INSTITUTE OF
BIOLOGY

NATIONAL CENTRE FOR SCIENTIFIC RESEARCH "DEMOKRITOS"

INSTITUTE OF BIOLOGY

2003
ANNUAL REPORT

INSTITUTE OF BIOLOGY

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P E R S O N N E L

INSTITUTE OF BIOLOGY

DIRECTOR

Iatrou Kostas	Professor of Biochemistry and Molecular Biology (1-6/2003)

ACTING DIRECTOR

Sekeri Kalliope	Biochemist (6-12/2003)

DIRECTOR/ELECT

Tsilibary Effie	MD, Cell Biologist (12/2003)

SCIENTIFIC STAFF

Research Directors

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

Senior Researchers

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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
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Tsolomiti-Gourgou Areti
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ADMINISTRATIVE STAFF

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Douvaras Panagiotis	Accountant
Papadaki Margarita	Secretary

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Mazomenos Vassilios (Dr. Chemical Ecologist)	Lab of Chemical Ecology and Natural Products
Merkouris Emmanouel (Dr. Chemist)	Georgoussi I.
Papageorgiou George (Dr. Biochemist)	Stamatakis K.
Papageorgiou Spyros (Dr. Physicist)	Almirantis I.
Sideris Eleftherios (Dr. Geneticist)	Sophianopoulou V.
Stathakos Dimitrios (Dr. Biochemist)	Kletsas D.
Sourlingas Thomae (Dr. Biologist)	Sekeri K.
Visvardis Evaggelos (Dr. Biologist)	Sophianopoulou V.

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Argyrou Eleftheria	Iatrou K. *(until 4/2003)* – Sophianopoulou V. (*from 5/2003*)
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Benaki Dimitra	Pelekanou M.
Douris Vassilios	Iatrou K.
Drossopoulou Garifallia	Tsilibari E.
Kafasla Panagiota	Sophianopoulou V.
Kenoutis Christos	Iatrou K.
Kitsiou Paraskevi	Tsilibary E.
Massas Ioannis	Skarlou V.
Mylonas Panagiotis	Lab of Chemical Ecology and Natural Products

Palaiomilitou Maria	Vlassi M.
Pratsinis Haris	Kletsas D.
Prinarakis Efthimios	Iatrou K.
Tsapali Dimitra	Sekeri K.
Zervolea Irene	Kletsas D.

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Handris Panagiotis	Kletsas D.
Kaldis Athanassios-Dimitrios	Prombona A. – **PhD obtained in 2003**
Karakatsanis Ioannis	Kletsas D.
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Lallas George	Havredaki M. *(retired)* – **PhD obtained in 2003**
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Mazarakou Georgia	Georgoussi I.
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Nikas Vassilios	*Rotation*
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Sdralia Konstantia	Iatrou K.
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Tzanopoulou |Stamatia	Pelekanou M.
Vieneratos Panagiotis	Tsilibari E.

GRADUATE RESEARCH ASSOCIATES

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COLLABORATING GRADUATE STUDENTS

Student (University)	**Supervisor**
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Fanourakis Galenos (Univ. of Athens)	Voutsinas G.
Fragopoulos Evaggelos (Univ. of Rome)	Tsilibari E.
Goudopoulou Athina (Univ. of Athens)	Voutsinas G.
Metaxatou Aghelina (Univ. of Eagean)	Iatrou K.
Moutzouris Dimitrios (Univ.of Athens)	Tsilibari E.

Papachristos Konstantinos (Univ. of Birmigham)	Georgoussi I.
Siskos Elias (Univ. of Cardiff)	Lab of Chemical Ecology and Natural Products
Spyrou Natalia (Univ. of Cardiff)	Voutsinas G.
Zacharioudakis Stilianos (Univ. of Athens)	Tsiropoulos G.

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Student (University)	**Supervisor**
Anagnostakis Nikos (Univ. of Athens)	Piperakis S.
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Beloukas Apostolos (Univ. of Athens	Prombona A.
Billini Maria (Univ. of Athens)	Sophianopoulou V. *(until 3/2003)*
Christopoulos George (Univ. of Athens)	Piperakis S.
Costa Sofia (Univ. of Lisbon)	Iatrou K.
Giakountis Antonios (Univ. of Thessaly)	Prombona A.
Kaloidas Konstantinos (Univ. of Larissa)	Tsilibari E.
Kanavetas Panagiotis (Univ. of Athens)	Piperakis S.
Karambelas Theodore (Univ. of Aberdeen)	Kletsas D.
Karabinas Theodoros (Univ. of Modena)	Prombona A.
Karanastassi Georgia (Univ. of Athens)	Piperakis S.
Karnavas Theodoros (Univ. of Athens)	Prombona A.
Kontogianni Nadia (Univ. of Athens)	Piperakis S.
Kontou Maria (Univ. of Ioannina)	Tsilibari E.
Lousa Diana (Univ. of Lisbon)	Sophianopoulou V.
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Maridaki Kiriaki (Univ. of Athens)	Piperakis S.
Melachrinou Sofia (Univ. of Athens)	Voutsinas G.
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Paparidou Olga (Univ. of Athens)	Voutsinas G.
Papaspiridakos Stefanos (Univ. of Athens)	Voutsinas G.
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Salpea Paraskevi (Univ. of Athens)	Sekeri K.
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Xedous Marios (Univ. of Athens)	Georgoussi I.

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 NCSR 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.
4. 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 of Technology.

Through 2003, the research directions of IB included three major Programs:

A. Biochemistry, Cellular and Molecular Biology
B. Structural Biology
C. Environmental Biology: the last researchers of this program have retired, therefore the IB programs are under reconsideration.

Through 2003, the main research themes have centered in the following fields:

1. Cellular functions in normal and pathological conditions
2. Structure of Biological and Bioactive Molecules
3. Biotechnology and Natural Products

As a large body of researchers have retired during the last 3 years, concomitant with the pending hiring of three new researchers in 2004, the targets for research and development of the IB will be re-evaluated. The new targets will be related to the interests of remaining active researchers, aiming at competitive research performance in fields reflecting the needs of the Hellenic and European societies.

