tissues

COLLECTIVE DATA

FINANCIAL REPORT 2005

1. Internal Funding from the Special Account Department

Income	**E u r o**
Carried over from 2004	114.361
Funding from NCSR “D”	0
Matching Funds	0
Income from services	0
Donations from companies	0
Transfer from other sources	24.761
TOTAL	**139.102**

Expenses	
Equipment	12.553
Supplies	33.643
Salaries	10.680
Travels	2.135
Other expenses	24.666
Committed	3.784
Supplies from “Demokritos”	8.900
TOTAL	**104.942**

2. Governmental Funding

	E u r o
Equipment maintenance	1.642
Xeroxing and similar supplies	500
TOTAL	**2.142**

2. **External Funding from the Programmes of the Institute**

SOURCE OF FUNDING (number of programmes)	**FUNDING (in EUROS)**			
	Programme A	**Programme B**	**Programme C**	**INSTITUTE**
European Union (5)	104.884	45.032	-	149.916
General Secretariat for Research & Technology (14)	324.929	30.000	8.267	363.196
Novartis Hellas SA (1)	11.698	-	-	11.698
International Atomic Energy Ageny (IAEA) (1)	-	-	333	333
“Chalips” Company	2.500	-	-	2.500
TOTAL	**444.011**	**75.032**	**8.600**	**527.643**

COLLECTIVE DATA ON PRODUCTIVITY OF SCIENTIFIC PROGRAMMES

	PROGRAMME			
	A	**B**	**C**	**INSTITUTE**
Researchers and Senior Research Specialists	9	6	3	18
Technical Specialist	1	1	2	5*
Collaborating Research Scientists & Emeritus Scientists	3	4	1	8
Postdoctoral Fellows	4	5	2	11
Graduate Students	18	6	3	27
Collaborating Graduate Students & Visiting Students	9	1	1	11
Graduate Research Associates	1	4	-	5
Undergraduate Students	11	7	2	20
Research Technicians	7 #	2	-	12 @
Administrative Staff	-	-	-	2
Total Personnel	**63#**	**36**	**14**	**119**
Publications in Peer-Reviewed Journals	**20+**	**8+**	**6**	**33**
Publications (Average) in Peer-Reviewed Journals per Scientist	2.22	1.33	2	1.83
Cumulative Impact Factor in Peer-Reviewed Journals (number of publications)	**91.613 (20)**	**29.868 (8)**	**20.01 (6)**	**141.491 (33)**
Average Impact Factor in Peer-Reviewed Journals	4.58	3.73	3.33	4.287
Cumulative Impact factor per Scientist	10.179	4.978	6.670	7.860
Proceedings to Conferences	**6$**	**7$**	**1**	**13**
Proceedings (Average) per Scientist	0.66	1.16	0.33	0.72
Total Publications	**26**	**15**	**7**	**46**
Publications (Average) per Scientist	2.888	2.5	2.33	2.555
International Patents	**-**	**-**	**-**	**-**
Greek Patents	**-**	**-**	**-**	**-**
Presentations to International Conferences	**12**	**6**	**9**	**27**
Presentations (Average) per Scientist to International Conferences	1.333	1	3	1.5
Presentations to Greek Conferences	**18**	**15**	**3**	**36**
Presentations (Average) per Scientist to Greek Conferences	2	2.5	3	2
Total Presentations to Conferences	**30**	**21**	**12**	**63**
Presentations (Average) per Scientist to Conferences	3.33	3.5	4	3.5

