INSTITUTE OF
BIOLOGY

NATIONAL CENTRE FOR SCIENTIFIC RESEARCH "DEMOKRITOS"

INSTITUTE OF BIOLOGY

2004
ANNUAL REPORT

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P E R S O N N E L

DIRECTOR

Effie Tsilibary	MD, Cell Biologist

ACTING DIRECTOR

Sekeri Kalliope	Biochemist

SCIENTIFIC STAFF

Research Directors

Iatrou Kostas	Professor of Biochemistry and Molecular Biology
Sekeri Kalliope	Biochemist
Tsilibary Effie	MD, Cell Biologist

Senior Researchers

Almirantis Yannis	Chemist
Georgoussi Zafiroula-Iro	Biochemist
Grammatikakis Nikolaos	Cell Biologist
Kletsas Dimitris	Biologist
Pelecanou Maria	Pharmacist
Piperakis Stelios	Biologist
Sophianopoulou Vassiliki	Biologist
Vlassi Metaxia	Physicist-Chrystallographer

Researchers

Labropoulou Vassiliki	Biochemist
Prombona Anastassia	Biologist
Stamatakis Konstantinos	Biologist
Voutsinas Gerassimos	Biologist
Swevers Luc	Biologist
Tzinia Athina	Biochemist

Senior Research Specialists

Skarlou-Alexiou Vassiliki	Agronomist

Technical Specialists

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

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ADMINISTRATIVE STAFF

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Douvaras Panagiotis	Accountant
Papadaki Margarita	Secretary

COLLABORATING RESEARCH SCIENTISTS & EMERITUS SCIENTISTS

Collaborating Research Scientist & Emeritus Sientists	**LLaboratory**
Ignatiadou Lydia (Dr. Hydrobiologist)- *Emeritus*	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.
Sourlingas Thomae (Dr. Biologist)- *Collaborating*	Sekeri K.

POSTDOCTORAL FELLOWS

Fellow	**Supervisor**
Andronopoulou Evi	Iatrou K.
Benaki Dimitra	Pelekanou M.
Douris Vassilios	Iatrou K.
Drossopoulou Garifallia	Tsilibari E.
Efrose Rodica	Iatrou K.
Kafasla Panagiota	Sophianopoulou V.
Kenoutis Christos	Iatrou K.
Kitsiou Paraskevi	Tsilibary E.
Malakassi Panagiota	Kletsas D.
Massas Ioannis	Skarlou V. *(Retired)*

Metaxatou Aghelina	Ignatiadou L. *(Emeritus)*
Palaiomilitou Maria	Vlassi M.
Pratsinis Haris	Kletsas D.
Teli Thalia	Georgoussi I.

GRADUATE STUDENTS

Student	**Supervisor**
Aliberti Sofia	*Rotation*
Argyrou Eleftheria	Sophianopoulou V. - **PhD obtained in 2004**
Billini Maria	Stamatakis K.
Bouzarelou Dimitra	Sophianopoulou V.
El Hamitie Abraam	*Rotation*
Erpapazoglou Zoi	Sophianopoulou V.
Georgomanolis Theodoros	Swevers L.
Giannouli Christina	Kletsas D.
Handris Panagiotis	Kletsas D.
Kaldis Athanassios-Dimitrios	Prombona A. – **PhD obtained in 2004**
Karakatsanis Ioannis	Kletsas D.
Konti Maria *(Until 7/2004)*	Prombona A.
Kypreou Aikaterini	Sekeri K. – **PhD obtained in 2004**
Leontiadis Leonidas	Georgoussi I.
Mazarakou Georgia	Georgoussi I.
Mavrogonatou Eleni	Kletsas D.
Morou Evaghelia	Georgoussi I.
Nikas Vassilios	Voutsinas V.
Nikolaou Christoforos	Almirantis Y.
Sdralia Konstantia	Iatrou K.
Talamagas Anargiros	Tsilbari E.
Tartas Athanassios	Vlassi M.
Tsagaraki Ioanna	Tzinia A.
Tzanopoulou |Stamatia	Pelekanou M.
Venieratos Panagiotis	Tsilibari E.
Xedous Marios	Sekeri K.

GRADUATE RESEARCH ASSOCIATES

Fellow	**Supervisor**
Kontogianni Nadia	Piperakis S.
Nikopoulos Kostas	Iatrou K.
Papaethimiou Ioannis *(MSc.)*	Iatrou K.
Tsikou Daniela *(MSc)*	Iatrou K.

COLLABORATING GRADUATE STUDENTS & VISITING GRADUATE STUDENTS

Student (University)	**Supervisor**
Anastasiou Dimitra (Univ. of Manchester)	Voutsinas G.
Bombori Anna (Univ. of Athens)	Sophianopoulou V.
Fanourakis Galenos (Univ. of Athens)	Voutsinas G.
Fragopoulos Evaggelos (Univ. of Rome)	Tsilibari E.

Goudopoulou Athina (Univ. of Athens)	Voutsinas G.
Litsiou Eleni (University of Athens)	Voutsinas G.
Manta Maria (Univ. of Athens)	Tzinia A.
Manye Daniel (Institut de Biologia, Molecular de Barcelona -CSIC-) – *Visiting Student*	Iatrou K.
Ninios Ioannis (Univ.of Athens)	Sekeri K.
Siskos Elias (Univ. of Cardiff)	Lab of Chemical Ecology and Natural Products
Soin Thomas (Univ. of Guent, Belgium) – *Viting Student*	Iatrou K.

UNDERGRADUATE STUDENTS AND OTHER IN TRAINING

Student (University)	**Supervisor**
Anagnostakis Nikos (Univ. of Athens)	Piperakis S.
Bandarra Sousana (Unin. of Lisboa, Portugal)	Iatrou K.
Boumlic Anissa (Ecole Superieure de Biotechnologie de Strasbourg, France)	Iatrou K.
Christopoulos George (Univ. of Athens)	Piperakis S.
Gerolimatou Eleni (Univ. of Ioannina)	Voutsinas G.
Giakountis Antonios (Univ. of Thessaly)	Prombona A.
Giarika Athina (Univ. of Thrace)	Voutsinas G.
Gioka Eleni (Univ. of Athens)	Piperakis S.
Goumas Vassilios (Univ. of Thessaly)	Vlassi M.
Gioni Eleni (TEi of Athens)	Kletsas D.
Glinou Vicky (Univ. of Athens)	Voutsinas G.
Drakopoulou Zacharoula (Univ. of Thessaly)	Vlassi M.
De Mendoca Cardoso Matias Ana Isabel Ayres (Universidade Nova de Lisboa)	Sophianopoulou V.
Lapa Stavriana (Univ. of Athens)	Voutsinas G.
Karabinas Theodoros (Univ. of Modena)	Kletsas D.
Karanastassi Georgia (Univ. of Athens)	Piperakis S.
Karnavas Theodoros (Univ. of Athens)	Prombona A.
Kotsogiannis Themistoklis (Univ. of Athens)	Georgoussi I.
Maridaki Kiriaki (Univ. of Athens)	Piperakis S.
Mavroudis Orestis (Univ. of Dundee)	Voutsinas G.
Panagouli Effie (Univ. of Athens)	Piperakis S.
Palaiologou Evagelia (Athens Polytechnic School)	Voutsinas G.
Papageorgiou Emilia (Univ. of London King's College)	Georgoussi I.
Papaioannou Danae (Univ. of Thrace)	Georgoussi I.
Paparidou Olga (Univ. of Athens)	Voutsinas G.
Papaspiridakos Stefanos (Univ. of Athens)	Voutsinas G.
Repouskou Anastasia (Univ. of Athens)	Prombona A
Salpea Paraskevi (Univ. of Athens)	Sekeri K.
Tsivola Lamprini (Univ. of Ioannina)	Voutsinas G.
Tsotakos Nick (Univ. of Thessaloniki)	Tzinia A.
Vaggelatos Ioannis (Univ. of Athens)	Sophianopoulou V.
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

About a year has elapsed since I was appointed as the new Head of the Institute of Biology. This year was not an easy one for "DEMOKRITOS", as it has been tainted by significant financial problems, rendering the smooth operation of this Research Center and the individual institutes harder than usual.

Nevertheless, 2004 has been a productive year: Three new Assistant Researchers were appointed, Drs. Kitsiou and Sourlinga, who have both been members of research teams of the Institute, contributed hard and effective work over a number of years, and earned their appointment based on merit. Additionally, a third Assistant researcher was elected, Dr. Chroni, who will join the Institute in 2005, and the previously elected Principal Researcher Dr. Grammatikakis, started his research in the Institute of Biology. The total gain in researchers was thus four new members during 2004. Warm wishes are extended from the Director to all the new members of the research faculty, for a successful career in their respective fields of interest. Early in 2004 Mrs. Skarlou retired, followed by Mr. Prassas. Thus, research related to earth sciences was completed without further renewal, while other existing and new biomedical directions keep developing, with help from the presence of new research faculty.

Despite ongoing and increasing financial difficulties, all researchers continued their efforts for contributing significant research results by publications in peer-reviewed and other journals, books, participation in research programs, etc. We all hope that 2005 will have bring happier developments for the Institute of Biology and DEMOKRITOS as a whole as well, which continuously fosters extremely competitive research efforts. Additionally, the largest part of the building housing the Animal Colony was renovated, despite the existing lack of funding, as well as laboratory space in some cases, while efforts for more renovations are in progress.

Furthermore, a proposal for funding of the Tissue and Experimental animal facilities was submitted, as well as an application for instrument funds (EPAN supporting infrastructure).

Once again, the retired researchers (in residence) contributed to the publication record of the Institute with their participation in existing research programmes, publications, chapters in books, reviews, etc. and with a valuable contribution to the Institute of Biology in general.

An overview of progress in 2004 lead to the conclusion that a significant increase in the average impact factor was achieved, compared to 2003. Even though we included papers "in press" this time, this increase was real and reflects all efforts of the vast majority of researchers, helping the overall research profile of the IB. More specifically, during 2004 the average impact factor per active researcher is 5.53, compared to 2.77 in 2003. The Director sincerely hopes that this effort will increase more during 2005, and is committed to the search of research resources. However,

it is emphasized that successful improvement of the Institute is not a matter of the Director's efforts only, but of all members of the research faculty.

With the completion of the first year of my tenure as ….. "head navigator" of the IB, I wish to express my most sincere gratitude to Dr. Sekeri, the Vice Director, and the members of the Scientific Advisory Committee whocontributed to the administrative tasks and decision making for the IB. Furthermore, I should whole-heartedly thank the researchers who contributed to the management of this Institute with prompt and enthusiastic participation in committees of various tasks, all aiming at the improvement and further development of the IB. Perhaps it should be mentioned that the support and confidence of the majority of researchers has been the main source of optimism during a year in which, all efforts could often be compared to the task of Sisyphus, and the general atmosphere was anything but encouraging as the lack of finances escalated. Nevertheless I firmly believe that, with continuous efforts and consensus we will manage to render the image of the IB that of an internationally competitive, cutting-edge research institution, based on funding, publications, impact factors and citations, in essence with our own research efforts. I am addressing to all of you, asking you to increase your efforts to participate in local or European programs, and fund your research adequately even through the private sector. I have confidence in the research potential of the IB faculty who prove their merit on a daily basis and keep trying for the best.

Moreover, I would like to express my gratitude to the employees in charge of the accounting services (Mrs. Bazigou and Mr. Douvaras, the latter mostly as a volunteer), who effectively contributed to the solution of technical and other problems, the disposition of old, useless equipment, and the preparation of IB space for renovation, so that we eventually obtain more useful laboratory space. I am also thankful for their regular and complete financial update.

Additionally, I would like to thank Mr. Zamanis for help with the graphics and preparation of the Greek version of the progress report, despite narrow deadlines and his beginning military service. Finally, the secretarial support of Ms. Margarita Papadaki is gratefully acknowledged. Ms. Papadaki has been in charge not only of coordinating the edition of the progress report, but daily and significantly contributes to the tasks of the Director.

To sum it up, I extend to all of you my sincere and warmest wishes for a productive research year, which will significantly promote the international image of the IB, and I hope there will be significantly more high visibility publications and adequate funding for all members of the research faculty, thus establishing a competitive identity of this Institution. Good luck to all of us!

Effie C. Tsilibary, MD, PhD

Director of IB
February 2005

PROGRAMME A:

REGULATION OF CELL FUNCTION

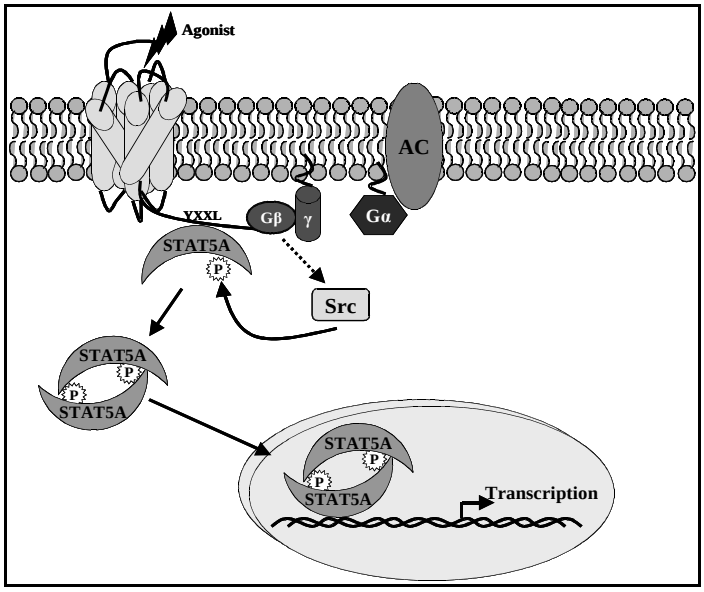
AGED-RELATED DISEASES

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
Themistoklis Kotsogiannis, Undergraduate Student
Emilia Papageorgiou, Summer Undergraduate Student
Danae Papaioannou, Summer Undergraduate Student

Research Interests



The overall objectives of our research group are focused:

a) on the understanding of the molecular mechanisms and the identification of the structural determinants of G protein coupled receptors in which opioid receptors belong

b) on the identification of distinct signaling circuits that occur in the proximity, or not of the receptor and on the determination of novel interactive proteins, which participate in those circuits

c) on the identification of transcription or mitogenic factors that are activated upon acute or chronic opioid exposure, with the ultimate goal to identify novel therapeutic targets

d) on the elucidation of the mechanisms of signal sorting and integration between different signaling pathways and the identification of the specific genes that are activated by the opioid receptors

e) on the development of High Throughput Screens (HTP) for GPCRs after heterologous expression in lepidopteran cells that lead in the identification of new analogs that bind in these receptors

2004 Findings

***Mapping the structural and functional domains of the opioid receptors*:** Based on our previous observations concerning the role and the significance of the third intracellular loop of the δ-opioid receptor (δ-OR), in G protein coupling and activation, a minigene and a fusion peptide encompassing this domain of the opioid receptor (δ-i3L) were tested as potential activators or inhibitors of opioid as well as α_2 and β2 adrenergic receptors' signaling cascades. Our results demonstrate that expression of the δ-i3L peptide in intact cells, expressing the opioid or the α2 adrenergic receptors, inhibits both PLC activation and ERK phosphorylation. These results demonstrate that a minigene encoding δ-i3L interacts with a specific population of Gi/Go protein(s) and impairs signaling for homologous and heterologous GPCRs.