One additional target of the IB is to provide services to society through the development of new knowledge, i.e. findings of basic research and also research aiming at development and interconnection with pharmaceutical companies. In this context, the IB historically maintains two units of services:

a) The Experimental Animal Colony, covering the needs of researchers from NCSR Demokritos and other Research and Scientific Institutions throughout Attica, as well as pharmaceutical companies and physicians, depending upon their needs. A detailed accounting of services provided is included in the related part of this progress report.

b) The Bank of tissue transplants, which makes available dry tissues, such as membranes (i.e. meninges), bone parts, etc. to Hospitals throughout Attica and Greece, to be used as transplants. The tissues available are described in the related part of this progress report. Recently, we have embarked in an effort to evaluate the possibilities of upgrading this facility, which is unique for Greece (availability of dry tissues for transplants), so it can become more efficient and productive.

During 2003 two novel Service Units were established:

c) Confocal Microscopy Unit, which renders services and help with the practice of related techniques to researchers of the IB and the general area of Attica who need to use this

facility, run by an expert Assistant Researcher (PhD). The aim is to make this technique available to scientists with related interests.

d) Structural Biology Unit, which, in collaboration with other Institutes of NCSR Demokritos is a unique center for Greece, with NMR spectrometers, area diffractometer for crystallographic analysis of small molecules and proteins as well, and equipment for micro-calorimetry. Scientists of this unit are members of a Hellenic Network of Structural Biologists and assist research colleagues with related interests.

Research activities of the IB were supported during 2003 from governmental funds allocated to NCSR Demokritos, as well as competitive programs from the EEC, the Hellenic Secretariat for Research and Development ("GGET") of the Ministry of Development, and other public and private sources. Additionally, three researchers of the IB were awarded grants of the type "PRAXE" aiming at the development of applicable research results. It is therefore evident that the IB nurtures the development of research and technology, to better service society in Greece, Europe and internationally.

During 2003, Dr. G. Tsiropoulos has retired, after a loyal service of many years, for the contributions of whom the personnel of the IB express their gratitude. With the retirement of Dr. Tsiropoulos (the last of the family of researchers in the field of Environmental Biology), the cycle of this research reached completion and subsided, only to be replaced by other, cutting-edge research directions representative of the remaining researchers and those who will be hired in 2004.

Special notion should be given to retired, collaborating researchers, still actively participating and continually contributing to the IB with peer-reviewed publications (Drs. L. Ignatiadou, V. Mazomenos, G. Papageorgiou, S. Papageorgiou, E. Sideris, D. Stathakos, H. Stassinopoulou) and funded research programs (Drs. V. Mazomenos, E. Sideris). The participation of all these researchers in ongoing research programs of the IB is inspirational and a role model model for new researchers. The Director of the IB extends her deepest gratitude and warm congratulations and wishes to these colleagues, to continue their extremely productive presence: Albeit typically retired, these colleagues are in essence active participants, thus representing a frontier continuation of a never ending effort to create new knowledge.

The Director of the IB also expresses her gratitude to Prof. K. Iatrou, the Director of the IB through 12/6/03 who during the last five years, worked hard and decisively in order to push this Institute forward, and change it according to his aspirations. Several of his achievements are mentioned here as examples of his contribution: 1) The establishment of a yearly, two-day retreat, which updates all researchers of the IB on recent developments and the evolution of different research teams: a retreat which is dear to almost everyone in this Institute, 2) The renewal of the series of graduate courses of the IB to "core courses", 3) the continuation of the memorable Summer School, which took place twice during his tenure, 4) the establishment of a series of graduate student seminars, which promoted critical evaluation of recent scientific literature, and the confidence of graduate students in their scientific presentations, 5) the effort to create inter-institutional collaborations and help the coming-together and uniting forces of researchers from different fields, by the additional funding of five bilateral collaborations during 2003. The first year of this experiment was completed, and participating investigators have been remarkably successful for the most part, thus mandating the continuation and extension of scientific inter-institutional collaborations.

Warm gratitude is also extended to the Acting Director Dr. K. Sekeri, who during her tenure worked hard and devoted a large amount of effort to solve the numerous problems that came up with objectiveness, reliability and cool wisdom: Her selfless attitude greatly accounted for the smooth operation of the IB, so that researchers could perform their tasks in agreement and without disruptions.

Finally, the contribution of the Research Advisory Council cannot be neglected: The members of this committee have continually contributed endless work, as well as extremely valuable input, advice, and opinion on important matters of the IB, and are a lively link between the Director and the body of researchers.

After all this, we finally came to the subject of the new Director of the IB, inaugurated in January 2004, who is committed to do her very best-and beyond that- to serve the existing goals of the IB and those that will arise along the way. I wish to sincerely thank all researchers for their trust in my person, their willingness to participate in endless committees aiming at our development, and their continuous efforts and contribution in matters concerning this Institute. As I mentioned to all of you and strongly believe, further achievements of our institute towards excellence is a common matter and can only be achieved with agreement and continuously escalating, shared efforts and service for the common goals, sometimes against or despite our own personal goals. We all have to bend over our problems with understanding and strict self-criticism, so we can make an accurate diagnosis of our weak and strong points, and together move towards a better future of this place and in general. We should not forget that despite many shortcomings, the government has endowed us with the responsibility to make important decisions and move towards better perspectives. It is sometimes extremely hard to abide to this goal of continuous evolution and development, because of the existing limitations. Nevertheless, this can be achieved with common effort and persistence. I am leaning to each one and all of you for the development of newer, better and harder-to-achieve goals, for a better IB with internationally recognised researchers, with important contributions and competitive research levels. Our progress will also contribute to recognition of NCSR Demokritos as a Center with undoubted, high-quality presence in response to the requirements of Research and Technology of our era. Good luck to all of us.

Effie C. Tsilibary, MD, PhD
Director of IB
February 2004

INSTITUTE OF
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A. BIOCHEMISTRY, CELL AND MOLECULAR BIOLOGY

Research Group: Theoretical Biology and Computational Genomics Group

Research Staff

Yannis Almirantis, Associate Research Scientist
Spyros papageorgiou, Collaborating Research 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.

2003 Findings

It is shown that, when examining a genomic sequence, the distribution of n-tuplet frequencies correlates with its functionality, whether this is done in a reading-frame specific or independent manner. The approaches adopted, apply a coarse-graining procedure, which is able to reveal aspects of n-tuplet usage that are related to the sequence's functionality, while at the same time remaining species-independent. They are based on a simple summation of n-tuplet frequencies after filtering the background nucleotide composition with suitable manipulations. Moreover, when examining triplets in a reading-frame specific mode, a significant distinction of coding and noncoding sequences is achieved, leading to the conclusion that the n-tuplet usage may be indicative of the sequence's coding potential.