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

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

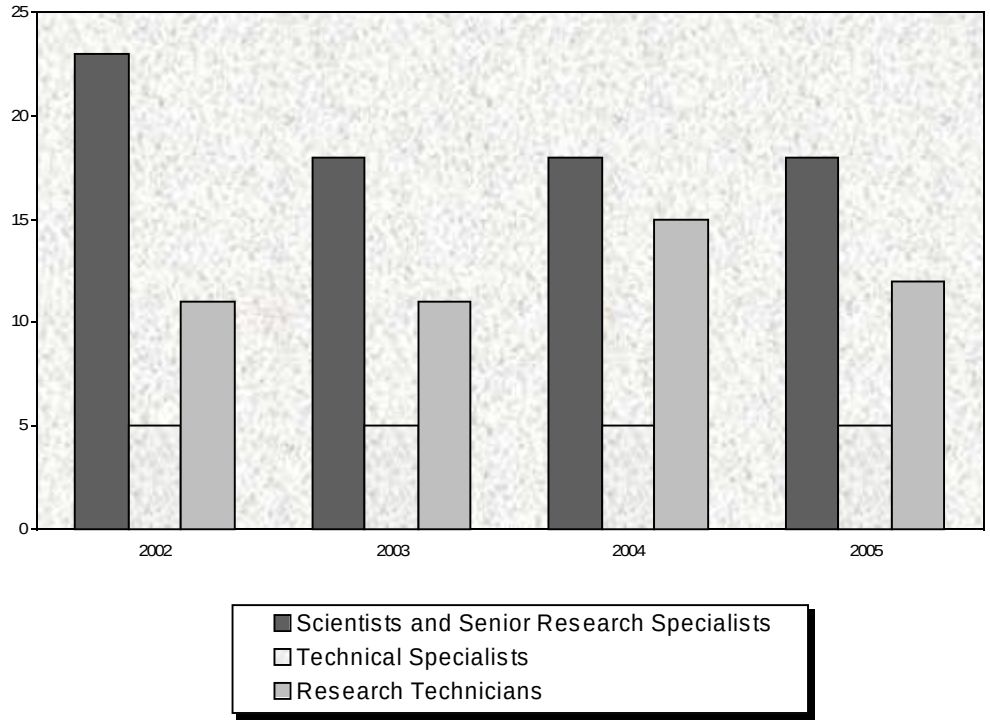
@ 2 Research Technicians who are occupied in Experimental Animal Colony and 1 Research Technician who is occupied in Human Tissue Bank are included

+1 publication common to A and B programme is included

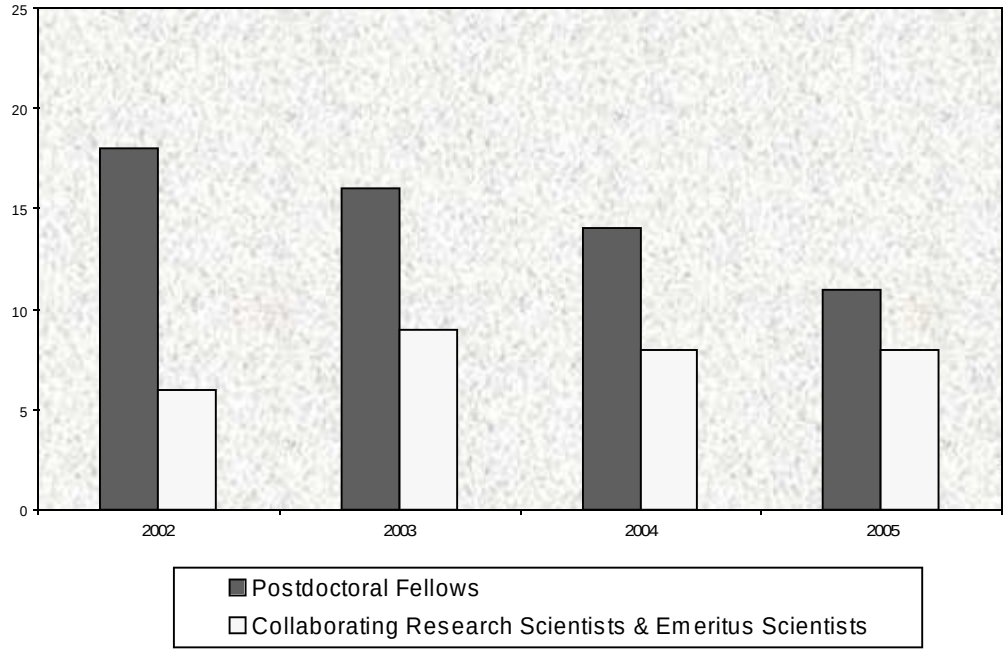
$ 1 publication in proceedings of international conference is included