Analyses of opioid receptor signaling pathways "beyond" G proteins and identification of modular interactive protein domains. To assess novel interacting partners for the opioid receptors, we have generated glutathione S-transferase (GST) or Maltose Binding Protein (MBP) fusion proteins of the third intracellular loop and the carboxyl-terminal regions of the δ- and μ-opioid receptors (δ-OR, μ-OR) as well as mutated versions of them, in order to use them as probes in pull down assays. In this regard, we demonstrate for the first time the ability of the C-terminal tail of the μ-OR, to form trimeric complexes with active Gαi and a novel interacting partner the RGS4 protein. Furthermore, expression of HA-RGS4 construct in COS-7 cells alters the signaling of μ-OR. (*Collaboration: Prof. Heidi Hamm, Department of Pharmacology Vanderbilt University School of Medicine, Nashville, TN, USA).*

Interactions between opioid receptors with other transmembrane proteins. We are interested in investigating the possible interaction or cross-talk between the μ-opioid receptor with other transmembrane proteins, which are implicated in neuronal function (e.g. glutamate transporters EAAT1, EAAT2, EAAC3). In this regard, by immunoprecipitation experiments we examined whether the myc-μ-OR could interact with the neuronal transporter EAAC1, in neuroblastoma C6 cells, which endogenously express this glutamate transporter. Furthermore, HEK293 cells were stably transformed to express the Flag-tagged μ- or the δ-OR and the cross-talk of these receptors with that of neurotensin is investigated.

Cellular signaling of opioid receptors leading to alterations in gene expression. Another area of our research activity covers the molecular signaling circuits of opioid receptors that lead to tolerance and dependence. Based on our previous results, demonstrating that activation of the μ-OR induces phosphorylation of STAT5A and STAT5B members of the family of Signal Transducers and Activators of Transcription, through the Src tyrosine kinase, in a Gi/Go independent manner, we propose a novel signaling pathway (Fig. 1) mediated by μ-OR. By using GST fusion proteins encompassing the wild type or mutated versions of the C-terminal tail of the μ-OR we determined the specific binding site of STAT5A on the μ-receptor. This direct interaction of STAT5A with μ-OR was confirmed by co-immunoprecipitation studies in COS-7 cells that express transiently the myc-tagged μ-OR and STAT5A.

Development of HighTroughput Screens (HTP): In collaboration with Professor K. Iatrou and Dr L. Swevers *(Laboratory of Insect Molecular Genetics and Biotechnology, Institute of Biology)* we developed HTP screening systems for ligands that bind to μ- and δ-ORs. The system is based on transformed insect cell lines (Bm5), which overexpress the δ- or/and the μ-ORs, in the presence or not, of the $G\alpha_{16}$ G protein. The functionality of the system is based on the ability of the expressed receptors to couple functionally either with the endogenous G protein population, or the overexpressed $G\alpha_{16}$ and the subsequent elevation of intracellular Ca^{2+}, which can be monitored by fluorescence techniques. These transformed cell lines can be used for the detection of new opioid agonists and/or antagonists present in natural products. Relevant studies are also performed using a new screening system expressing the human serotonin receptor (5-HT4) in lepidopteran Bm5 cells.

2004 Publications

Mazarakou G. and Georgoussi Z. STAT5A interacts with and is phosphorylated upon activation of the μ-opioid receptor. J. Neurochem. In press

Swevers L*., Morou E*., Balatsos N., Iatrou K. and Georgoussi Z. 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, in press, * authors with equal contribution

2004 Presentations at International Scientific Conferences

Mazarakou G. and Georgoussi Z. (2004) "Direct binding of STAT5A to multiple sites of the μ-opioid receptor reveals novel regulatory signaling pathways mediated upon activation of this receptor" Signal Transduction Pathways as Therapeutic Targets Jan. 25-28, 2004, Kirchberg-Luxembourg

Mazarakou G. and Georgoussi Z. "STAT5A interacts with the μ-opioid receptor and reveals novel regulatory signaling pathways", 35th International Narcotic Research Conference (INRC) Kyoto, Japan, 18-23 July 2004.

Morou E. and Georgoussi Z. "Expression of a peptide encoding the third intracellular loop of the delta opioid receptor alters G protein signaling and ERK activation", 35th International Narcotic Research Conference (INRC) Kyoto, Japan, 18-23 July 2004.

Leontiadis L., Hyde K., Hamm H. and Georgoussi Z. "Direct interaction of the third intracellular loop of the delta opioid receptor with G protein subunits", 35th International Narcotic Research Conference (INRC) Kyoto, Japan, 18-23 July 2004.

Swevers, L., Farrell, P.J.F., Douris, V., Andronopoulou, E., Stefanou, D., Labropoulou, V., Morou, E., Balatsos, N., Georgoussi, Z., and Iatrou, K. (2004). Transformed lepidopteran cell lines: from genomics to functional genomics and biotechnology. Lepidopteran Genomics Workshop, XXII International Congress of Entomology, August 15-21, Brisbane, Australia.

Impact Factors (for 2 publications): 9,958

Citations 2004 (without self- citations): 11

Total Citations 2002-2004 (without self- citations): 33

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

Research Staff
Nikos Grammatikakis, Senior Researcher
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.

2004 Findings

The first part of our work in 2004 was carried in our lab in the US (Harvard University/ BIDMC). Upon transferring to the Institute of Biology at the NCSR Demokritos and following the initial period of adjustment, we continued our signaling work. The results of the work we have carried out both in the US and in Greece during the past year can be summarized as follows:

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.

2004 Publications

Wang X, Grammatikakis N, Siganou A, Stevenson MA, Calderwood SK. J Biol Chem. 2004 Nov 19;279(47):49460-9. Epub 2004 Sep 10. Interactions between extracellular signal regulated protein kinase 1 (ERK1), 14-3-3 epsilon and heat shock factor 1 during stress. (First two authors had equal contribution)

Zhang H, Wu W, Du Y, Santos SJ, Conrad SE, Watson JT, Grammatikakis N, Gallo KA. Hsp90/p50cdc37 is required for mixed-lineage kinase (MLK) 3 signaling. J Biol Chem. 2004 May 7; 279(19):19457-63. Epub 2004 Mar 04.

2004 Presentations at International Scientific Conferences

Invited Speaker at EMBO workshop and 2nd International Conference "The HSP90 Chaperone Machinery, September 27-29, Gwatt, Switzerland.

Invited speaker at 6th Annual Signal Transduction Longwood Medical Area Meeting, April 15-16, Boston, USA.

Impact Factors (for 2 publications): 14,06

Citations 2004 (without self- citations): 139

Total Citations 2002-2004 (without self- citations): 351

Research Group: Growth Factors and the Regulation of Tissue Homeostasis-Cellular Aging

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
Eleni Gkioni, Undergraduate Student
Theodoros Karabinas, Undergraduate 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. The mechanism of their action on cell proliferation and extracellular matrix production is 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. Furthermore, the structural and functional characteristics of the senescent (non-proliferating) cell – in comparison to the cancer cell – are also investigated, aiming at the elucidation of the mechanisms underlying ageing and longevity, as well as malignant transformation.

2004 Findings

During 2004 we have continued our studies on the action of the multifunctional growth factor TGF-β on human fibroblasts. We have shown that TGF-β regulates the proliferation of human fibroblasts in a manner depending on the developmental stage of the donor, as it stimulates the proliferation of adult cells while it inhibits embryonic fibroblasts. This transition seems to be accomplished within the third trimester of gestation. These findings are in accordance with relevant alterations of tissue physiology, such as the strategies followed by wound healing in the various developmental stages. Our goal was to elucidate the mechanism underlying this differential action. Accordingly, we have studied the activation of the signaling pathways regulated by TGF-β and have shown that SMAD proteins are equally activated in both cell types. Concerning the stimulatory action of TGF-β in adult fibroblasts, we have shown that it is mediated by the formation of an autocrine loop: TGF-β induces the synthesis and release of FGF-2 which stimulates cell proliferation by the activation of the MEK-ERK pathway. In contrast, in fetal fibroblasts TGF-β inhibits proliferation via the activation of protein kinase A (PKA) and the subsequent upregulation of the cyclin-dependent kinase inhibitors $p21^{WAF1}$ and $p15^{INK4B}$. Finally, we have shown that, in contrast to TGF-β, the fetal environment, i.e. amniotic fluid, activates several parameters of the healing process, such as cell proliferation, migration and collagen synthesis in both fetal and adult fibroblasts.

We have also continued our investigations on the structure and function of senescent cells. In particular, the studies of the morphological and functional changes of the senescent-cell nucleus were continued, with emphasis in alterations of the nuclear lamina proteins, i.e. lamins, at the levels of expression and post-transcriptional modifications. Furthermore, we

have investigated the contribution of the senescent cell to organismal ageing and the development of various age-related disorders. In this vein, we have shown that the onco-suppressor protein p53, which is over-activated in senescent cells leading to their growth arrest, can induce directly the expression of ICAM-1, a main player in the immune response, at both the mRNA and protein levels. This was shown not only on the classical system for the study of senescence, i.e. normal human fibroblasts, as well as in a novel system of conditionally immortalized human vascular smooth muscle cells developed in our Laboratory. In addition, we have demonstrated in a classical inflammatory and age-related disease, i.e. atherosclerosis, the parallel expression of activated p53 and ICAM-1, as well as the presence of senescent cells, indicating the involvement of cellular senescence in this disorder. These findings demonstrate that the p53-mediated ICAM-1 expression may represent a beneficial stress response that activates immune reaction, however the continuous ICAM-1 over-expression can contribute to age-related pathologies, such as atherosclerosis (hypothesis of "antagonistic pleiotropy"). In addition, our laboratory is currently working on the role of senescent stromal cells in the progression of carcinogenesis in both breast and lung.

One of the tissues severely affected by the aging process is the intervertebral disc. Our laboratory has focused in the examination of the senescence of disc cells, as well as in their response to various stresses. Accordingly, we have developed primary cultures of both human and bovine cells from different parts of the disc, i.e. the annulus fibrosus and the nucleus pulposus, and we are currently studying the features of young vs. senescent cells (both after ageing in vitro and derived from aged donors). In the course of these studies, the above cell strains were also characterized in terms of their proliferative responses to exogenous and endogenous growth factors, since they are crucial in tissue repair processes, and several of the implicated signalling pathways were studied. Finally, the cell responses to several stresses (extreme pH, osmolarity, hypoxia, etc.), as well as to mechanical forces were also examined.

Finally, we have investigated the mechanism underlying the function of known anti-cancer drugs and we are also continuing our studies on the cytostatic/cytotoxic, anti-ageing and the would healing activity of natural and new synthetic compounds.

2004 Publications

Pratsinis, H., Giannouli, C.C., Zervolea, I., Psarras, S., Stathakos, D., Kletsas, D. (2004). Differential proliferative response of fetal and adult human skin fibroblasts to TGF-β. Wound Rep. Reg. 12, 374-383.

Kletsas, D., Li, W., Han, Z., Papadopoulos, V. (2004). Peripheral-type benzodiazepine receptor (PBR) and PBR drug ligands in fibroblast and fibrosarcoma cell proliferation: role of ERK, c-Jun and ligand-activated PBR-independent pathways. Biochem. Pharmacol. 67,1927-1932.

Eliades, T., Pratsinis, H., Kletsas, D., H., Eliades, G., Makou, M. (2004). Characterization and cytotoxicity of ions released from stainless steel and nickel-titanium orthodontic alloys. Am. J. Orthod. Dentofacial Orthop. 125, 24-29.

Athanasas, K., Magiatis, P., Fokialakis, N., Skaltsounis, A.-L., Pratsinis, H., Kletsas, D. (2004). Hyperjovinols A and B: two new phloroglucinol derivatives from Hypericum jovis with antioxidant activity in cell cultures. J. Nat. Prod. 67, 973-977.

Kletsas, D., Pratsinis, H., Mariatos, G., Zacharatos, P., Gorgoulis, V.G. (2004). The pro-inflammatory phenotype of senescent cells: the p53-mediated ICAM-1 expression. Ann. N.Y. Acad. Sci. 1019, 330-332.

Roussidis, A.E., Mitropoulou, T.N., Theocharis, A.D., Kiamouris, C., Papadopoulos, S., Kletsas, D., Karamanos, N.K. (2004). STI571 as a potent inhibitor of growth and invasiveness of human epithelial breast cancer cells. Anticancer Res. 24,1445-1447.

Bakas, P.G., Liapis, A.E., Zervolea, I., Voutsinas, G., Kletsas, D., Creatsas, G. (2004). mRNA assessment for procollagen production in women with genuine stress urinary incontinence. Int. Urogynecol. J. Pelvic Floor Dysfunct. 15, 429-431.

Carrieri, G., Marzi, E., Olivieri, F., Marchegiani, F., Cavallone, L., Cardelli, M., Giovagnetti, S., Stecconi, R., Molendini, C., Trapassi, C., De Benedictis, G., Kletsas, D., Franceschi. C. (2004). The G/C915 polymorphism of transforming growth factor beta1 is associated with human longevity: a study in Italian centenarians. Aging Cell 3, 443-448.

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).

Gorgoulis V., Vassiliou L.-V., Karakaidos P., Zacharatos P., Kotsinas A., Liloglou T., Venere M., DiTullio R.A., Kastrinakis N.G., Levy B., Kletsas D., Yoneta A., Herlyn M., Kittas C., Halazonetis T.D. "Activation of the DNA damage checkpoint and genomic instability are early events in the natural history of human cancer" Nature (in press).

2004 Presentations at International Scientific Conferences

T. Kundakovic, N. Fokialakis, O. Kretsi, H. Pratsinis, D. Kletsas, N.Kovacevic, I. Chinou (2004). Naphthazarine derivatives from Onosma leptantha (Boraginaceae). 4th International Symposium on Chromatography of Natural Products, June 14-17, 2004, Lublin-Kazimierz Dolny, Poland.

D. Kletsas, H. Pratsinis, E. Mavrogonatou, E. Sklavounou, H. Evans, S. Roberts (2004). Replicative senescence of intervertebral disc cells. Mid-Term EURODISC Workshop, June 25-26, 2004, Ulm, Germany.

E. Mavrogonatou, H. Pratsinis, D. Kletsas (2004). The effect of physical stresses on the proliferation of intervertebral disc cells. Mid-Term EURODISC Workshop, June 25-26, 2004, Ulm, Germany.

H. Pratsinis, E. Mavrogonatou, D. Kletsas (2004). Proliferative effects and intracellular signaling of exogenous and autocrine growth factors on intervertebral disc cells. Mid-Term EURODISC Workshop, June 25-26, 2004, Ulm, Germany.