In another work, we have applied two recently formulated quantities, strongly correlated with the coding character of a sequence, as an additional "module" on GeneMark, in a three-criterial method. The difference in the statistical approaches implicated by the combined methods, is expected to contribute to an efficient assignment of functionality to un-annotated genomic sequences. 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.

2003 Publications

C.Nikolaou & Y.Almirantis. (2003) Mutually symmetric and complementary triplets: Differences in their use distinguish systematically between coding and non-coding genomic sequences. *Journal of Theoretical Biology* 223, 477-487.

S.Papageorgiou. A cluster translocation model may explain the collinearity of Hox gene expressions. Accepted for publication, *BioEssays*.

Impact Factors (for 1 publication): 1,414

Citations 2001- 2003 (without self-citations): 6

Research Group: Signal Transduction Mechanisms –Molecular Pharmacology

Research Staff
Iro Georgoussi, Associate Research Scientist
Emmanouel Merkouris, Collaborating Research Scientist
Nikolaos Balatsos, Postodoctoral Fellow
Georgia Mazarakou, Graduate Student
Evaghelia Morou, Graduate Student
Leonidas Leodiadis, Graduate Student
Konstantinos Papachristou, Collaborating Graduate Student
Marios Xidous, Undergraduate Student

Research Interests

The overall objective of our research interests 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 which will lead in changes of certain transcription or mitogenic factors with the ultimate goal to identify novel therapeutic targets, c) on the elucidation of the mechanisms of signal sorting and integration between different signaling pathways d) on the development of High Throughput Screens HTP for GPCRs, that will lead in the identification of new analogs.

2003 Findings

Mapping the structural and functional domains of the opioid receptors.

Based in our previous observations concerning the role and the significance of the third intracellular loop of the δ-opioid receptor, in G protein coupling and activation, a minigene encoding this domain of the opioid receptor (pDORi3) was tested, as potential activator or inhibitor of GPCR signaling cascade. Expression of this minigene in intact HEK 293 cells, stably expressing μ- and δ- opioid receptors resulted in a significant inhibition in ERK activation by DAMGO and DSLET respectively. Moreover functional assays have indicated that the presence of pDORi3 in the activated receptor results in inhibition of PLC and adenylyl cyclase activities. Supplementary studies of DAMGO stimulated [^{35}S]GTPγS binding indicated that the presence of pDORi3 also interferes in G protein activation. The presence of the minigene in Rat-1 fibroblasts expressing the α2 adrenergic receptor, which couples with the same Gi/o proteins as the opioid receptors, blocks the signaling cascade from this receptor. This result indicates, that the minigene is functional in heterologous systems in a similar manner as that observed in homologous systems.

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 S-glutathione (GST) fusion proteins of the third intracellular loop and the carboxyl-terminal regions of the delta opioid receptors 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 and the third intracellular loop, of the δ-opioid receptors, to interact with recombinant heterotrimeric Gαβ1γ1, active GαGTPγS, or inactive GαGDP. Moreover, we demonstrate that both fusion proteins interact with novel interacting partners one of which is the RGS4 protein. The role of this RGS4 protein in opioid receptor function is under investigation. *Collaboration: Prof. Heidi Hamm, Department of Pharmacology Vanderbilt University School of Medicine, Nashville, TN, USA.*

Cellular signaling of opioid receptors leading to alterations in gene expression.

Another area of our research activity covers the molecular signaling circuits that lead opioid receptors to tolerance and dependence. In this regard, we observed for the first time that acute exposure to opioid agonists, of COS-7 cells transiently transfected with the μ-opioid receptor and STAT5A or STAT5B members of the Signal Transducers and Activation of Transcription, lead to their phosphorylation. In order to examine which protein kinases are responsible for opioid agonist induced STAT5A phosphorylation selective inhibitors for various kinases are used. Our data suggest that morphine phosphorylation of STAT5A is due to a Src kinase. Moreover, activation of HEK293 cells stably expressing the μ- opioid receptor, with various opioid agonists, stimulated the expression of a STAT5A responsive reporter gene. GST-pull down experiments, revealed binding of the STAT5A to the carboxyl-terminal tail of the μ-opioid receptor. Our data reveal novel signaling pathways through which μ- opioid receptor regulate transcriptional activity, probably altering gene expression in specific target neurons and thereby inducing tolerance and dependence.

Development of HighTroughput Screens (HTP).

Efforts are being made to develop high-throughput screening tools for rapid detection of novel opioid receptor ligands using insect cell-based assays that express these receptors In this regard, we have generated two stable cell lines expressing, either, the rat δ1-opioid receptor (δ1OR), or the δ1OR with the human $G\alpha_{16}$ in Bm5 cells. Our data demonstrate high expression levels and functional coupling between the δ1-opioid receptor and the G proteins present in Bm5 cells, that lead to PLC stimulation and subsequent changes in the levels of intracellular Ca^{2+}. *Collaboration: Laboratory of Insect Genetics and Biotechnology Prof. K. Iatrou and Dr L. Swevers.*

2003 Publications

Vassilaki T. Georgoussi Z**.** and Thermou K. (2003) Somatostatin receptors (sst2) are coupled to Go and modulate GTPase activity in the rabbit retina. J. Neurochem. 84:625-63

2003 Presentations at International Scientific Conferences

Swevers, L., Ciolfi, S., Lioupis A., Morou E., Balatsos N.A.A., Farrell, P.J., Georgoussi Z. and Iatrou K. Lepidopteran Cell- based High Throughput Screens for the Identification of Ligand Mimentics for Insect and Mammalian receptors.Sixth Conference on Protein Expression in Animal Cells, September 7-11, 2003, Mont-Tremblant, Quebec, Canada

Impact Factors (for 1 publication): 4,834

Citations 2003 (without self- citations): 37

Research Group: Molecular Genetics of Insects and Biotechnology

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Eleftheria Argyrou, Postodoctoral Fellow
Vassilios Douris, Postdoctoral Fellow
Christos Kenoutis, Postdoctoral Fellow
Efthimios Prinarakis, Postdoctoral Fellow
Konstantia Sdralia, Graduate Student
Theodoros Georgomanolis, Graduate Student
Aghelina Metaxatou, Collaborating Graduate Student
Sofia Costa, Undergraduate Student
Kallia Apazoglou, 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 of differentiation programs induced by ecdysteroid hormones (b) Expression and characterization of hymenopteran parasitoid polydna virus-encoded proteins and their molecular interactions with hemocyte proteins of their lepidopteran hosts (c) Control mechanisms of olfactory function in the 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 mammalian gene therapy and cellular immunization.