CHANGES OF IB STAFF DURING 2002-2005

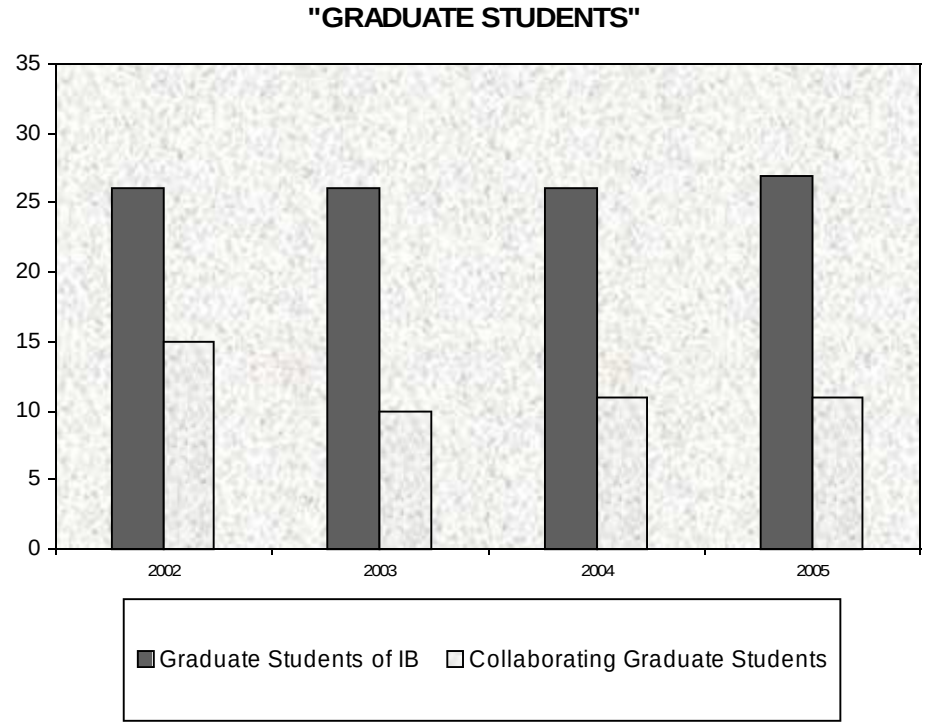
"TENURED EMPLOYEES"

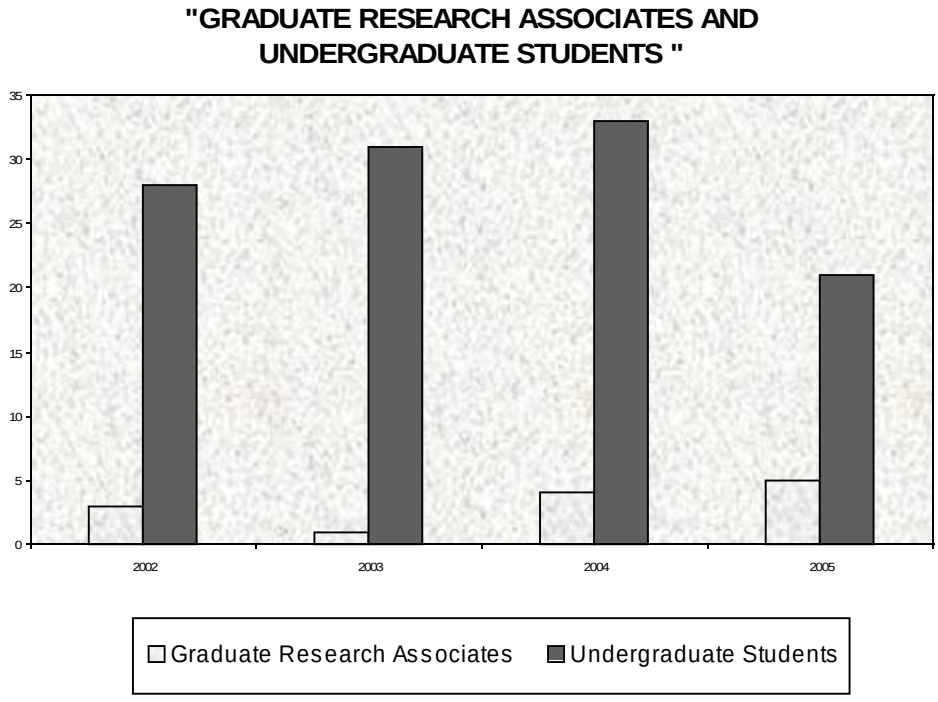


"POSTDOCTORAL FELLOWS, COLLABORATING RESEARCH SCIENTISTS & EMERITUS SCIENTISTS"