D. Kletsas, H. Pratsinis, E. Mavrogonatou (2004). Pivotal signalling pathways in the regulation of cellular homeostasis. Mid-Term EURODISC Workshop, June 25-26, 2004, Ulm, Germany.

H. Pratsinis, E. Mavrogonatou, D. Kletsas (2004). Proliferative response of intervertebral disc cells to growth factors. XIXth Meeting of the Federation of the European Connective Tissue Societies, July 9-13, 2004, Taormina-Giardini Naxos, Italy.

D. Kletsas, H. Pratsinis, P. Zacharatos, A.G. Papavassiliou, V.G. Gorgoulis (2004). p53-dependent ICAM-1 over-expression in senescent human cells and tissues. Implication for age-related pathologies. XIXth Meeting of the Federation of the European Connective Tissue Societies, July 9-13, 2004, Taormina-Giardini Naxos, Italy.

E. Mavrogonatou, H. Pratsinis, G. Sapkas, S. Roberts, J. Urban, D. Kletsas (2004). Senescence of intervertebral disc cells. XIXth Meeting of the Federation of the European Connective Tissue Societies, July 9-13, 2004, Taormina-Giardini Naxos, Italy.

C.C. Giannouli, D. Kletsas (2004). The role of PKA and FGF-2 in the differential response of fetal and adult skin fibroblasts to TGF-β. Bioscience 2004 – From molecules to organisms, July 18-22, 2004, Glasgow, UK.

D. Kletsas, H. Pratsinis, P. Zacharatos, V.G. Gorgoulis (2004). p53-dependent ICAM-1 over-expression in senescence. A putative role in atherosclerosis. 4th European Congress of Biogerontology, November 7-10, 2004, Newcastle upon Tyne, UK.

D. Kletsas (2004). Disc cells and cell signaling. Back-to-Back Scientific Meeting, November 11, 2004, RJAH Orthopaedic Hospital, Oswestry, UK (invited speaker).

Impact Factors (for 11 publications): 25,64

Citations 2004 (without self- citations): 74

Total Citations 2002-2004 (without self- citations): 177

Research Group: DNA Repair Systems and Cancer

Research Staff
Stelios Piperakis, Senior Researcher
Nadia Kontogianni, Graduate Research Associate
Nikolaos Anagnostakis, Undergraduate Student
Elli Gkioka, Undergraduate Student
Panagiotis Kanavetas, Undergraduate Student
Georgia Karanastassi, Undergraduate Student
Kyriaki Maridaki, Undergraduate Student
Effie Panagouli, Undergraduate Student
George Christopoulos, Undergraduate Student

Research Interests

The repair of DNA must be regarded along with replication and recombination as our essential transaction of the genetic material in all life forms. The study of DNA damage and the biological responses to such damage has undergone massive expansion during the recent years. Much of the excitement in this field was derived from the evident relevance of DNA repair to human health. Damage of DNA has been clearly implicated in cancer and there have been suggestions that it may be a component in the biology of aging as well.

The laboratory is involved in Molecular Biology studies on DNA damage and repair including Molecular Epidemiology.

2004 Findings

1. The study “DNA damage and repair in schizophrenic patients” was published.
2. A study (in cooperation with the University of Thessaly) of Greek primary school children dietary habits, was accepted for publication.
3. The study “effects of electric fields on the DNA of human lymphocytes” was accepted for publication.
4. The study “Effects of antioxidants (vitamins C, E) in human lymphocytes” was completed and is prepared for publication.
5. The study “diabetes mellitus - DNA damage and repair” was completed and is prepared for publication.
6. The study “Effects of alcohol and hydrogen peroxide in breast cancer patients” was completed and is prepared for publication.
7. The study «Obstractive sleep apnoea, DNA damage-repair was completed and is prepared for publication.
8. The study “European network on children’s susceptibility and exposure to environmental genotoxicants” is been continued.
9. The study “Lung cancer patients, DNA damage and repair” is in progress.
10. The study “effects of common variable immunodeficiency in DNA repair” is in progress.
11. The study “, DNA damage and repair in pancreas cancer patients” is in progress.
12. The study “stomach cancer patients, DNA damage and repair” is in progress.

2004 Publications

D. Psimadas, N. Messini-Nikolaki, M. Zafiropoulou, A. Fortos, S. Tsilimigaki, S.M. Piperakis, 2004. DNA damage and repair efficiency in lymphocytes from schizophrenic patients. Cancer Letters. Cancer Letters v. 204, p. 33-40.

V. Papadimitriou, S.M. Piperakis. An investigation into the Greek secondary school graduates' knowledge and awareness of healthy diet and nutrition. Journal of Science Education. In press.

J. Delimaris, S. Tsilimigaki, N. Messini-Nicolaki, E. Ziros and S.M. Piperakis Effects of pulsed electric fields on DNA of human lymphocytes. Mutation Research. In press.

S. M. Piperakis, V. Papadimitriou, M. Zafiropoulou and P. Zisis. Dietary Habits of Greek Primary School Children. Journal of Human Nutrition. In press.

2004 Presentations at International Scientific Conferences

3rd International Conference on Children's Health and the Environment", London, April 2004. "Comparison of DNA sensitivity, repair efficiency, apoptosis and Necrosis between lymphocytes from child and adult populations exposed to external agents".

EEMS Conference, Maastricht, September 2004. "DNA damage-repair, apoptosis and necrosis in children, adults and old humans".

EEMS Conference, Maastricht, September 2004. "Breast cancer patients DNA damage and repair efficiency".

EEMS Conference, Maastricht, September 2004. " Lung cancer patients DNA damage and repair efficiency".

EEMS Conference, Maastricht, September 2004. " Vitamin's C and E "protection" of lymphocytes exposed to external agents".

EEMS Conference, Maastricht, September 2004. " Apnea patients lymphocytes DNA damage and repair capacity".

EEMS Conference, Maastricht, September 2004. "DNA damage and repair efficiency in common variable immunodeficiency patients lymphocytes".

Impact Factors (for 4 publications): 7,9

Citations 2004 (without self- citations): 25

Total Citations 2002-2004 (without self- citations): 71

Research Group: Nuclear Proteins and Chromatin Function

Research Staff
Kalliopi Sekeri, Research Director
Thomais Sourlingas, Collaborating Research Scientist
Aikaterini Kypreou, Graduate Student
Marios Xydous, Graduate Student
Giannis Ninios, Collaborating Graduate Student
Niki Varouxi, Undergraduate Student
Paraskevi Salpea, 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 are 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 are also being studied. The aim of these studies is to find molecules and/or factors which may have a functionally active involvement during the course of apoptosis.

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.

2004 Findings

The effects of the histone deacetylase inhibitor, trichostatin A, on linker histone H1o expression, histone H4 acetylation and apoptosis were studied in peripheral blood lymphocytes as a function of donor age. It was found that the effect of trichostatin A was dependent on the age of the donor. More specifically, the effect of the histone deacetylase inhibitor increased with increasing age of the donor. From these results it was concluded that histone deacetylases showed differential sensitivity to the inhibitor as a function of increasing age. These results may possibly be due to the appearance of different deacetylase molecules during senescence from those that are found in young cell types. 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 almost been completed by the identification of almost all the H1 somatic subtypes in human peripheral blood lymphocytes and the partial identification of the H1 subtype that decreases during ageing (in collaboration with Prof. D. Doenecke, Institut für Biochemie und Molekulare Zellbiologie, Georg-August-Universität Göttingen).

Also under investigation is the acetylation of cytoplasmic proteins as potential signaling factors in physiological peripheral blood lymphocytes in comparison to leukemic cells. The

first results show 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 which were studied, differential responses to treatment with the inhibitor were found, i.e., different, non physiological levels of tubulin acetylation. 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 after treatment with these inhibitors are also being studied in these leukemic cell lines. 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. The in vitro cell systems that are being used for this work are immortalized mouse fibroblasts as well as mouse cancer cells. Circadian rhythm was induced in the immortalized fibroblast mouse cell line, NIH3T3. This cell line under normal cell culture conditions does not have a circadian rhythm.

2004 Publications

Kypreou, K.P., Sourlingas, T.G. and K.E. Sekeri-Pataryas. Age-dependent response of lymphocytes in the induction of the linker histone variant, H1o and histone H4 acetylation after treatment with the histone deacetylase inhibitor, trichostatin A. *Exp. Gerontol.,* 39: 469-479, 2004.

Wagner W.M., Ouyang, Q., Sekeri-Pataryas K., Sourlingas T.G., and G. Pawelec. Basic biology and clinical impact of immunosenescence. *Biogerontology,* 5: 63-66, 2004.

Sourlingas T.G. and K.E. Sekeri-Pataryas. Major psychoses and the immune system: cell cycle related histone expression of peripheral blood lymphocytes. Invited review article, submitted.

2004 Presentations at International Scientific Conferences

Kypreou K.P., Sekeri-Pataryas K.E. and T.G. Sourlingas. Linker histones and apoptosis. European Cell Death Organization (ECDO), 12th Euroconference on Apoptosis. Chania, Crete, 2004.

Impact Factors (for 2 publications): 5,7

Citations 2004 (without self- citations): 13

Total Citations 2002-2004 (without self- citations): 43

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

Research Group: Chemical Ecology and Natural Products

Research Staff

Vassilios Mazomenos, Emeritus Scientist
Maria Konstantopoulou, Technical Specialist

Dimitra Stefanou, Technical Specialist
Elias Siskos, Collaborating Graduate Student
Anastassia Pantazi-Mazomenou, Research Technician

Research Interests

- Isolation and characterization of biological active chemicals of agricultural and pharmaceutical interest
- Development of pheromone formulation technologies, and biotechnological methods for pest control.
- Study of the molecular mechanisms involved in insect chemical communication

2004 Findings

- The toxicity effect of metabolites that are produced by the fungus *Mucor hiemalis* was evaluated on adults of *Bactrocera oleae* and *Ceratitis capitata*. Two types of bioasssays were used: a) oral and b) contact. The toxicity levels of metabolites in each type of bioassay and for each dipteran species were investigated. The methodology of the isolation of bioactive substances of *M. hiemalis* was improved and with the utilisation of modern organology (HPLC, NMR, MS), the determination of their structure is very closed to be identified.
- The kairomonal effect of *Prays oleae,* and *Palpita unionalis* sex pheromone components in different concentrations, was investigated on the searching activity of the egg-parasitoid, *Trichogramma cordubensis*.
- The study of the formulation method for the *P. oleae* and *P. unionalis* pheromone was completed and adequate quantities were prepared for the application of mating disruption method in pilot field plots in the olive produced countries that participate in EC project, INCO-2000 Cont. No ICA4-CT2001-10004. (Egypt, Tunisia, Portugal and Greece).
- Monitoring systems based on pheromones (different concentrations and trap types) were developed for the almond wasp *Eurytoma amygdali* and the Jasmine moth *Palpita unionalis* and were tested in several regions in Greece and other Mediterranean countries.
- Mating disruption method and parasitoid releases were applied in olive fields for third year in pilot plots for the control of Lepidoptera olive pests.

2004 Publications

Athanassiou, Ch. A., N.G. Kavallieratos, and B.E. Mazomenos (2004). Effect of trap type, trapcolor, trapping location, and pheromone dispenser on male captures of *Palpita unionalis* (Lepidoptera: Pyralidae). J. Econ. Entomol. 67, 321-329.

Mazomenos, B.E., Ch. A. Athanassiou, N. Kavallieratos and P. Milonas (2004). Evaluation of the major female *Eurytoma amygdali* sex pheromone components, (*Z,Z*)-6,9-tricosadiene and (*Z,Z*)-6,9-pentacosadiene for male attraction in field tests. J. Chem Ecol. 30, 1245-1255.

Konstantopoulou, M.A, F. D. Krokos, and B.E. Mazomenos (2004). Chemical composition of corn leaf essential oil; and its role in the oviposition behavior of *Sesamia nonagrioides* (Lef) females. J. Chem Ecol. 30, 2243-2256.

Konstantopoulou, M.A. and B.E. Mazomenos. Biological activity of fungi species isolated from field-collected diseased insects against adults of two fruit fly species the *Ceratitis capitata* and *Bactrocera oleae* (Diptera: Tephritidae) *Biocontrol* (in press, 2005).

Schwartz, B.D., Ch. S.P. McErlean, M.T. Flecher, B.E. Mazomenos, M.A. Konstantopoulou, W. Kitching and J.J. De Voss. Spiroacetal biosynthesis in *Bactrocera* Fruit Fly sp. Further Definition of the Pathway towards (±)-1,7-Dioxaspiro(5,5) undecane in *B cacuminata* and *B. oleae* (olive fruit fly). *Org. Lett*. (in press, 2005).

Kavallieratos, N.G., Ch. A. Athanassiou, B.E. Mazomenos, G. N. Balotis and G. TH. Tatsi. Factors affecting male *Prays oleae* (Lepidoptera: Yponomeutidae) captures in pheromone-baited traps in olive orchards. *J Econ. Entomol.* (in press, 2005).

Konstantopoulou M., H. Pratsinis, D. Kletsas, F. Krokos, and Mazomenos B., 2005. Pheromone binding protein and general odorant binding protein of *Sesamia nonagrioides* (Lepidoptera: Noctuidae): Sex- and Light-dependent expression. *Ent. Exp. Appl*. (in press, 2005).

2004 Presentations at International Scientific Conferences

B.E. Mazomenos and A. Mazomenou-Pantazi. Integrated pest management (IPM) for the major olive pests based on semiochemicals. XVTH International Plant Protection Congress Beijing, China, May 11-16, 2004. (Invited).

M. A. Konstantopoulou, and B.E. Mazomenos. Toxic metabolites to *Bactrocera oleae* from *Mucor hiemalis*, SMU-21 Isolate. XVTH International Plant Protection Congress Beijing, China, May 11-16, 2004.

E.M. Hegazi, B.E. Mazomenos, P. Milonas, W.E. Khafagi, A. Zitoun, S. Showel, L. Abo-Abdalla, A., El-Shazly, A. Eli-Minshawy, M. Hafez, S., El-Kenny, N. Khamis: Evaluation of mating disruption for the control of the jasmine moth *Palpita unionalis* (Lepidoptera: Pyralidae) in Egypt. XXII Intern. Congress of Entomology, Brisbane Queensland Australia 15-21 August 2004.

L. M. Torres, A. Bento, J.A. Pereira, and B.E. Mazomenos. Flight phenology of *Palpita unionalis* (Hübn) (Lepidoptera: Pyralidae) in the northeast Portugal. XI Iberic Congress of Entomology Madeira Sept. 13-17, 2004.

E. Agamy, A. Bento, B. Hafez, S.A. Hassan, E. Hegazi, A. Herz, T. Jardak, M. Konstantopoulou, M. Ksantini, B. Mazomenos, T. Moschos, F. Nasr, J.A. Pereira, L. Torres, B. Wührer, A. Youssef 2004. Triphelio – An international research project for sustainable control of lepidopterous pests in olive groves. XXII International Congress of Entomology, 15-21 August, 2004, Brisbane, Australia.