3. Functional genomics: (a) Systems for production of proteins of economic importance in insect cell lines (b) High throughput screening systems for bioactive substances (activators and inhibitors of pharmacological targets) in natural products (plants and microorganisms)

2003 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. Efforts are in progress for (i) the recovery of the complete open reading frames (ORFs) for these two proteins from cDNA libraries, and (ii) the development of reproducible methodologies allowing selective and tissue-specific gene silencing and an assessment of the consequences of the silencing of NTP 1-4 and CTP 15-1 encoding genes on the differentiation program of the follicular cells.

The characterization of the BmSH3 protein, a regulatory factor of the follicular cells that is characterized by the presence of three SH3 domains and one lipid raft-targeting “SoHo” domain and interacts with the orphan nuclear receptor BmE75C, was also continued. For this study, several analyses were carried out to verify the existence of different BmSH3 isoforms, protein fragments were produced in bacteria for the generation of specific antibodies, and efforts were

made for the construction of BmSH3/yellow fluorescent protein (YFP) fusions to be used for localization studies in silkmoth-derived cell lines.

Parasitization and immunosuppression of lepidopteran insects by hymenopteran parasitoids: molecular interactions between polydna virus-encoded proteins and proteins of the parasitized host hemocytes

Insect parasitoids represent an as yet unexploited source of factors that are capable of inducing severe pathological syndromes in their insect hosts, and provide capabilities for the development of new "natural" insecticides. In the context of a research program that is carried out in collaboration with other European research groups and aims in the development of new, effective and environmentally friendly insecticides, two proteins that are expressed in the hemocytes of the tobacco hornworm *Manduca sexta* and interact with the hymenopteran parasitoid *Cotesia congregatα* polydna virus-encoded protein (CcV1), have been identified and cloned. These two hemocyte proteins play an important role in the immune response of the host and are likely to represent targets, through which the immunosuppresion of the host is achieved during parasitization. The effort for a more detailed analysis of the interactions and the functional characterization of the two *Manduca* hemocyte proteins is in progress.

Under the same research program, we also optimised conditions for expression of two polydna virus proteins (TnBV1, EP1) as fusions with relevant secretion modules using an expression-secretion system derived from lepidopteran insect cells. The optimal purification procedure for each of these proteins was also standardized. Moreover, an additional polydna virus protein, cystatin (Cyst1), was expressed in its native form, purified to high purity and proved to have cysteine protease inhibitor activity. This protein represents the first viral cystatin to be expressed and characterized to date.

Finally, cDNA clones derived from polydna viruses and encoding an aspartyl protease, two tyrosine phosphatases and a novel protein with an as yet unidentified biological role, were over-expressed in bacteria in order to produce correctly folded and biologically active proteins in amounts and purity required for X-ray crystallography studies. Following the initial screening for the determination of optimum induction, solubility and folding conditions for the expressed proteins, our effort was focused on two of them, one of the tyrosine phosphatases and the novel protein. The optimization of the purification procedure is still in progress.

Regulation of mosquito olfactory function

Under a collaborative research program with Dr Harald Biessmann, University of California, Irvine, USA, three *Anopheles gambiae* odorant binding proteins (OBPs) were cloned in appropriate plasmid vectors and transformed into yeast cells to be used as baits in a yeast two hybrid system for screening proteins of the mosquito olfactory neurons (e.g. olfactory receptors), with which the specific OBPs interact during the signal (olfaction) transduction process from the mosquito's antenna to its brain. Screening experiments for interactions are in progress. In parallel, expression of one of the *Anopheles gambiae* OBP proteins above was achieved into insect cell lines and isolation and purification the protein is in progress in order to examine its ability to bind specific olfactory molecules.

Molecular biology and genetic engineering of insect nucleopolyhedrosis viruses

Nucleopolyhedrosis viruses (NPVs or baculoviruses) have features that have sparked interest for their possible development as gene therapy vectors (e.g. non-specific mode of entry, absence of toxicity and endogenous viral gene expression in the mammalian cells, including human ones, and large packing capacity of foreign genetic material). However, baculovirus production (as well as recombinant protein production via baculovirus-based expression vectors), occurs most efficiently in insect cell lines grown in the presence of fetal bovine serum (FBS), an additive that may potentially contain harmful pathogens such as viruses, mycoplasmas and prions. For this

reason, we have genetically engineered silkmoth-derived cell lines that are adapted to growth in serum-free medium (Bm5-SF) cells, to constitutively express the "promoting protein" (PP), a secreted factor of the silkmoth fat body, whose presence in the cells and their growth medium enhances baculovirus infectivity of and productivity by the engineered cells. The PP-expressing Bm5-SF cell lines display a 100-1,000 fold higher productivity relative to non-engineered cells, comparable to the productivity of cells growing in serum-containing media. The engineered cell lines can now be used for high-level production of baculovirus vectors (and recombinant proteins derived from them) in cells grown in serum-free media, and both the virus vectors (and recombinant proteins produced by them in insect cells) can be used for gene therapy applications without the risk of appearance of secondary infections caused by infectious factors that may be present in FBS.

For the construction of inactivated nuclear polyhedrosis viruses as vectors for genetic transformation of lepidopteran insects (insect baculovirus-derived artificial chromosomes or iBVACs), we are in the process of generating stably transformed "rescue" cell lines for *Bombyx mori* NPVs (BmNPVs) that are deficient for the LEF8 protein (viral RNA polymerase subunit) and cannot complete the infection cycle of normal host cells and, thus, behave in them as iBVACs. The expression of the LEF8 gene of BmNPV under the control of different inducible promoters has been tested and our effort for the transformation of silkmoth cell lines with the relevant expression constructs inserted into various transformation vectors is in progress. a variety transformation systems is currently tested.

Finally, the construction of plasmid transfer vectors allowing exchange of genetic information with NPV genomes in cultured cells has started with the aim of constructing baculovirus-based gene therapy vectors, which will allow the permanent introduction into and stable expression of selected genes in mammalian cells.