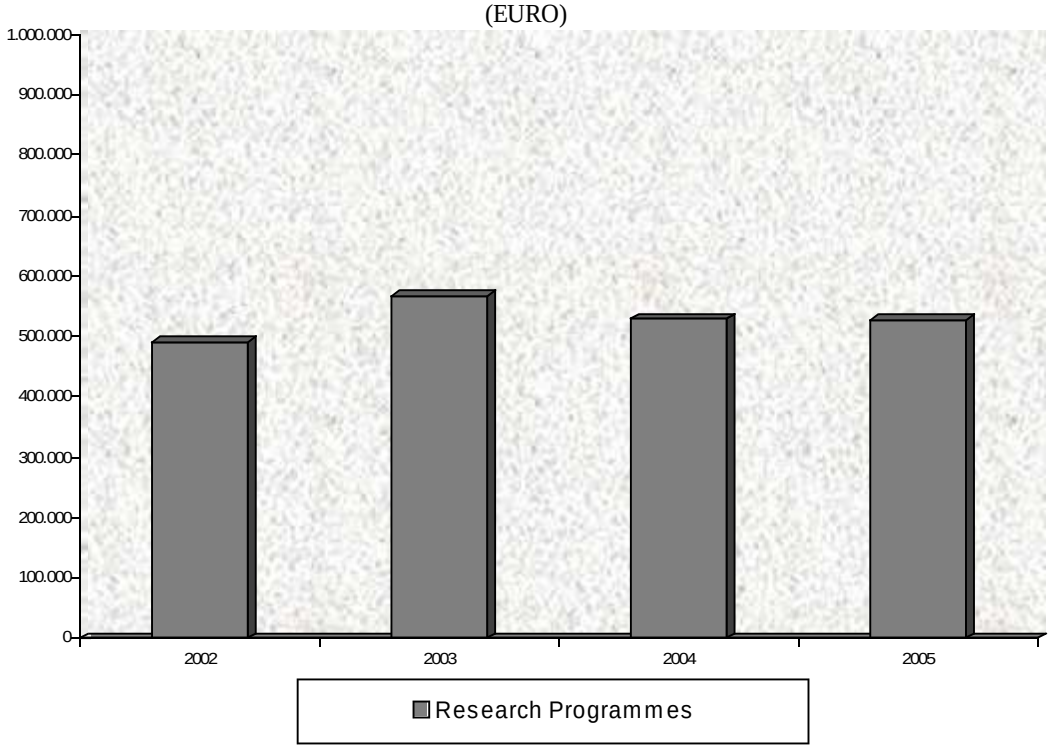
CHANGES OF IB STAFF
DURING 2002-2005





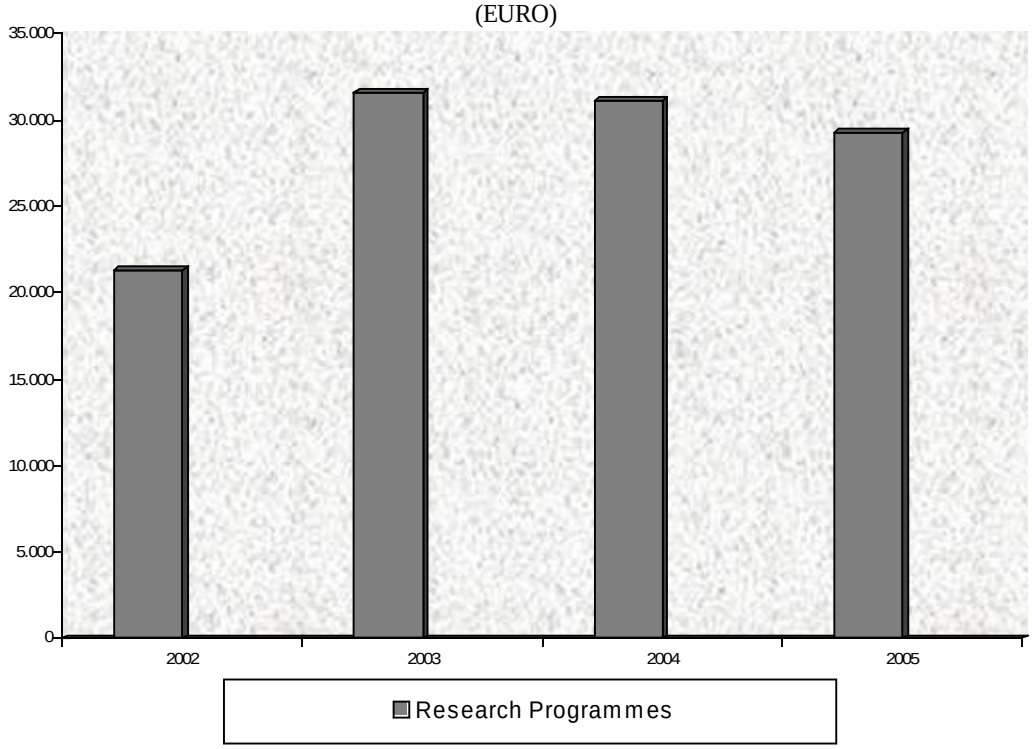
CUMULATIVE EXTERNAL FUNDING OF THE INSTITUTE DURING 2002-2005

(EURO)



EXTERNAL FUNDING OF THE INSTITUTE PER SCIENTIST DURING 2002 - 2005

(EURO)



PUBLICATIONS IN PEER-REVIEWED JOURNALS
AND CUMULATIVE IMPACT FACTOR DURING 2002-2005