Herz A., Hassan S., Torres L., Bento A., Pereira J.A., Ksantini M., Jardak T., Konstantopoulou M., Mazomenos B., Hegazi E., Hafez B., Nasr F., Youssef A., Agamy E. 2004. Nachhaltiger Pflanzenschutz im Olivenanbau: mogliche Anwendung biologischer und biotechnischer Kontrollmethoden am Beispiel der Olivenmotte, *Prays oleae* (Lepidoptera, Plutellidae). 54 Deutsche Pflanzenschutztagung, 20-23, September 2004, Hamburg, Germany.

Herz, A., Hassan S.A., Hafez B., Hegazi E., Nasr F., Youssef A., Agamy E., Jardak T., Ksantini M., Konstantopoulou M., Mazomenos B., Torres L., Bento A., Pereira J.A., 2004: Prospects for sustainable control of Lepidopterous olive pests by pheromones and egg parasitoids. Workshop, Biological Control of plant, medical and veterinary pests“, 15-17 November 2004, Wetzlar, Germany.

Impact Factors (for 3 publications): 4,657

Citations 2004 (without self- citations): 12

Total Citations 2002-2004 (without self- citations): 65

Research Group: Pathobiology of the 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 interests of this laboratory focus on regulation of cell functions which are related mainly to matrix components, and matrix-mediated signaling via specialized integrin receptors (integrins, growth factor receptors such as insulin receptor, etc,) in normal and pathological conditions, in which matrix is involved (i.e., conditions mimicking the metabolic disease of diabetes, Alzheimer's neurodegeneration, etc.). The laboratory's research directions follow:

- Regulation of cell function insofar matrix and matrix receptor turnover are concerned, in diabetic conditions (in the presence of increased glucose concentrations). The cell types used include glomerular epithelial cells/podocytes, β-pancreatic islet cells, etc.), and the goal is to prevent the development of diabetic complications, in which the connective tissue (matrix) is involved.
- Regulation of gene expression of transmembrane sialoprotein podocalyxin, in cultured, immortalized renal glomerular epithelial cells and also *in situ*, during chick development. The aim is to understand the mechanisms involved in up- and down-regulation of podocalyxin expression, therefore the controlled expression of this sialoprotein in renal pathology, such as diabetic nephropathy, minimal change disease, etc., in which decreased expression of podocalyxin results in modulation of podocytic function and morphology.
- Regulation of the function of renal glomerular epithelial cells in conditions of chronic inflammation which occurs in glomerulonephritis. The aim is to prevent the results of chronic inflammation in the matrix of the kidneys.
- Examination of functional properties of neuroblastoma cells in Alzheimer's conditions (culture in the presence of amyloid αβ), with the aim to prevent the extracellular accumulation of the amyloid protein which is involved in neuronal degeneration.
- Functional properties of glutamate transporters in normal and neuro-degenerative conditions, with the aim to understand the role of these transporters in neurodegenerative conditions, and the regulation of transporter function in these conditions.

- Regulation of osteoblastic cell function in inflammatory conditions, with the aim to prevent inflammation-generated modulation of matrix composition and function in conditions such arthritis and osteoporosis.

2004 Findings

- ***Regulation of gene expression of transmembrane sialoprotein podocalyxin in renal glomerular epithelial cells***: The role of transcription factor WT1 was examined, since it has been reported to be involved in the regulation of podocalyxin expression. Under conditions in which podocalyxin expression was dramatically decreased, in the presence of increased glucose concentrations, WT1 epxression was unchanged, indicating the participation of additional transcriptional factors. The expression of p53 was next examined, the levels of which remained unchanged in the presence of high glucose concentrations. However, in these conditions the binding of p53 to WT-1 was increased, indicating that WT1-p53 interactions result in downregulation of podocalyxin expression. Alternatively, WT1-p53 interactions modulate the trans-activation of podocalyxin gene by WT1.
- ***Regulation of pancreatic islet β-cell function in the presence of increased glucose concentrations***: Increased glucose concentrations in the media of cultured murine β-cells, apparently modulated the phosphorylation and activation of insulin receptor (IR), IR-substrates (IRS1, IRS2), and the subsequent activation of PI-3 kinase. In addition, the phosphorylation of Akt kinase, which becomes activated via the above-mentioned signaling pathways (IR, IRS1/IRS2, PI-3 kinase) was substantially decreased in the presence of high glucose concentrations. The mechanisms via which high glucose eventually results in apoptosis of β- islet, insulin-secreting cells are being investigated, since β-cell apoptosis leads to permanent hyperglycemia of diabetes mellitus.
- ***Functions of Neuroblastoma cells in Alzheimer's conditions*** (***in the presence of amyloid αβ***): Previous results of the lab indicate that collagenase MMP-9 exrpression is induced in SK-N-SH neuroblastoma cells by amyloid αβ, and acts as neuroprotective α-secretase. This enzyme prevents the extracellular aggregation of amyloid, in areas surrounding neuronal cells. Increased extracellular deposition of amyloid is directly related to Alzheimer's disease and is a diagnostic histopathologic finding. To obtain further evidence of the role of MMP-9 as α- secretase, the effect of this collagenase was studied in the presence of phorbol esters which activate protein kinase C and thus results in enjanced effects of α- secretases, with a corresponding increase of soluble amyloid, sAPPa, which does not aggregate in the extracellular space. In the presence of 1 μM PMA, soluble amyloid sAPPa was increased by ~ 2.5 times in the cultured medium of HEK/APP-695 cells, which were transfected to secreted amyloid. Furthermore, the effect of activated MMP-9, which was isolated from HEK/MMP-9 cells, in APP metabolism was examined. The addition of 5μg/ml activated MMP-9 in the culture medium of HEK/ APP-695 cells, resulted in an increase of soluble APP, sAPP by 10 times. Finally an interaction between pro-MMP-9 and APP695 was observed by immunoprecipitation, indicating that amyloid αβ binds to MMP-9 and thus is a specific substrate for this collagenase.

2004 Publications

Economou CG, Kitsiou, PV, Tzinia, AK, Panagopoulou Marinos E, Kershaw, DB, Effie C. Tsilibary EC Enhanced podocalyxin expression alters the structure of podocyte basal surface *J Cell Sci* 117: 3281-3294, 2004

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* (In Press).

Impact Factors (for 2 publications): 11,695

Citations 2004 (without self- citations): 74
(Tsilibary EC, Tsilibary E, Tsilibary PC): 65
(Tzinia A, Tzinia AK): 9

Total Citations 2002-2004 (without self- citations): 219
(Tsilibary, EC. Tsilibary E, Tsilibary PC):193
(Tzinia A, Tzinia AK): 26

Research Group: Environmental Mutagenesis-Carcinogenesis

Research Staff

Gerassimos Voutsinas, Researcher
Vassilis Nikas, Graduate Student
Dimitra Anastasiou, Collaborating Graduate Student
Athina Goudopoulou, Collaborating Graduate Student
Eleni Litsiou, Collaborating Graduate Student
Galenos Fanourakis, Collaborating Graduate Student
Eleni Gerolimatou, Undergraduate Student
Vicky Glinou, Undergraduate Student
Stavrianna Liapa, Undergraduate Student
Olga Paparidou, Undergraduate Student
Stefanos Papaspiridakos, Undergraduate Student
Lamprini Tsivola, Undergraduate Student
Athina Giarika, Summer Undergraduate Student
Evaggelia Palaiologou, Summer Undergraduate 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

2004 Findings

FLIP expression in bladder urothelial carcinomas: its role in resistance to Fas-mediated apoptosis and clinicopathological correlations
The expression of Fas, FasL, and c-FLIP was quantified immunohistochemically in paraffin-embedded tissues from 53 patients for whom clinical information was available. Positive immunostaining was detected in 72%, 66%, and 81% of cases, respectively. Concurrent expression of Fas and FasL was seen in 27 samples (51%), of which 22 (81.5%) also displayed c-FLIP positivity. FasL and c-FLIP expression increased with advancing stage but was absent from normal urothelium. None of the 53 urothelial carcinoma samples analyzed showed evidence of mutations by polymerase chain reaction single-strand conformation polymorphism and direct sequencing. Survival analysis demonstrated that although both FasL and c-FLIP expression adversely affected survival, only c-FLIP remained statistically significant on multivariate analysis.

No association between *RNASEL* Arg462Gln variant and breast cancer risk
A total of 453 breast cancer patients and 382 age- and sex-matched controls from Greece and Turkey were analyzed. Genotyping for the RNASEL G1385A variant was performed using an Amplification Refractory Mutation System (ARMS). Statistical evaluation of the RNASEL G1385A genotype distribution among breast cancer patients and controls revealed no significant association between the presence of the risk genotype and the occurrence of breast cancer.

Mononucleotide markers of microsatellite instability in carcinomas of the urinary bladder
Seventy-two cases of primary TCC were screened for the presence of alterations in MSI markers by molecular techniques and evaluated immunohistochemically for the expression of

hMLH1 and hMSH2 proteins. The percentage of MSI rose to 16.6%. Reduced (<20%) hMLH1 expression was closely related to the presence of MSI (p=0.0004). Neither MMR proteins nor MSI was associated with grade, stage or papillary status. Clinical outcome analysed as a function of MSI did not show significant differences in terms of both disease-free and overall survival. Reduced hMLH1 expression was a significant predictor of shorter disease-free survival in univariate and multivariate analysis.

mRNA assessment for procollagen production in women with genuine stress urinary incontinence

The aim of the study was to examine changes in the levels of mRNA for procollagen type I and III in women with or without genuine stress incontinence. We did not find statistically significant difference between the two groups of patients in relation to the amount of mRNA of procollagen type I and III, but the quantity of collagen type I and III was significantly reduced in patients with stress incontinence. The possible cause for the reduction in the amount of collagen in women with GSI could be attributed to either a disturbance in the translation of mRNA to protein (collagen) or increased catabolism of collagen by its collagenase.

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.

2004 Publications

Korkolopoulou, P., A. Goudopoulou, G. Voutsinas, E. Thomas-Tsagli, E. Patsouris and A. Saetta (2004) FLIP expression in bladder urothelial carcinomas: its role in resistance to Fas-mediated apoptosis and clinicopathological correlations, Urology 63, 1198-1204.

Sevinç, A., D. Yannoukakos, I. Konstantopoulou, E. Manguoglu, G. Lüleci, T. Çolak, C. Akyerli, G. Çolakoglu, M. Tez, I. Sayek, G. Voutsinas, G. Nasioulas, E. Papadopoulou, L. Florentin, E. Kontogianni, B. Bozkurt, N.A. Kocabaş, A.E.Karakaya, I.G. Yulug and T. Özçelik (2004) No association between *RNASEL* Arg462Gln variant and breast cancer risk, Anticancer Research 24, 2547-2549.

Saetta, A.A., A. Goudopoulou, P. Korkolopoulou, G. Voutsinas, E. Thomas-Tsagli, N.V. Michalopoulos and E. Patsouris (2004) Mononucleotide markers of microsatellite instability in carcinomas of the urinary bladder, European Journal of Surgical Oncology 30, 796-803.

Bakas, P.G., A.E. Liapis, I. Zervolea, G. Voutsinas, D. Kletsas and G. Creatsas (2004) mRNA assessment for procollagen production in women with genuine stress urinary incontinence, International Urogynecological Journal of Pelvic Floor Dysfunctions 15, 429-431.

Poulaki, V., C.S. Mitsiades, C. McMullan, G. Fanourakis, J. Negri, A. Goudopoulou, I.X. Halikias, G. Voutsinas, S. Tseleni-Balafouta, J.W. Miller and N. Mitsiades (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 5 publications): 10,801

Citations 2004 (without self- citations): 8

Total Citations 2002-2004 (without self- citations): 33

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 t
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)
Kostas Nikopoulous, Graduate Research Assosiate
Daniel Maye, Visiting Graduate Student
Thomas Soin, Visiting Graduate Student
Anissa Boumlic, Summer Undergraduate Student
Susanna Bandarra, Summer Undergraduate Student
Dimitra Stefanou, Technical Specialist
Dimitrios Kopanelis, Research Technician

Research Interests

1. **Regulatory mechanisms controlling insect physiological functions:** (a) Oogenesis in lepidopteran insects: a model for differentiation programs induced by ecdysteroid hormones (b) Mechanisms of immunosuppression in lepidopteran insects following parasitization by hymenopteran endoparasitoids: the role of the interactions between proteins produced by hymenopteran endosymbiotic polydna viruses and hemocyte proteins of the lepidopteran hosts (c) Mechanisms controlling olfactory function in the malaria mosquito *Anopheles gambiae*.
2. **Molecular Biology and genetic manipulation of insect nuclear polyhedrosis viruses:** (a) Viruses expressing proteins harmful to the insect hosts (b) Incapacitated viruses as vectors for insect genetic transformation (c) Modified viruses as vectors for human gene therapy and cellular immunization.
3. **Functional genomics:** (a) Systems for production of proteins of economic importance in lepidopteran insect cell lines (b) High throughput screening systems for bioactive substances (activators and inhibitors of pharmacological targets) in chemical libraries and collections of natural products (plants and microorganisms).

2004 Findings

Regulators of lepidopteran insect oogenesis

The characterization of two proteins, NTP 1-4 and CTP 15-1, that are involved in the terminal differentiation of the ovarian follicular cells of the silkmoth *Bombyx mori*, via interactions with the transcription factor BmGATAβ, is in progress. Specific polyclonal antibodies have been generated and used for protein localization studies in the follicular cells. Comparisons with sequences deposited in Genbank by the silkworm genome project revealed that the isolated

cDNA sequences encompass the complete ORFs of both proteins and allowed the deduction of the structure (exon-intron organization) of the corresponding genes. The analysis of the functional role of the two proteins with respect to the follicular cell differentiation program is in progress.

The characterization of BmSH3, a regulatory factor of the follicular cells which (i) interacts with the orphan nuclear receptor BmE75C, (ii) becomes specifically expressed during the transition from vitellogenesis to choriogenesis and (iii) is characterized by the presence of three protein interaction SH3 domains and one lipid raft-targeting "SoHo" domain, was also continued. A specific antibody for the protein was raised and expression constructs were generated to over-express BmSH3 proteins that are fused with the "yellow fluorescent protein" (YFP) to reveal the subcellular localization of the BmSH3 protein. Fluorescence microscopy revealed that BmSH3 is targeted to focal points at the cell surface suggesting a role in the organization of the cytoskeleton and/or interactions with the extracellular matrix.

Molecular mechanisms of parasitization of lepidopteran insects by hymenopteran parasitoids.

In the context of a European research program, we continued the study of a system of endoparasitization, which consists of three biological components: (1) the lepidopteran insect *Manduca sexta* that is parasitized by a hymenopteran insect, the wasp *Cotesia congregata*; (2) *C. congregata* whose the embryos develop in the hemocoelic cavity of *M. sexta*; and (3) an endosymbiotic virus of *C. congregata*, CcBV, which is introduced together with the embryos of *Cotesia* and contributes to the immunosuppression of the host and the development of the parasitoid embryos.