Functional genomics

In the framework of the development of new vectors for recombinant protein expression in insect cells, we have constructed vectors that can be used for expressing heterologous secreted proteins with a modified C-terminus (double tag) that facilitates the ease of quantification of the expression levels of the recombinant proteins and their purification. Expression trials of an odorant binding protein of the mosquito *Anopheles gambiae* were performed with very encouraging results, thus enabling a wider application of the new vectors for the expression of other secreted proteins. Meanwhile, the development and construction of additional insect cell expression vectors for proteins with different properties is in progress.

We have previously developed a transformed Bm5 cell line that responds to substances with insect moulting hormone (ecdysteroid) activity through induction of intense green fluorescence. This cell line was used to screen a chemical library of dibenzoyl hydrazines, provided by Dr. Y. Nakagawa, Kyoto University, Japan. Besides the detection of a few very active compounds, which have potential for development as new environmentally friendly insecticides (endocrine disruptors), detailed measurements have allowed the determination of the half-maximal response for each compound. These data will be used for "quantitative structure-activity relation (QSAR) analysis" to model new and improved compounds that will be used in a second round of testing on the transformed cell line. In collaboration with Dr. G. Smagghe, University of Ghent, Belgium, the activity of the most active compounds was also tested in larval toxicity assays and their insecticidal efficacy on whole animals was confirmed.

Finally, in collaboration with the laboratory of Dr. Z. Georgoussi, transformed Bm5 cell-derived lines were developed that over-expresses the murine μ- or δ-opioid receptor, together with the human $G\alpha_{16}$ protein, and can be used for the screening of the presence of opioid agonists and antagonists in complex mixtures. Ligand binding studies, G-protein coupling assays and second messenger inositol trisphosphate (IP3) assays showed functional coupling of and signaling by the

mammalian opioid receptors to the IP3/calcium pathway of silkmoth cell lines. Because addition of the opioid ligand also induces a calcium response in the cells, that can be easily detected by fluorometric methods, the transformed cell lines can be used as screening tools for the fast detection of opioid mimetics (agonists and antagonists) in natural products.

2003 Publications

Swevers, L. and Iatrou, K. (2003). The ecdysone regulatory cascade and ovarian development in lepidopteran insects: the silkmoth paradigm. Insect Biochem. Mol. Biol. 33, 1285-1297.

Swevers, L., Kravariti, L., Ciofli, S., Xenou-Kokoletsi, M., Ragousis, M., Smagghe, G., Nakagawa, Y., Mazomenos, B. and Iatrou K. (2003). A cell-based high-throughput screening system for detecting ecdysteroid agonists and antagonists in plant extracts and libraries of synthetic compounds. *FASEB J* 17 (14): U218-U243.

Metaxatos, A., C. Panagiotopoulos and L. Ignatiades, 2003. Monosaccharide and aminoacid composition of mucilage material produced from a mixture of four phytoplanktonic taxa. J. Exp. Mar. Biol. Ecol. 294, 203-217.

2003 Presentations at International Scientific Conferences

Ignatiades L. Bioassay experiments using toxic *Alexandrium* species. Second Workshop, Stragegy Project, January 21-24, 2003, Rome, Italy.

Swevers L. and Iatrou K. Generation of a Transformed Silkmoth Cell Line that Displays Enhanced Susceptibilitry to Baculovirus (BmNPV) Infection and Supports the Production of High Titers of Budded Virus in Serum-Free Media. Sixth International Workshop on the Molecular Biology and Genetics of Lepidoptera, August 25-30, 2003, Kolymbari, Crete, Greece.

Sdralia N., Glushek M., Ito K. and Iatrou K. Yeast Two Hybrid Screens for the Identification of Follicular Cell Proteins Interacting with the Silkmoth Transcription Factor BmGATAβ: Characterization of cDNAs Encoding Proteins that Interact with BmGATAβ in Follicular and Bm5 Tissue Culture Cells. Sixth International Workshop on the Molecular Biology and Genetics of Lepidoptera, August 25-30, 2003, Kolymbari, Crete, Greece.

Douris V., Farell P.J., Swevers L. and Iatrou K. Expression of polyDNA virus genes in lepidopteran insect cell lines. Sixth International Workshop on the Molecular Biology and Genetics of Lepidoptera, August 25-30, 2003, Kolymbari, Crete, Greece.

Farrell P.J., Swevers L. and Iatrou K. (2003). An Expression Cassette for Recombinant Protein Expression and High-Throughput Screening for Bioactive Compounds in Insect Cells. Sixth Conference on Protein Expression in Animal Cells, September 7-11, 2003, Mont-Tremblant, Quebec, Canada.

Swevers L., Ciolfi S., Lioupis A., Morou E., Balatsos N.A., Farrell P.J., Georgoussi Z. and Iatrou K. Lepidopteran Cell-Based High Throughput Screens for the Identification of Ligand Mimetics for Insect and Mammalian Receptors. Sixth Conference on Protein Expression in Animal Cells, September 7-11, 2003, Mont-Tremblant, Quebec, Canada.

Ignatiades, L., O. Gotsis – Skretas and A. Metaxatos, 2003. Nutritional and ecological factors affecting the growth of the toxic algal species *Alexandrium minutum* in Greek coastal waters. International Conference on the Scientific and Policy Challenges Towards an effective management of the Marine Environment, 13-18 October, Varna, Bulgaria.

Gotsis-Skretas O., A. Pavlidou and L.Ignatiades, 2003. Spatial and temporal variability of phytoplankton communities in the Aegean Sea, with emphasis to the toxic dinoflagellate genus *Alexandrium*. International Conference on the Scientific and Policy Challenges Towards an effective management of the Marine Environment. 13-18 October, Varna, Bulgaria.

Swevers, L., Farrell, P.J., Kravariti, L., Xenou-Kokoletsi, M., Sdralia, N., Lioupis, A., Morou, E., Balatsos, N.A.A., Douris, V., Georgoussi, Z., Mazomenos, B., and Iatrou., K. (2003). Transformed insect cells as high throughput screening tools for the discovery of new bioactive compounds. 17th Forum for Applied Biotechnology, Ghent, Belgium September 2003.

Impact Factors (for 3 publications): 12,225

Citations in total 2001- 2003 (without self citations): 298

Iatrou K. (Swevers' publications are included): 144
Swevers L.: 8
Lampropoulou V.: 66
Ignatiades L.: 80

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

Research Staff
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Christina Giannouli, Graduate Student
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Theodoros Karabelas, 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 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.