During 2004, we completed, through the use of the yeast two-hybrid system, the screening for interacting partners of two CcBV proteins, CcV1 (a protein homologous to CrV1 of the polyDNA virus of *C. rubecula*, which interferes in the process of aggregation of the hemocytes of the host); and Cactus 2 (a protein homologous to IκB, an inhibitor of the transcription factor NFκB, which is involved in the immunological response of metazoans), with proteins of *Manduca* hemocytes. For CcV1 we detected and confirmed the interactions with two *Manduca* hemocyte proteins, hemolin (a member of the immunoglobulin superfamily) and SPH2 (a serine protease homolog), both of which are involved in the immunological responses of *Manduca* following infection with bacteria or fungi. For Cactus 2, we detected interactions with the hemocyte proteins serpin 2 (a protease inhibitor involved in the breakdown of the cell wall of bacteria), hemolin (as in the case of CcV1), scolexin (homolog of the anticoagulation serine proteases), arylphorin (a glycoprotein that is downregulated during parasitization), lebocin (an antibacterial peptide), transferrin (an iron binding protein that is downregulated during infection) and a non-characterized protein that has homology to protein kinases.

In the framework of the same program we also completed the functional characterization of the protein Cystatin 1 of CcBV. After overexpression in lepidopteran cell lines, it was shown that Cystatin 1 acts as a specific inhibitor of cystein proteases. Finally, the expression of another protein of polydna viruses, TnBV1, in the same system showed that this protein induces cell death similar to apoptosis.

Regulation of mosquito olfactory function

In the context of a collaborative project with the University of Irvine (USA), we continued the functional characterization of odorant-binding proteins (OBPs) from the antennae of the malaria mosquito *Anopheles gambiae*. The characterization comprised the over-expression of

three OBPs in lepidopteran cell lines, their purification from the culture media and the subsequent use of one of them (OBP8) in binding assays of olfactory molecules using physicochemical techniques (Biacore plasmon resonance analysis). In addition we completed the detection and confirmation of the interactions of OBP8 with other proteins of the mosquito antenna through the use of the yeast two-hybrid system. Using this system it was found that OBP8 forms homodimers and heterodimers with other OBPs but also interacts with two enzymes that possibly are involved in the inactivation of OBP8 (OBP degrading enzymes) as well as three other proteins of unknown function, which structurally resemble carrier proteins.

Molecular biology and genetic engineering of insect nucleopolyhedrosis viruses

For our effort to generate incapacitated baculoviruses (nuclear polyhedrosis viruses or NPVs) for use as vectors of genetic transformation of insects, we generated stably transformed "rescuing" cell lines for that may be capable rescuing *Bombyx mori* (silkworm) NPVs (BmNPVs) deficient for the production of LEF8 protein (subunit of viral RNA polymerase) and, thus, unable to complete their lifecycles and functioning as artificial chromosomes (baculovirus-derived artificial chromosomes or BVACs). In addition, a transfer vector has been generated, which contains a fluorescent expression cassette replacement of the LEF8 ORF in the baculovirus genome with. This vector has been used for the generation of a recombinant baculovirus in which the LEF8 gene is replaced with the fluorescent expression cassette. This recombinant virus will be used as the basis for the construction of artificial chromosomes that will direct the production of recombinant proteins of economic importance in lepidopteran cell lines as well as in silkworm larvae.

In the context of the same project, baculoviruses have been also generated that have a mammalian expression cassette for green fluorescent protein (GFP) incorporated into their genomes. Mammalian cell lines "infected" with such viruses express GFP, thus validating the use of recombinant baculoviruses as vectors for gene transduction in mammalian systems. The generation of recombinant baculoviruses that encompass expression cassettes for proteins with potential therapeutic action against neurodegenerative diseases and a transposition system that may direct permanent cell transformation with the therapeutic genes is currently under development.

Functional genomics

In collaboration with the University of Kyoto in Japan we completed the analysis of data regarding function-structure relationships [quantitative structure-activity relation (QSAR) analysis] for a collection of dibenzoyl hydrazine compounds. These compounds were shown to have insect-specific ecdysteroidal toxic activities through the use of a cell-based high throughput screening (HTS) system for ecdysteroid mimics developed by us previously.

In collaboration with the University of Ghent in Belgium, we initiated the first phase for the development of a similar HTS system based on dipteran insect cell lines, with the aim to use it in parallel with the lepidopteran HTS system to screen for ecdysteroidal compounds that target specific insect orders. Ecdysteroid-responsive reporter plasmids based on dipteran promoters were generated and tested in transient expression assays using *Drosophila* S2 cells. The construct with the highest induction level is currently used for the development of stably transformed ecdysteroid-responsive S2 cell lines.

Finally, in collaboration with the laboratory of Dr. Z. Georgoussi, we completed the development of a HTS system for opioid mimics that is specific for the mammalian δ-opioid receptor (DOR). The system is based on transformed lepidopteran cell lines that over-express

DOR either alone or in combination with an auxillary factor, the human Gα16 protein. Biochemical experiments have shown that efficient activation of DOR (coupling to G proteins, production of inositol trisphosphate, calcium release) takes place after agonist challenge. The release of calcium upon agonist binding was determined quantitatively with fluorescent methods. Thus, this system can be used for HTS of chemical libraries and collections of natural products for compounds with opioid agonist or antagonist activity.

2004 Publications

G., Nakagawa, Y., Mazomenos, V., and Iatrou, K. (2004). A cell-based high-throughput screening system for detecting ecdysteroid agonists and antagonists in libraries of synthetic compounds and complex mixtures derived from natural sources. FASEB J, 18, 134-136.

Farrell, P.J. and Iatrou, K. (2004). Transfected insect cells in suspension culture rapidly yield moderate quantities of recombinant proteins in protein-free culture medium. Prot. Expr. Pur. 36, 177-185.

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. (2004). Expression of a *Toxoneuron nigriceps* polydnavirus (TnBV) encoded protein, TnBV1, causes apoptosis-like programmed cell death in lepidopteran insect cells. J. Gen. Vir. In Press.

Swevers L*., Morou E*., Balatsos N., Iatrou K. and Georgoussi Z. 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, in press, * authors with equal contribution

Espagne, E., Douris, V., Lalmanach, G., Provost, B., Cattolico, L., Iatrou, K., Drezen, J-M., and Huguet, E. (2004). A virus required for hymenopteran parasite survival into lepidopteran host expresses genes encoding cystatins. J. Vir. Accepted for publication (pending revision).

Metaxatos A. (2004) Population dynamics of the venerid bivalve Callista chione (L.) in a coastal area of the eastern Mediterranean. J. Sea Res. 52, 293– 305.

Ignatiades L. (2004) Scaling the trophic status of the Aegean Sea, Eastern Mediterranean. J. Sea Res. In Press.

2004 Presentations at International Scientific Conferences

Swevers, L., Sdralia, N., Georgomanolis, Th., Glushek, M., Ito, K., and Iatrou, K. (2004). The transition from vitellogenesis to choriogenesis during silkmoth oogenesis. XXII International Congress of Entomology, 15-21 August 2004, Brisbane, Australia.

Swevers, L., Farrell, P.J., Douris, V., Andronopoulou, E., Stefanou, D., Labropoulou, V., Morou, E., Balatsos, N., Georgoussi, Z., and Iatrou, K. Transformed lepidopteran cell lines: from genomics to functional genomics and biotechnology. Lepidopteran Genomics Workshop, XXII International Congress of Entomology, 15-21 August 2004, Brisbane, Australia.

Ignatiades L, Gotsis-Skretas, O., and Metaxatos A. Isolation and cultivation of the toxic species Alexandrium minutum from the Greek coastal waters. International Workshop on Toxic Algae, 31st March, -2nd April 2004, Athens, Greece.

Gotsis-Skretas, O., Ignatiades L, Pavlidou, A., Papadopoulos A. and Metaxatos A. STRATEGY results in Greek network. Workshop on Harmful Algal Blooms in the Mediterranean Sea, 23-26 October 2004, Majorka, Spain.

Gotsis-Skretas O., Ignatiades L. and Metaxatos A. The distribution of the toxigenic species A*lexandrium minutum* in the Aegean Sea (Eastern Mediterranean). 11th International Conference on Harmful Algae, Nov. 15-19, 2004, Cape Town, S.Africa.

Impact Factors (for 7 publications): 24,642

Citations 2004 (without self- citations): 52
Iatrou K. (Swevers' publications are included): 144
Swevers L.: 2
Lampropoulou V.: 25
Ignatiades L.: 19

Total Citations 2002-2004 (without self- citations): 288
Iatrou K. (Swevers' publications are included): 140
Swevers L.: 7
Lampropoulou V.: 66
Ignatiades L.: 75

Research Group: Transcriptional Regulation by the Biological Clock

Research Staff
Anastassia Prombona, Researcher
Athanasios-Dimitrios Kaldis, Graduate Student
Maria Konti, Graduate Student *(until 7/04)*
Antonios Giakountis, Undergraduate Student
Theodoros Karnavas, Undergraduate Student
Anastasia Repouskou, Undergraduate 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.
- ***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.

2004 Findings

I. Our progress in the study of the plant biological clock function regards the cloning of the 3' fragment of *PvTOC1* cDNA and study of *PvTOC1* rhythmic gene expression profile under different photoperiods. The 3'cDNA end was cloned by the adaptor-ligated PCR method from bean genomic DNA, as this region does not contain any introns. The available *PvTOC1* cDNA is 1254 bp long and the deduced polypeptide sequence is 417 amino acids long. The cloning of the 5' cDNA fragment is in progress. The expression profile of *PvTOC1* gene was studied in leaves of 10 days-old bean seedlings under three photoperiods that is, under equal day-night length, under long days (18 hours) and under short days (6 hours). The steady-state levels of *PvTOC1* mRNA were monitored at different times of the day for two consecutive days. Our results show that 1. *PvTOC1* expression is independent of phytochrome B signaling (no acute response at exposure of etiolated leaves to light), 2. the peak in *PvTOC1* expression is during the night, that is the increase of expression starts at the evening and 3. the peak in the expression levels is reached 12 to 18 hours after dawn, in dependence of the applied photoperiod.

II. Regarding the study of the biological clock involvement in carcinogenesis, we have cloned the cDNAs of the mouse genes that will be used as reporters of the cell cycle and the biological clock function in mouse cell lines. In addition, the laboratory of Histone Biochemistry has achieved the induction of the biological clock function in mouse NIH3T3 fibroblasts, an arrhythmic cell line under normal culture conditions. This project is accomplished in collaboration with the group of Histone Biochemistry and is realized by a PhD student (M. Xydous).

2004 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 (for 1 publication): 1,556

Citations 2004 (without self- citations): 9

Total Citations 2002-2004 (without self- citations): 22

Research Group: Microbial Molecular Genetics and Radiation Genetics

Research Staff
Vassiliki Sophianopoulou, Senior Researcher
Eleftherios Sideris, Emeritus Scientist
Panagiota Kafasla, Postodoctoral Fellow
Zoi Erpapazoglou, Graduate Student
Dimitra Bouzarelou, Graduate Student
Anna Bombori, Collaborating Graduate Student
Ioannis Vaggelatos, Undergraduate Student
Orestis Mavroudis, Summer Undergraduate Student
Ana Isabel Ayres de Mendoca Cardoso Matias, Summer Undergraduate Student
Areti Tsolomiti-Gourgou, Research Technician

Research Interests

- Study of the molecular mechanisms involved in the transport of nucleobases, ascorbate and amino acids across the plasma membrane, mediated by specific transmembrane transporters.
- Structure-function analysis of the above transporters.
- Cloning and functional characterization of putative nucleobase/ascorbate transporter genes from plants and humans using *A. nidulans* as a novel model system.
- Study of Expansin-like proteins of *A. nidulans.*

2004 Findings

- We studied the regulation of expression of purine transporter genes (*uapA, uapC and AzgA*) of *A. nidulans*, during conidiospore germination and the onset of mycelium development. Our studies have shown that both mRNA steady state levels and purine transport activities increase substantially during the isotropic growth phase of conidial germination. Both processes occur in the absence of purine induction, independently of the nitrogen source present in the medium and also independently of the presence of a carbon source in the medium. The pathway-specific transcriptional activator UaY is dispensable for the germination-induced expression of the three transporter genes. AreA, the general GATA factor of *A. nidulans*, on the other hand, is essential for the expression of *uapA*, but not for *azgA* or *uapC*, during germination. Our results establish the presence of a novel system triggering purine transporter transcription dubring germination.
- We studied the function of the ShrA protein of *A. nidulans*. Our studies have shown that ShrA protein is an ER component and is specifically required for the topogenesis of the major proline ransporter to the plasma membrane of *A. nidulans*.
- We studied the role of native Cys residues of PrnB on its structure/function, using a constructed strain of *A. nidulans* expressing a functional Cys-less proline (PrnB) transporter and the methodology of Cys-scanning mutagenesis. Our studies have shown that none of the three native Cys residues of PrnB is directly involved in proline binding and/or transport.
- We studied the regulation of expression of an expasin-like gene, *An-ExpA*, of *A. nidulans*. Our studies have shown that expression of this gene is under carbon catabolite repression.

2004 Publications

S. Amillis, G. Cecchetto, V. Sophianopoulou, M. Koukaki, C. Scazzocchio and G. Diallinas (2004). Transcription of purine trasporter genes is activated during the isotropic growth phase of *Aspergillus nidulans* conidia. Mol. Microbiol. 52 (1), 205-216.

E. Tsoulou, C. A. Kalfas and E. G. Sideris (2004). Conformational properties of DNA after exposure to γ-rays and neutrons Radiat. Res. 163: 90-98.

Impact Factors (for 2 publications): 8,9

Citations 2004 (without self- citations): 36
V. Sophianopoulou:23
E. Sideris: 13

Total Citations 2002-2004 (without self- citations): 80
V. Sophianopoulou:54
E. Sideris: 26

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. Thermotropic behavior of cyanobacteria with, or without polyunsaturated fatty acids in their membranes. Relevance of plasma membrane fluidity to osmotic adaptation of cells.

2004 Findings

Cells of fresh water cyanobacterium *Synechococcus* sp. PCC 7942 import NaCl passively and export Na^+ actively, primarily *via* Na^+/H^+ antiporter. During 2004 a gene encoding Na^+/H^+ antiporter 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 gene *(SycNaH1)*. Functional analysis of the Na^+/H^+ antiporter (SycNaH1) shows high affinity to Na^+ (*Km* = 0.5 mM), pH-dependent activity, and ability to transport also Li^+ and K^+ ions.

In 2004 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.

2004 Publications

Stamatakis, K., Ladas, N., and Papageorgiou, G.C. (accepted in 2004 - in press, 2005) Facilitated water transport in cyanobacterium *Synechococcus* sp PCC 7942 studied by means of phycobilisome-sensitized chlorophyll a fluorescence. Photosynthesis Res

2004 Presentations at International Scientific Conferences

K. Stamatakis, N. Ladas, G. C. Papageorgiou (2004). A model fluorometric assay of facilitated water transport in cyanobacteria. Photosynthesis and Post-genomic Era, 25-28 August 2004, Trois-Rivieres, Quebec, Canada.

Impact Factors (for 1 publication): 2,239

Citations 2004 (without self- citations): 4

Total Citations 2002-2004 (without self- citations): 16

PROGRAMME C:

STRUCTURAL AND COMPUTATIONAL BIOLOGY

Research Group: Theoritical Biology and Computational Genomics Group

Research Staff
Yannis Almirantis, Senior Researcher
Spyros papageorgiou, Emeritus Scientist
Christoforos Nikolaou, Graduate Student

Research Interests

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

- Deviations from randomness at the level of nucleotide n-tuplets.
- Long and short range correlations.
- Genome linguistics – DNA sequences seen as genomic text – Zipf's laws.
- Genomic evolution.

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

- Early development – Left-right asymmetries – Limb development.
- Reaction-diffusion systems – Spontaneous symmetry breakings.
- Prebiotic and early evolution as a complex self-organization procedure. .

2004 Findings

A. The distribution of n-tuplet frequencies is shown to strongly correlate with functionality when examining a genomic sequence in a reading-frame specific manner. The developed method applies a coarse-graining procedure, which is able to reveal aspects of triplet usage that are related to protein coding, while at the same time remaining species-independent, based on a simple summation of suitable triplet occurrences measures. These quantities are ratios of simple frequencies over suitable mononucleotide-frequency products promoting the incidence of the RNY motif, preferred in the most widely used codons. A significant distinction of coding and noncoding sequences is achieved.

B. Two recently formulated quantities, strongly correlated with the coding character of a sequence, have been applied as an additional "module" on GeneMark, in a three-criterial method. The developed combined algorithm is used to fractionalize a collection of GeneMark-predicted exons into sub-collections of different expectation to be coding. A further modification of the algorithm allows for the assignment of an improved estimation of the probability to be coding, to GeneMark-predicted exons. This is on the basis of a suitable training set of GeneMark-predicted exons of known functionality. The obtained algorithm is found to improve the efficiency of GeneMark in the task of assignment of functionality to unannotated genomic sequences.

C. A model was proposed explaining Hox gene collinearity along the primary and secondary axes of developing vertebrates. According to the model and in response to an extracellular morphogen gradient, physical forces act on the Hox cluster and translocate sequentially the genes from inside the chromatin territory toward the interchromatin domain where the genes are exposed to transcription factors for activation. The above mechanistic approach can successfully reproduce the existing data of spatial, temporal and quantitative collinearities at the initial stages of Hox gene activation. Some recent experiments verify the predicted sequential gene shifts.

Furthermore, it seems that attractive (Coulomb?) forces act on the 3' end of the Hox cluster and pull the genes out of the chromatin territory.

2004 Publications

C.Nikolaou & Y.Almirantis. (2004). Measuring the Coding Potential of Genomic Sequences through a Combination of Triplet Occurrence Patterns and RNY Preference. *Journal of Molecular evolution.* 59, 309-316.

Y.Almirantis & C.Nikolaou(2004). Multi-criterial coding sequence prediction. Combination of GeneMark with two novel, coding-character specific quantities.*Computers in Biology and Medicine. In Press, Available online.*

S.Papageorgiou. (2004). A cluster translocation model may explain the collinearity of Hox gene expressions. BioEssays 26, 189-195.

2003 Presentations at International Scientific Conferences

S. Papageorgiou. Tinkering or physical laws underlie Hox gene collinearity? British Society for Developmental Biology Annual Meeting, 14-16 March 2004. University of Warwick.

Impact Factors (for 3 publications): 13,109

Citations 2004 (without self- citations): 15

Total Citations 2002-2004 (without self- citations): 52

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

Structural, conformational and dynamic studies using NMR and other spectroscopic methods of compounds with pharmacological and biological interest as well as of their interaction with other bioactive molecules. Two types of compounds are mainly considered:

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

2004 Findings

Our research efforts in the field of Alzheimer's disease in which our team is actively involved since 2001 continue through diverse approaches. In 2004, the structural study of humanin, a peptide with neuroprotective action against the insults of Alzheimer's disease was completed and submitted to *Biochemical and Biochemical Research Communications* (Dimitra Benaki, Christos Zikos, Alexandra Evangelou, Evangelia Livaniou, Metaxia Vlassi, Emmanuel Mikros, Maria Pelecanou "Solution Structure of Humanin, a Peptide against Alzheimer's Disease-Related Neurotoxicity"). In the process of submitting is our study on the interaction of β-amyloid peptide (β-AP) of Alzheimer's disease with Congo red, an azo dye known to inhibit toxicity of β-AP in cultures of nervous cells *in vitro*. In parallel, in the effort to develop a radiodiagostic agent for *in vivo* imaging of Alzheimer's amyloid plaques, a series of complexes of oxorhenium and oxotechnetium have been synthesized and completely characterized based on the structure of the histological dye thioflavin T which possesses affinity for amyloid plaques. This study was the subject of the Master's of Science Dissertation S. Tzanopoulou submitted to the Division of Inorganic Chemistry and Technology of the University of Athens in July 2004. A publication on the same subject titled "Synthesis and characterization of novel oxorhenium(V) and oxotechnetium(V) complexes of 2-pyridin-2′-yl-benzothiazole towards the development of Alzheimer's disease radiodiagnostics" is being prepared.

In the area of complexes of technetium and rhenium as potential radiopharmaceuticals for diagnosis and therapy, the NMR structural study of the oxorhenium core with the decapeptide RGDSCRGDSY for imaging of metastatic melanoma was completed and appeared in *Inorganic Chemistry* (2004, **43,** 5598-5602). In addition, our study on complexes of Re(I) and Tc(I) with S-derivatives of cysteine was submitted to the same journal. S-derivatization of cysteine is considered a useful way for attaching a bioactive molecule with affinity for specific receptors on a metal chelator that will direct the radionuclide to the target tissue. Finally, in 2004 progress has been made in the NMR study of the structure of a number of derivatives of the neuropeptide bombesin and of their oxotechnetium complexes, for imaging of small cell lung carcinoma and breast cancer.

2004 Publications

Kostopoulos, B. Benaki, D., Pelecanou, M., Mikros, E. Stassinopoulou, C. I., Varvarigou, A. D., Archimandritis, S. C. (2004) Structural study by NMR of an oxorhenium-RGDdecapeptide complex for application in radiotherapy. Inorg. Chem. 43**,** 5598-5602

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" Biochemical and Biophysical Research Communications, in press

Constantinou, M. A., Papakonstantinou, E., Benaki, D., Spraul, M., Shulpis, K., Koupparis, M. A., Mikros, E. (2004) Application of NMR combined with principal component analysis in detecting inborn errors of metabolism using blood spots: a metabonomic approach. Analytica Chimica Acta 511, 303-312.

2004 Presentations at International Scientific Conferences

S. Tzanopoulou, G. Patsis, I. C. Pirmettis, M. Papadopoulos, M. Pelecanou (2004). Synthesis and characterization of oxorhenium(V) complexes of 2-pyridin-2'-yl-benzothiazole towards the development of Alzheimer's disease radiodiagnostics. 12th European Symposium on Radiopharmacy and Radiopharmaceuticals, September 9-12, Sopot, Poland.

D. Benaki, Ch. Zikos, H. Gourni, M. Pelecanou (2004). Characterization of an oxorhenium bombesin-derivative complex with NMR spectroscopy. 12th European Symposium on Radiopharmacy and Radiopharmaceuticals, September 9-12, Sopot, Poland.

Impact Factors (for 3 publications): 8

Citations 2004 (without self- citations): 12

Total Citations 2002-2004 (without self- citations): 47

Research Group: Protein Structure by Crystallography and Theoretical Modeling

Research Staff
Metaxia Vlassi, Senior Researcher
Maria Palaiomilitou, Postodoctoral Fellow
Athanassios Tartas, Graduate Student

Research Interests

Our current research activities are focused on 1) structural studies of protein-protein interaction modules (TPRs, BRCTs) that are involved in many important biological functions, 2) structure-based (rational) design of inhibitors of the enzyme GAPN of pathogenic bacteria and 3) elucidation of the role of specific residues in the function of a peptide (humanine) with neuroprotective activity against Altzheimer's desease. The approach we follow comprises a combination of 3D-Modelling and biophysical methods (x-ray Crystallography, Circular dichroism (CD).

2004 Findings

1. Structural studies of protein-protein interaction modules:
   - *TPRs*: In order to study the structure of TPR mediated protein-protein interactions we use as system-model the Ssn6 protein (see previous annual reports). In 2004, we over-expressed in *E.coli* a deletion mutant of Ssn6 comprising a TPR domain that we have previously predicted as structurally stable. We then purified the mutant and studied its structure and thermal stability using circular dichroism spectropolarimetry (CD) The CD spectra were subsequently compared with the CD spectra of previous Ssn6 mutants comprising various TPR lengths (see previous annual reports). Crystallization experiments of the mutant are in progress.
   - *BRCTs*: In order to study the structural and thermal stability of the BRCT protein-protein interaction module, we use as system-model the BRCT domain of human BRCA1 (BRCT-tan) protein that is related to familial breast/ovarian cancer. We have performed a structure-based estimation of thermodynamic parameters of the BRCT-tan region of BRCA1 based upon a 3D-model of a dimeric form of the protein that we have constructed. Comparison of the theoretic parameters with those obtained experimentally by Differential Scanning Calorimetry (DSC) (from the group of G. Nounenis, IRRP), leads to the identification of a region of the BRCT-tan molecule that is essential for its thermodynamic stability. This work is in press in Biophysical Chemistry (see publications 2004).
2. Concerning the structure-based (rational) design of inhibitors of the non-phosphorylating glyceraldehyde–3-phosphate dehydrogenase (GAPN), we have previously modeled the 3D structure of two GAPNs from pathogenic bacteria identified by prof. Soukri's group (Hassan II University, Morocco). During 2004, we modeled the structure of both GAPNs in the presence of their cofactor and substrate. The 3D-models allowed us to analyze the specific interactions of these molecules with the enzyme. Based on the 3D-models of the complexes we designed analogues as potential inhibitors of GAPNs. Energy minimization and molecular dynamics simulations *in silico* of the above GAPN complexes are in progress. This work is funded by GSRT (02-PRAXE-197).
3. In the framework of intra-institutional collaboration with M. Pelecanou (NMR group) in the field of Alzheimer disease (AD), we have previously produced a theoretical 3D-model of the peptide humanine which shows a neuroprotective function against the disease. The 3D-model was in perfect agreement with the NMR data in aqueous solution. During 2004, we used the

3D model in order to elucidate the role of specific humanine residues, mutations of which are known to alter its neuroprotective function against AD. This work is submitted for publication to Biochem. Biophys. Res. Comm [Benaki, D., Zikos, C., Livaniou, E., Vlassi, M., Mikros, E., Pelecanou, M. Solution structure of humanin, a peptide against Alzheime's disease-related neurotoxicity. Submitted to BBRC].

2004 Publications

Pyrpassopoulos, S, Ladopoulou, A., Vlassi, M., Papanikolau, Y., Vorgias, C.E., Yannoukaκos, D. and Nounesis, G (2004). Thermal denaturation of the BRCT tandem repeat region of human tumour suppressor gene product BRCA1. In press in Biophys. Chem. 114.

Benaki, D., Zikos, C., Evangelou, A., Livaniou, E., Vlassi, M., Mikros, E., Pelecanou, M. Solution Structure of Humanin, a Peptide against Alzheimer's Disease-Related Neurotoxicity. *Biochemical and Biophysical Research Communications*, in press.

2004 Presentations at International Scientific Conferences

M. Palaiomylitou, A. Tartas, G. Kefala, N. Gounalaki, D. Tzamarias, M. Vlassi (2004) Circular Dichroism studies on the SSN6/TUP1 complex: Indications for a conformational change. 29th Congress of the Federation of European Biochemical Societies (FEBS Meeting), June 2004, Warsaw, Poland.

A. Kosmopoulou, A. Staurakoudis, M. Vlassi, M. Sakarellos-Daitsiotis, Constantinos Sakarellos (2004). Detection of T-cell epitopes and modeling of DQ2 and DQ7 molecules using computational methods. 12th International Conference on Intelligent systems for Molecular Biology & 3rd European Conference on Computational Biology, July 31-August 4, 2004, Glasgow, Scotland.

Impact Factors (for 2 publications): 4,628

Citations 2004 (without self- citations): 13

Total Citations 2002-2004 (without self- citations): 51

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 2004

The numbers of the various types of grafts, which were produced and delivered to Hospitals, during 2004, are listed in the following table.

GRAFTS	DELIVERY
Cancellous Bone	622
Cortical bone	2
Mixed bone	1
Dura mater	144
Cranium bone	17
Collaborations	28

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 2004, the Animal Facility made available the following animals:

Users	***Rats***	***Mice***	***Rabbits***
Institute of Biology	34	13	9
Institute of Radioisotopes & Radiodiagnostics	80	380	2
University of Athens – Dpt Biology	347	26	
University of Thessaloniki		40	
"ELPEN" Pharmaceuticals	220		
IIBEAA		125	
Doctors (2)	53		
Total of animals provided	**734**	**584**	**11**

In addition, there are constantly in the Facility experimental animals of various age and weight, according to demand from users, and there exist animals in stock for reproduction, colony renewal and programming, and response to immediate needs.

The personnel of the Facility helped with animal maintainance, and performed the required immunizations and blood sampling.

In addition, there is ongoing research collaboration with a team of external scientists/physicians, headed by Prof. Tsigganos.

LASER CONFOCAL MICROSCOPY

Research Staff

Marina Sagnou, Technical Specialist

Description

The current Unit activities include:

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

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

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

2004 Findings

During the year 2004, 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, Associate Research Scientist
Maria Pelecanou, Associate Research Scientist
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.

20034Findings

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 2004, 14 scientists were trained at the postdoctoral level at our Institute. Furthermore, 26 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 2004, three of our graduate students finished their thesis work and became PhDs.

Moreover, 35 students from the University are carrying out their pre-graduate project thesis work at the Institute. Additionally, 8 students from Universities abroad did practical lab training in laboratories at the Biology Institute as required by their corresponding Universities abroad. Also opportunity was given for students from Greek Universities join the Summer Training Programme to work in labs of the IB and four students from Greek Universities participated.

In the framework of Graduate Programme, during the year 2004 the Biology Institute organized two courses 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:

- *Chromatine structure and regulation of exression* [course lecturers: L. Swevers, V. Sophianopoulou (course coordinator), A. Prombona, and K. Stamatakis].
- *Structural Biology and Theoretical Approaches* [course lecturers: M.Vlassi (course coordinator), Y.Almirantis, and M.Pelekanou].