2003 Findings

During 2003 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. Our goal is to elucidate the mechanism underlying this differential action. Accordingly, we have studied the activation of the SMAD and MAPK signaling pathways by TGF-β, as well as of cell cycle regulators and it has been found that the TGF-β-mediated growth inhibition is accompanied by the overexpression of cyclin-dependent kinase inhibitors. Furthermore, its stimulatory action on cell proliferation has also been examined and found that, at least in part, is mediated via an autocrine mechanism. Concerning the role of the extracellular environment we have also shown that the polymerized collagen can reverse the action TGF-β, through the activation of integrins and the ERK pathway. In parallel, we are investigating (in humans and other mammals) the developmental stage where the switch in the response of fibroblasts to TGF-β (from inhibitory to stimulatory) takes place, in order to associate this with physiological alterations, such as differences in wound healing strategies.

We have studied the involvement of the mitochondrial protein PBR (Peripheral Benzodiazepine Receptor) in proliferation and ageing. By using RNA interference (RNAi) we have shown that this protein is crucial for the proliferation of epithelial but not of mesenchymal cells.

We have also continued our investigations on the structure and function of the senescent (non-proliferating) cell:

We are currently studying morphological and functional changes of the senescent-cell nucleus and in particular of the nuclear lamina and we have characterised alterations in lamins and lamin-

associated proteins (thymopoeitins), in terms of expression and post-transcriptional modifications.

In addition, developmental and age-related alterations in crucial regulators of cellular homeostasis, such as intracellular calcium concentrations, have also been investigated. We have observed significant differences between fetal and adult fibroblasts, depending on the tissue of origin. On the other hand, the major alteration found in senescent cells is their inability for capacitative cell entry after depletion of intracellular pools.

One of our main goals is the understanding of the contribution of the senescent cell to ageing and the development of various age-related disorders. In this vein, we have shown that the onco-suppressor p53, that is over-activated in senescent cells, can induce directly the expression of ICAM-1, a main player in the immune response. Inhibition of p53 function results in a reduction of ICAM-1 expression in several models of senescent human cells. 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. Furthermore, the role of cellular senescence in the progression of carcinogenesis is also investigated.

One of the tissues whose aging can lead to severe functional disorders is the intervertebral disc. Accordingly, we are currently studying the features of young vs. senescent cells from different parts of the disc, i.e. the annulus fibrosus and the nucleus pulposus, and and their response to several stresses (pH, osmolarity, hypoxia, etc.), as well as to mechanical forces.

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

2003 Publications

Papazafiri P, Kletsas D. (2003) Developmental and age-related alterations of calcium homeostasis in human fibroblasts. Exp Gerontol. 38, 307-11.

Gorgoulis VG, Zacharatos P, Kotsinas A, Kletsas D, Mariatos G, Zoumpourlis V, Ryan KM, Kittas C, Papavassiliou AG. (2003) p53 activates ICAM-1 (CD54) expression in an NF-kappaB-independent manner. EMBO J. 22, 1567-78.

Mitropoulou TN, Tzanakakis GN, Kletsas D, Kalofonos HP, Karamanos NK (2003) Letrozole as a potent inhibitor of cell proliferation and expression of metalloproteinases (MMP-2 and MMP-9) by human epithelial breast cancer cells. Int J Cancer. 104, 155-60.

Kolokythas G, Kostakis IK, Pouli N, Marakos P, Kletsas D, Pratsinis H. (2003) Synthesis and cytotoxic activity of some new azapyranoxanthenone aminoderivatives. Bioorg Med Chem. 11, 4591-8.

Eliades T., Pratsinis H., Kletsas D., 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.

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. (in press).

Pratsinis H, Giannouli Ch.C, Zervolea Ir, Psarras S, Stathakos D, Kletsas D. (2004) Differential proliferative response of fetal and adult human skin fibroblasts to TGF-β. Wound Repair & Regeneration (in press).

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. (in press).

Gioka C., Bourauel C., Hskia A., Kletsas D., Eliades T., Eliades G. (2004) Light-cured vs. chemically-cured orthodontic adhesive resins: a comparative assessment of the degree of cure, monomer leaching and cytotoxicity. Am. J. Orthod. Dentofacial Ortop. (in press)

2003 Presentations at International Scientific Conferences

Kletsas D. "Conventional" and "alternative" modes in the regulation of tissue homeostasis. 2nd EURODISC Meeting. Helsinki, Finland.18-19 June, 2003.

Kletsas D., Pratsinis H., Mariatos G., Kittas Ch., Papavassiliou A.G., Gorgoulis V.G. Cellular senescence and inflammation: The p53-mediated ICAM-1 expression. 4th International Conference on Basic Biology and Clinical Aspects of Immunosenescence. Kolymbari, Crete. May 21-25, 2003 (invited speaker).

Kletsas D., Pratsinis H., Mariatos G., Gorgoulis V.G. On the pro-inflammatory phenotype of senscent cells: The p53-mediated ICAM-1 expression. 10th Congress of the International Association of Biomedical Gerontology. Cambridge, UK, September 19-23, 2003.

Kletsas D. Cellular senescence and carcinogenesis: Antagonism, synergism and therapeutic perspectives. 4th Conference "Medicinal chemistry: drug design and metabolism". Patras, 13-14 March, 2003 (invited speaker).

Impact Factors (for 4 publications): 20,983

Citations2001- 2003 (without self-citations): 133

Research Group: Transcriptional Regulation by the Biological Clock

Research Staff
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Michalis Barkoulas, Undergraduate Student
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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 dual role of the factor as positive and negative regulator in rhythmic transcription. Investigation of PvLHY and PvTOC1 roles in the function of the central oscillator of bean.

2003 Findings

I. The ongoing research regards the study of PvLHY, a putative transcription factor and component of the central oscillator in bean. Towards the isolation of the *PvLHY* gene promoter, we achieved the cloning of a 667 bp long 5' UTR region of the *PvLHY* gene. The theoretical study of the obtained sequence did not reveal any known promoter elements (e.g. G-Box, TATA-Box). Moreover, we cloned an approx. 2 kb long fragment from the further upstream region, which is under analysis. The second project aims the study of the PvLHY protein function. Thus, towards the production of polyclonal antibodies, we cloned three fragments from the PvLHY cDNA in pGEX expression vector. These fragments are translated to hydrophilic peptides of 150, 150 and 450 amino acids length, respectively. We further tested the conditions for optimal production of all three GST-PvLHY peptide fusion proteins.