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)
- *Cell cultures – Tissue cultures* (**Dr. D. Kletsas**, Department of Biology, University of Athens).
- *General Biology* (**Dr. S.Piperakis**, School of Humanities, University of Thessalia)
- *Cell Cycle: Checkpoints and Consequences for Physiological Cell Function* (**Dr. K. Sekeri** and **Dr. Th. Sourlinga** Department of Biology, University of Athens).
- *Matrix-mediated signal transduction: The role of proteoglycans, growth factor and integrin receptors* (**Dr. E. Tsilibari**, Department of Biology, University of Athens)
- *Pathobiology of Pancreatitis and Diabetes Mellitus* (**Dr. E. Tsilibari**, Department of Biology, University of Athens)
- *Insect molecular biology and insect pest control* (**Prof. K. Iatrou**, Department of Biology, University of Athens)
- *The example of the use of fungi in cloning and studying the role of genes from microbial pathogens, plants and human species* (**Dr. V. Sophianopoulou**, Department of Biology, University of Athens)

- *Teaching of the course "Introduction to computational biology" of the "Bioinformatics Postgraduate Programme"*, **(Dr. I. Almyrantis,** Department of Cell Biology and Biophysics, Faculty of Biology, University of Athens)
- *Teaching in the framework of the postgraduate courses: "Bioinformatics" and "Clinical Bichemistry & Molecular Diagnostics" (***Dr. M. Vlassi**, Department of Biology, University of Athens)

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 (2004) 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 2004

GRADUATE STUDENT	TITLE OF DOCTORAL THESIS	ADVISOR (in Institute of Biology)	UNIVERSITY
Eleftheria Argyrou	Expression and structure-function analysis of nucleobase transporters: the filamentous fungus *Aspergillus nidulans* as model system.	Vassiliki Sophianopoulou	Biology Dpt., University of Athens
Athanasios Kaldis	Study of the *Lhcb* genes' expression that code for the LHCII apoproteins of the light-harvesting complex of photosystem II as influenced by endogenous and exogenous factors.	Anastasia Prombona	Biology Dpt., University of Athens
Aikaterini Kypreou	The role of histones during the *in vitro* ageing and apoptosis of lymphocytes	Kalliope Sekeri	Biology Dpt., University of Athens

SEMINAR PROGRAMME 2004

DATE	SPEAKER	TITLE
8/1/04	**L. Leodiadis** Institute of Biology NCSR "Demokritos"	The GBeta5 subunit that associates with the R7 subfamily of RGS proteins regulates mu-opioid effects
8/1/04	**M. Konti** Institute of Biology NCSR "Demokritos"	Targeted degradation of TOC1 by ZTL modulates circadian function in *Arabidopsis thaliana*
15/1/04	**A. Talamagas** Institute of Biology NCSR "Demokritos	Platelet-derived growth factor induces the beta-gamma-secretase-mediated cleavage of Alzheimer's amyloid precursor protein through a Src-Rac-dependent pathway.
15/1/04	**Th. Georgomanolis** Institute of Biology NCSR "Demokritos"	The Focal Adhesion Protein Vinexin α Regulates the Phosphorylation and Activity of Estrogen Receptor α
22/1/04	**E. Mavrogonatou** Institute of Biology NCSR "Demokritos	Calcium induces cell survival and proliferation via the activation of MAPK in the human hormone-dependent leukemia cell line TF-1
29/1/04	**M. Xedous** Institute of Biology NCSR "Demokritos	Hypertension and prolonged vasoconstrictor signaling in RGS2 – deficient mice
29/1/04	**V. Nikas** Institute of Biology NCSR "Demokritos	Progesterone crosstalks with insulin-like growth factor signaling in breast cancer cells via induction of insulin receptor substrate -2
5/2/04	**D.Bouzarelou** Institute of Biology NCSR "Demokritos"	Riboswitches control fundamental biochemical pathways in Bacillus subtilis and other bacteria.
5/2/04	**N. Sdralia** Institute of Biology NCSR "Demokritos"	GATA-1-dependent transcriptional repression of GATA-2 via disruption of positive autoregulation and domain-wide chromatin remodeling
12/3/04	**Prof. I Nikolis** University of Patras	Structure and dynamics of complex systems
15/3/04	**Dr. K. Charalambidis** University of Athens	The molecular basis of the antimicrobial, plant protecting function of Avenacins
22/3/04	**Prof. N. Mantzaris** Rice University	BioSystems Engineering: Designing single-cell genetic architecture for controlling cell population phenotypes
16/4/04	**Prof. Z. Vogel** Weitzmann Institute of Science	Regulation of Adenylyl Cyclase by Acute and Chronic Exposure to Opiates and Cannabinoids
19/4/04	**Prof. G. Manetas** University of Patras	The adaptive significance of leaf anthocyanins: why some leaves transiently form anthocyanins and why these anthocyanins are always red
28/4/04	**P. Venieratos** Institute of Biology NCSR "Demokritos	Effect of high glucose concentration on insulin signaling pathway in cultures of mouse pancreatic beta cells
28/4/04	**M. Billini** Institute of Biology NCSR "Demokrito"s	Study of Na+/H+ antiporters in the freshwater cyanobacterium *Synechococcus sp.* PCC 7942
31/4/04	**I. Tsagaraki**	Osteoblasts: apoptotic-antiapoptotic mechanisms

	Institute of Biology NCSR "Demokritos"	in the presence of cytokines
5/5/04	**D.Bouzarelou** Institute of Biology NCSR "Demokritos"	Cloning and molecular characterization of a gene similar to expansin genes in *Aspergillus nidulans*
12/5/04	**M. Konti** Institute of Biology NCSR "Demokritos	Molecular mechanisms of the biological clock function in *Phaseolus vulgaris*
19/5/04	**C. Nikolaou** Institute of Biology NCSR "Demokritos"	A study on the usage of oligonuclotide "words" in genomic sequences
26/5/04	**Th. Georgomanolis** Institute of Biology NCSR "Demokritos"	Molecular and functional characterization of the BmSH3 of *Bombyx mori*
26/5/04	**V. Nikas** Institute of Biology NCSR "Demokritos"	Deactivation of molecular targets involved in signaling pathways and in regulation of apoptosis: Towards a targeted therapy of epithelial ovarian cancer.
2/6/04	**A. Talamagkas** Institute of Biology NCSR "Demokritos"	Putative function of matrix metalloproteinaseBb (MMP-9) as APP a-secretase
9/6/04	**Z. Erpapazoglou** Institute of Biology NCSR "Demokritos"	Study of the topogenesis mechanism of transmembrane transporters in *Aspergillus nidulans*
15/6/04	**Dr. A. Chroni** Boston University	The role of apolipoprotein A-I in the biogenesis and biological effects of HDL
16/6/04	**I. Karakatsanis** Institute of Biology NCSR "Demokritos"	Response of fetal and adult human fibroblasts to amniotic fluid and TGF
16/6/03	**E. Mavrogonatou** Institute of Biology NCSR "Demokritos"	Response to stress and senescence of intervertebral disc cells
18/6/04	**Dr. E. Stratikos** Boston University	Kinetic traps in protein-protein interactions: Examples from inhibition of proteases and presentation of antigenic peptides
23/6/04	**L. Leodiadis** Institute of Biology NCSR "Demokritos"	Functional interactions of the opioid receptors with various proteins
23/6/04	**T. Tzanopoulou** Institute of Biology NCSR "Demokritos"	Oxorhenium and oxotechnetium complexes of thioflavin T derivatives for radiodiagnosis of Alzheimer's disease
28/6/04	**Dr. A. Tsadili** Institute of Biology NCSR "Demokritos"	Σχεδιασμός ανοσφαιρινών τροποποιημένων με Γενετική Μηχανική: Μελέτη της συγγένειας και Ειδικότητάς τους
7/7/04	**N. Sdralia** Institute of Biology NCSR "Demokritos	Identification and Characterization of Proteins that Interact with the Transcription Factor BmGATAb, which is Expressed at Specific Stages during Oogenesis in the Silkmoth *Bombyx mori*
12/7/04	**Dr. P. Kitsiou** Institute of Biology	Effect of glucose concentration on cells: A sweet result?

	NCSR “Demokritos”	
24/9/04	**Prof. G. Chaconas** University of Calgary, Canada	The unusual genome structure and replication mechanism of Borrelia burgdorfesis, the Lyme disease spirochaete: potential therapeutic targets and strategies for control
1/12/04	**V. Nikas** Institute of Biology NCSR “Demokritos”	A new form of constitutively active farnesylated Akt1 supresses anoikis and chemotherapy induced apoptosis.
1/12/04	**M. Xedous** Institute of Biology NCSR “Demokritos”	Viruses and Schizophrenia: a focus on Herpes Simplex Virus
9/12/04	**C. Nikolaou** Institute of Biology NCSR “Demokritos "	Practicing oblivion: The chemical accident in Bhopal and the reaction by the scientific community
9/12/04	**Th. Georgomanolis** Institute of Biology NCSR “Demokritos”	Axin and the Axin/Arrow-binding protein DCAP mediate glucose–glycogen metabolism
16/12/04	**I. Tsagaraki** Institute of Biology NCSR “Demokritos”	c-jun N-terminal kinase is required for metalloproteinase expression and joint destruction in inflammatory arthritis
16/12/04	**E. Mavrogonatou** Institute of Biology NCSR “Demokritos”	p38 MAPK determines the common signaling cascade leading to cell senescence

COLLECTIVE DATA

FINANCIAL REPORT 2004

1. Internal Funding from the Special Account Department

Income	**E u r o**
Carried over from 2003	100.116
Funding from NCSR "D"	50.000
Matching Funds	67.597
Income from services	5.569
Donations from companies	800
Transfer from other sources	5.064
TOTAL	**229.146**

Expenses	
Equipment	16.303
Supplies	30.854
Salaries	17.176
Travels	2.193
Supplies from "Demokritos"	8.900
Other expenses	27.277
Committed	119.438
TOTAL	**148.160**

2. Governmental Funding

	E u r o
Equipment maintenance	420
Animal chow	4.485
Xeroxing and similar supplies	420
TOTAL	**5.325**

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

SOURCE OF FUNDING (number of programmes)	**FUNDING (in EUROS)**			
	Programme A	**Programme B**	**Programme C**	**INSTITUTE**
European Union (4)	128.000	102.650	-	230.650
General Secretariat for Research & Technology (15)	155.486	109.732	23.584	288.802
Novartis Hellas SA (1)	8.860	-	-	8.860
National Bank of Greece (1)	-	-	1.455	1.455
TOTAL	**292.346**	**212.382**	**25.039**	**529.767**

COLLECTIVE DATA ON PRODUCTIVITY OF SCIENTIFIC PROGRAMMES

	PROGRAMME			
	A	**B**	**C**	**INSTITUTE**
Researchers and Senior Research Specialists	8	6	3	18**
Technical Specialist	1	1	2	5*
Collaborating Research Scientists & Emeritus Scientists	4	3	1	8
Postdoctoral Fellows	5	7	2	14
Graduate Students	13	8	3	26$
Collaborating Graduate Students & Visiting Students	8	3	-	11
Graduate Research Associates	1	3	-	4
Undergraduate Students	23	8	2	33
Research Technicians	8 #	4	-	15 @
Administrative Staff	-	-	-	3
Total Personnel	**68#**	**44**	**13**	**134**
Publications in Peer-Reviewed Journals	**35+**	**11+**	**8**	**53**
Publications (Average) in Peer-Reviewed Journals per Scientist	4.37	1.83	2.66	2.94
Cumulative Impact Factor in Peer-Reviewed Journals (number of publications)	**97.292 (35)**	**37.337 (11)**	**25.737 (8)**	**160.366 (53)**
Average Impact Factor in Peer-Reviewed Journals	2.779	3.394	3.217	3.025
Cumulative Impact factor per Scientist	12.161	6.222	8.579	9.433
Proceedings to International Conferences	**10**	**8**	**2**	**20**
Proceedings (Average) per Scientist	1.25	1.33	0.66	1.17
Total International Publications	**45**	**19**	**10**	**74**
International Publications (Average) per Scientist	5.625	3.166	3.333	4.352
Publications to Greek Journals, Books or Proceedings	1	1	-	2
Total Publications	**46**	**20**	**10**	**76**
Publications (Average) per Scientist	5.75	3.33	3.33	4.470
International Patents	**1**	**-**	**-**	**1**
Greek Patents	**-**	**-**	**-**	**1**
Presentations to International Conferences	**31**	**5**	**6**	**42**
Presentations (Average) per Scientist to International Conferences	3.875	0.833	2	2.470
Presentations to Greek Conferences	**31**	**5**	**7**	**43**
Presentations (Average) per Scientist to Greek Conferences	3.875	0.833	2.33	2.529
Total Presentations to Conferences	**62**	**10**	**13**	**85**
Presentations (Average) per Scientist to Conferences	7.75	1.66	4.33	5

*** 1 Senior Research Specialist, who is recently retired, is included*

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

$ 2 Graduate Students who are in rotation are included

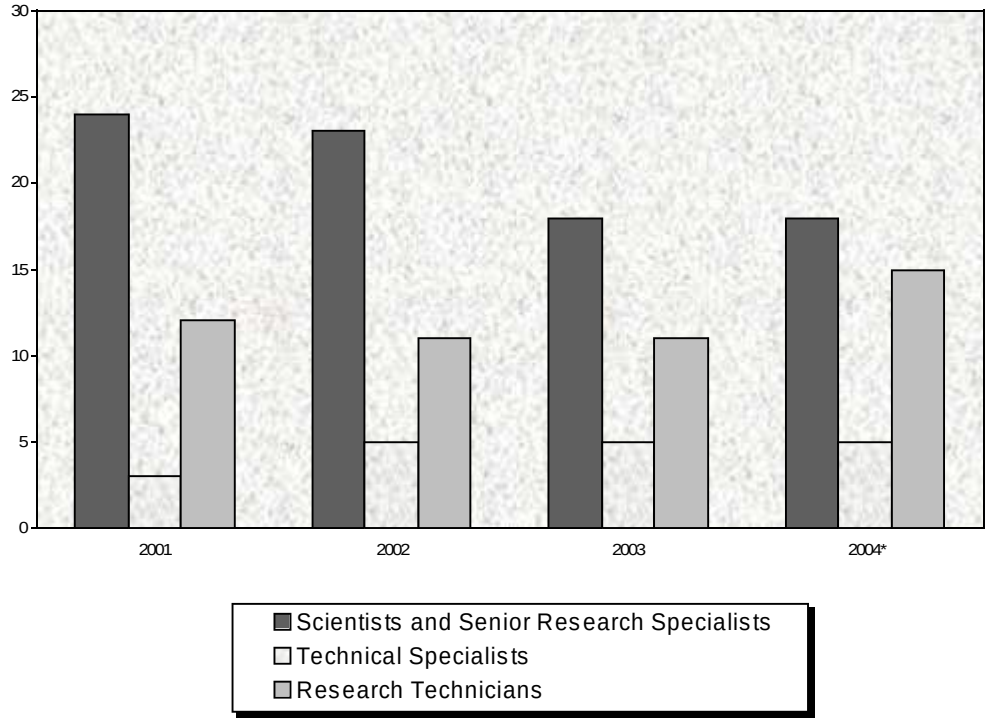
#1 Research Technician who is occupied in other programme also is included

@ 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

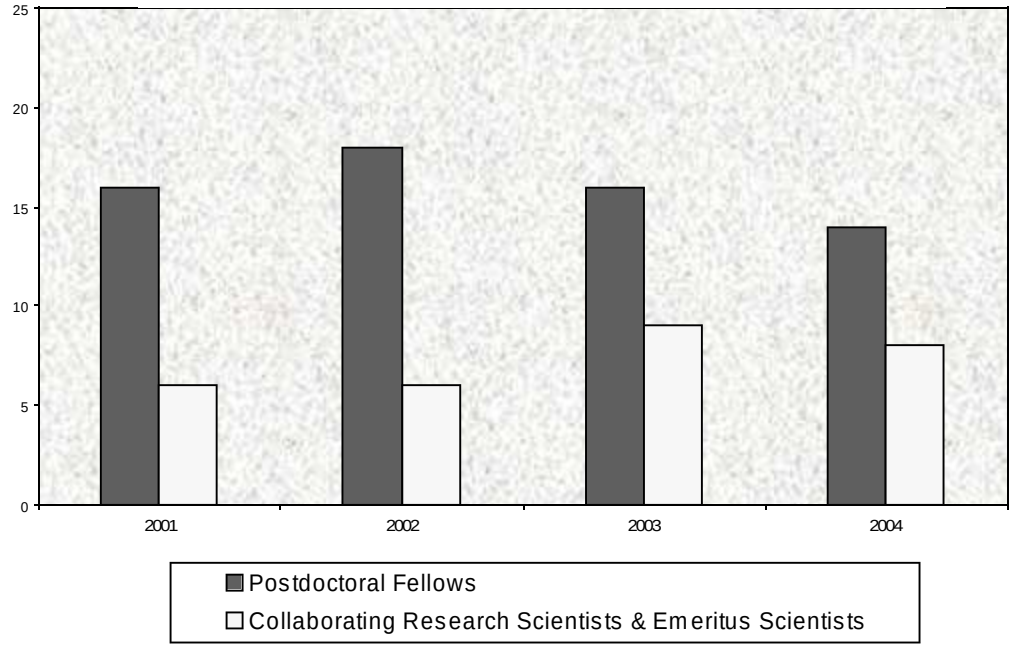
CHANGES OF IB STAFF
DURING 2001-2004

"TENURED EMPLOYEES"

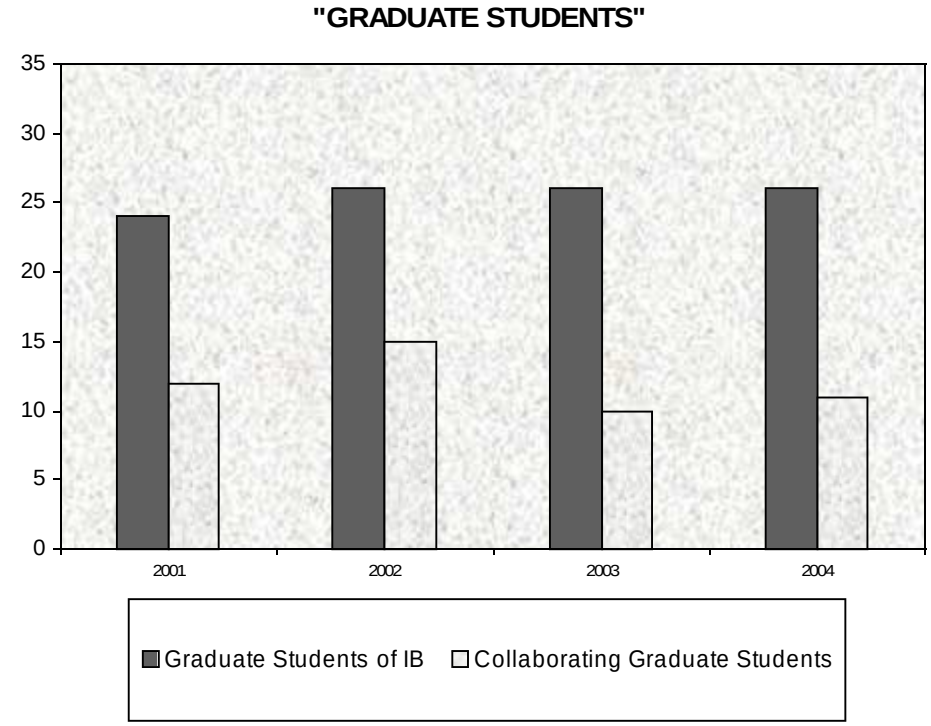


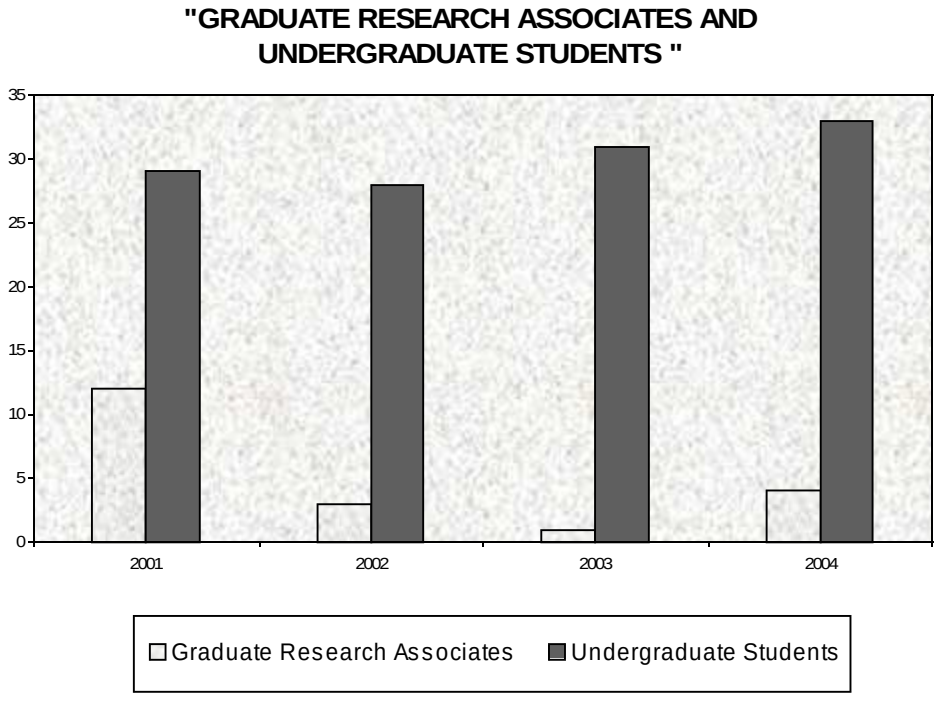
* During 2004, 4 Research Technicinas are simbasiouxoi

"POSTDOCTORAL FELLOWS, COLLABORATING RESEARCH SCIENTISTS & EMERITUS SCIENTISTS"



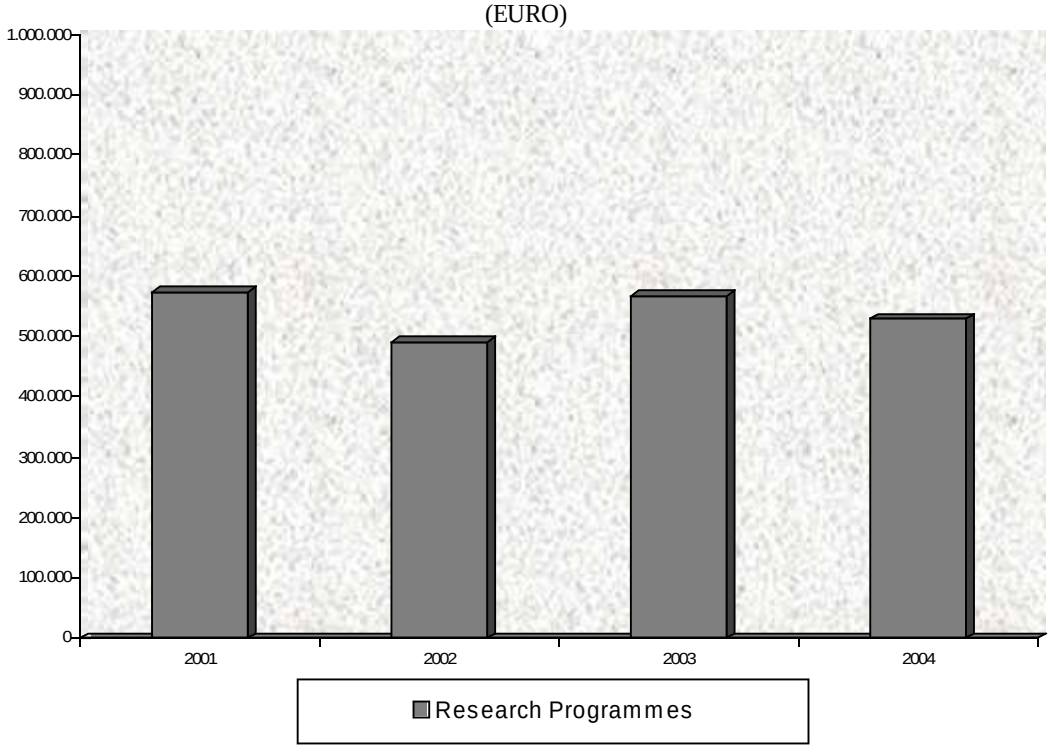
CHANGES OF IB STAFF
DURING 2001-2004





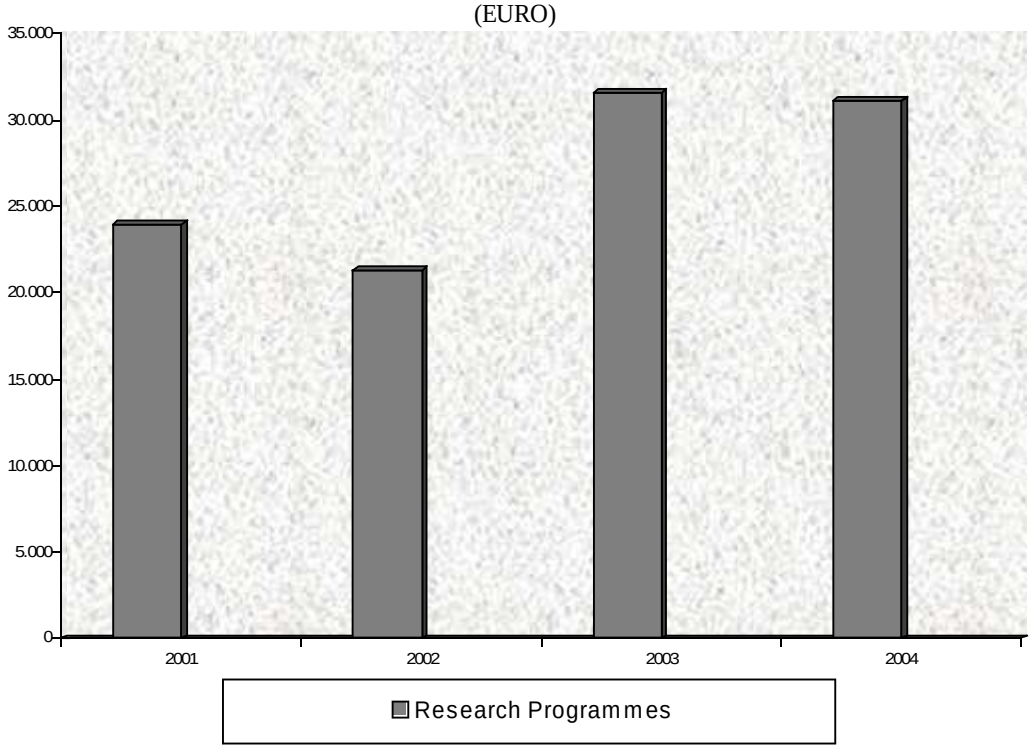
CUMULATIVE EXTERNAL FUNDING OF THE INSTITUTE DURING 2001-2004

(EURO)

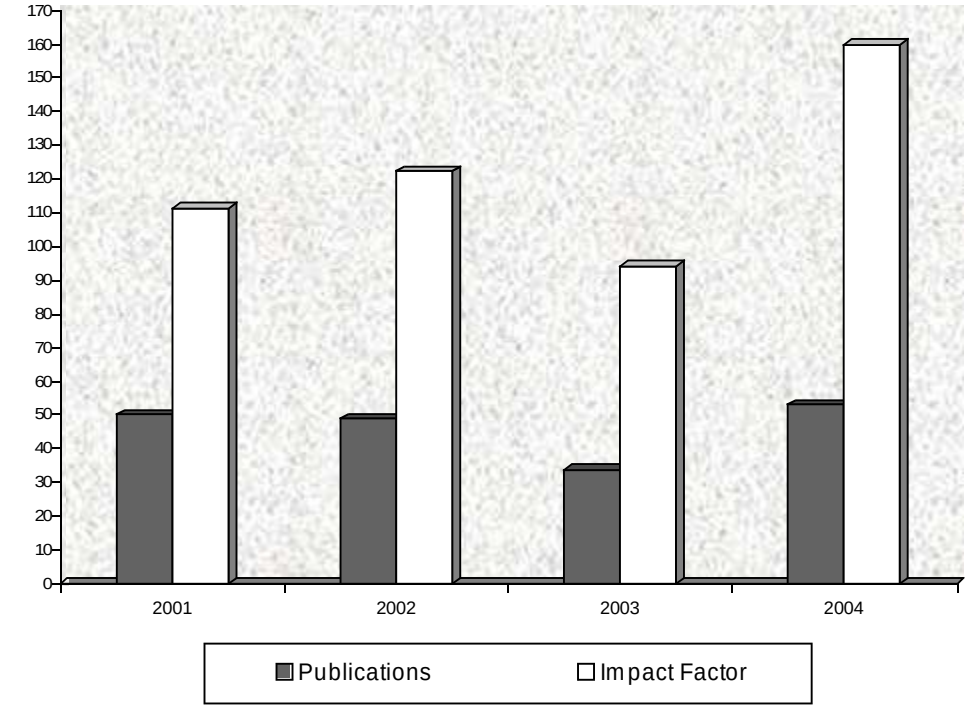


EXTERNAL FUNDING OF THE INSTITUTE PER SCIENTIST DURING 2001 - 2004

(EURO)



PUBLICATIONS IN PEER-REVIEWED JOURNALS AND CUMULATIVE IMPACT FACTOR DURING 2001-2004



PUBLICATIONS IN PEER-REVIEWED JOURNAL AND AVERAGE IMPACT FACTOR PER SCIENTIST DURING 2001-2004