II. Towards the isolation of new clock components of *Phaseolus vulgaris*, we isolated from total RNA of bean leaves by RT-PCR and cloned a cDNA of 1078 bp length. The deduced amino acid sequence (358 amino acids) exhibits 35% identity and 47% similarity to a partial sequence of the TOC1 protein from the plant *Arabidopsis thaliana*. For this reason the *Phaseolus vulgaris* cDNA was designated *PvTOC1*. The regulation of *TOC1* gene expression by light and the circadian clock is under study.

2003 Publications

A.-D. Kaldis, P. Kousidis, K. Kesanopoulos and A. Prombona (2003) Light and circadian regulation in the expression of *LHY* and *Lhcb* genes in *Phaseolus vulgaris*. Plant Mol. Biol. 52, 981-997

Impact Factors (for 1 publication): 3,592

Citations 2001- 2003 (without self-citations): 20

Research Group: Nuclear Proteins and Chromatin Function

Research Staff
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Thomais Sourlingas, Collaborating Research Scientist
Dimitra Tsapali, Postdoctoral Fellow
Aikaterini Kypreou, Graduate Student
Paraskevi Salpea, Undergraduate Student
Kalliopi Kalokyri-Stylianidi, Research Technician

Research Interests

Studies regarding changes in the constitution of chromatin related to histone variant expression and histone acetylation using different cellular systems in order to obtain information which will relate the histone constitution of chromatin to its biological functional state. The systems used are ageing cell systems such as fibroblasts and lymphocytes as well as leukemic cell lines.

2003 Findings

The effects of the histone deacetylase inhibitor, trichostatin A, on 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 obtained so far 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. In continuation we will proceed to identify all the somatic subtypes of the H1 family in human lymphocytes and especially the specific subtype in question which showed this change in the older age groups.

2003 Publications

Sourlingas, T.G., Issidorides, M.R., Alevizos, B., Kontaxakis, V..P., Chrysanthou-Piterou, M., Livaniou, E., Karpouza, A. and K.E. Sekeri-Pataryas. Lymphocytes from bipolar and schizophrenic patients share common biochemical markers related to histone synthesis and histone cell membrane localization characteristic of an activated state. *Psychiatry Res.*, 118: 55-67, 2003.

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.*, accepted

2003 Presentations at International Scientific Conferences

Sourlingas, T.G., Doenecke, D., Happel, N., Albig, W., Kypreou, K.P., Tsapali, D.S. and K.E. Sekeri. Changes in the H1 linker histone constitution of chromatin in peripheral blood lymphocytes as a function of donor age. Immunology and Ageing in Europe (ImAginE). Fourth International Conference on Basic Biology and Clinical Impact of Immunosenescence, Crete, 2003.

Sourlingas, T.G., Kypreou, K.P., Tsapali, D.S. and K.E. Sekeri. An introduction to the role of histones in chromatin remodeling during ageing. . Immunology and Ageing in Europe (ImAginE). Fourth Conference on Basic Biology and Clinical Impact of Immunosenescence,

Crete, 2003.

Kypreou, K.P., Sourlingas, T.G. and K.E. Sekeri. Effect of histone deacetylase inhibitors on histone H1o expression and histone H4 acetylation during senescence of T lymphocytes. Immunology and Ageing in Europe (ImAginE). Fourth Conference on Basic Biology and Clinical Impact of Immunosenescence, Crete, 2003.

Kypreou, A.P., Sourlingas, T.G., and K.E. Sekeri-Pataryas. The role of histone H1o and histone H4 acetylation during senescence and apoptosis of T lymphocytes. 25th Panhellenic Society for Biological Sciences, Mytilini, 2003.

Tsapali, D.S., Sourlingas, T.G., Kypreou, A.P. and K.E. Sekeri-Pataryas. Regulation of histone H1o expression by retinoic acid and histone deacetylase inhibitors. 25th Panhellenic Society for Biological Sciences, Mytilini, 2003.

Sourlingas, T.G., Kypreou, A.P., Happel, N., Doenecke, D., Albig, W. and K.E. Sekeri-Pataryas. Analysis of the H1 linker histone subtype pattern in peripheral blood lymphocytes as a function of donor age. Hellenic Society for Biochemistry and Molecular Biology. Athens, 2003.

Impact Factors (for 1 publication): 1,775

Citations 2001- 2003 (without self-citations): 33

Research Group: Microbial Molecular Genetics and Radiation Genetics

Research Staff
Vassiliki Sophianopoulou, Associate Research Scientist
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Eleftherios Visvardis, Collaborating Research Scientist
Panagiota Kafasla, Postodoctoral Fellow
Eleftheria Argyrou, Postodoctoral Fellow
Zoi Erpapazoglou, Graduate Student
Dimitra Bouzarelou, Graduate Student
Vassilis Nikas, Graduate Student (rotation)
Maria Billini, Undergraduate Student
Ioannis Vaggelatos, Undergraduate Student
Diana Lousa, Undergraduate Student
Eleni Tripodaki, 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.*

2003 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. This work establishes the presence of a novel system triggering purine transporter transcription during germination.
- We have constructed and characterized a functional Cys-less proline (PrnB) transporter of *A. nidulans* and investigated the role of native Cys residues of PrnB on its structure-function using Cys-scanning mutagenesis.
- We have functionally expressed and partially characterized at the molecular, the human ascorbate transporter (SVCT2) in *Aspergillus nidulans.*
- We have cloned and characterized at the molecular level a gene, *shrA,* of *A. nidulans* encoding a protein that complements an *shr3*- strain of *S. cerevisiae*, suggesting that the product of this gene is required for localization of amino acid permeases in the ER membrane of *A. nidulans*.
- We have cloned, over-expressed and partially characterized at the molecular level a gene, An-ExpA, encoding a putative Expansin-like protein of *A. nidulans*.

2003 Publications

S. N. Tavoularis, U. H. Tazebay, G. Diallinas, M. Sideridou, A. Rosa, C. Scazzocchio and V. Sophianopoulou (2003). Mutational analysis of the major proline transporter (PrnB) of *Aspergillus nidulans*. Mol. Membr. Biol. 20, 285-297.

A. Tsoulou, C. A. Kalfas and E .G. Sideris (2003). Changes in DNA flexibility after irradiation with γ rays and neutrons studied with the perturbed angular correlation method. Radiat. Res. 159, 33-39.

S. Amillis, G. Cecchetto, V. Sophianopoulou, M. Koukaki, C. Scazzocchio and G. Diallinas (2003). Transcription of purine trasporter genes is activated during the isotropic growth phase of *Aspergillus nidulans* conidia. Mol. Microbiol. (in press).

2003 Presentations at International Scientific Conferences

P. Kafasla, S. Frillingos and V. Sophianopoulou (2003). Structure-function analysis of the major proline transporter of Aspergillus nidulans (PrnB) using Cys-scanning mutagenesis. Abstract of the XXI International Conference on Yeast Genetics and Molecular Biology, Goteborg, Sweden, page 233.

E. E. Visvardis, K. S. Haveles, A. G. Georgakilas, E. G. Sideris and V. Sophianopoulou, (2003). Patterns of DNA DSBs SSBs and alkali-labile sites induction and repair in gamma-irradiated human lymphocytes using alkaline and neutral pulsed field gel electrophoresis. Abstract of the EU-US Workshop on Molecular signatures of DNA damage induced stress responses. Cortona, Italy, 26-30 September, page 104.

K. Stamatakis and V. Sophianopoulou (2003). Cloning and molecular characterization of a gene encoding a putative $N\alpha^+/H^+$ antiporter in *Synechococcus sp*. PCC 7942. Abstract of the 25^o Panhellenic Conference of HSBS, Mytilini, Greece 302, 303.

D. Bouzarelou, G. Diallinas and V. Sophianopoulou, (2003). Cloning and molecular characterization of an expasin-like gene from *Aspergillus.* Abstract of the 25^o Panhellenic Conference of HSBS, Mytilini, Greece p. 222, 223.

Impact Factors (for 2 publications): 6,830

Citations 2001- 2003 (without self-citations): 62

Research Group: Biophysics and Biotechnology of Membranes

Research Staff
Kostas Stamatakis, Assistant Research Scientist
George Papageorgiou, Collaborating Research 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.

2003 Findings

Cells of fresh water cyanobacterium *Synechococcus* sp. PCC 7942 import NaCl passively and export Na^+ actively, primarily *via* Na^+/H^+ antiporter. During 2003 a gene encoding a putative Na^+/H^+ antiporter of freshwater cyanobacterium *Synechococcus* sp. PCC 7942 was cloned and partially characterized at the molecular level. Cloning of this gene was achieved screening a genomic cosmid library of *Synechococcus* sp. PCC 7942. The screening procedure was performed using as probe a PCR product amplified from *Synechococcus* sp. PCC 7942 genomic DNA using degenerate oligo's. These oligo's were constructed from regions shown, by *in silico* analysis, to be highly conserved among known Na^+/H^+ antiporters. The screening procedure was resulting in the isolation of one positive clone. DNA of this clone was digested by different restriction enzymes and hybridized with the product of the PCR reaction. A positive DNA fragment of 4.3 kb was then cloned and sequenced. This fragment contains an ORF encoding a protein of 715 amino acids. *In silico* analysis shown that the resulting protein has 54% identity and 62% similarity with known Na^+/H^+ antiporters. Finally we have shown that the gene is expressed constitutively under different salt conditions.

2003 Publications
George C. Papageorgiou (2003). Photosynthesis Research in Greece: A Historical Snapshot (1960-2001). Photosynth Research 76: 427-433

2003 Presentations at International Scientific Conferences

G. C. Papageorgiou (2003). Chlorophyll a fluorescence: a reporter of osmotic volume changes and solute/water transport in cyanobacteria. Oxidants and Antioxidants in Biology, 6-9 Feb 2003, Cadiz, Spain. (invited speaker)

Impact Factros (for 1 publication): 1,739

Citations 2001- 2003 (without self citations): 27

Research Group: Pathobiology of the Exracellular Matrix

Research Staff
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Kostas Economou, Graduate Student
Argiris Talamaghas, Graduate Student
Panagiotis Vevieratos, Graduate Student
Ioanna Tsagaraki, Graduiate Student
Evaggelos Fragopoulous, Collaborating Graduate Student
Dimitrios Moutzouris, Collaborating Graduate Student
Ioanna Nikitopoulou, Undergraduate Student
Ioardanis Theodosiadis, Undergraduate Student
Kostas Kaloeidas, Graduate Research Associate
Maria Kontou, Graduate Research Associate

Research Interests

The interests of this laboratory focus on integrin-mediated regulation of gene expression in different cell types, with emphasis on cell-matrix interactions in normal and pathological conditions, such as Diabetes Mellitus, Alzheimer's Disease, etc. The team examines molecular mechanisms of function of receptors, which serve for binding to the matrix, and basement membranes, as well as surface sialoproteins which antagonize matrix binding. As a model, cell cultures are used which simulate diabetic conditions, peripheral blood cells from diabetic patients, and neuroblastoma cell cultures mimicking the conditions of Alzmeimer's disease. Interactions between matrix, integrins and the sialoprotein podocalyxin which is important for cytoskeletal re-organization of the basal surface of renal glomerular epithelial cells are examined. The regulation of expression of podocalyxin is being examined. Recent approaches include the study of integrin-related mechansisms which sither lead to cell apoptosis or prevent this process in insulin-secreting β-pancreatic cells, osteoblasts, etc. Furthermore, glucose-mediated dysfunction of β-cells is examined. The aim is to unravel signaling pathways regulating gene expression via interactions with matrix components, for example, collagen IV and TIN antigen, novel functions (enzymatic activity) of which are being examined. Additionally, ways to over-express cell surface components which serve for matrix binding or inhibition of this process, with the aim to be used in pathological conditions, for example, over-expression of anti-apoptotic integrins in cases of undesired cell apoptosis (pancreatic β-cells, osteoblasts, etc.), over-expression of podocalyxin and non-functional mutations to prevent tumor cell implantation in secondary loci, induction of wound healing during the first phases, when increased cell motility is required, etc.

2003 Findings

1. Secretase-related functions of collagenase MMP-9 were examined. HEK-293 cells, permanently overexpressing APP were additionally transfecetd in the presence of lipofectin, with cDNA of MMP-9. Cell culture supernatants containing APP fragments were examined by western analysis. There was an increase of the sαAPP fragment, indicating that overexpression of MMP-9 had a secretase α-like effect, and should inhibit amyloid plaque formation.
2. Renal glomerular epithethelial cells (podocytes) exposed to increased glucose concentrations almost totally suppress the expression of podocalyxin, even when cultured on substrates of basement membrane components which enhance the expression of this sialoprotein up to ~65%, in normal conditions. Podocalyxin-deficient glomerular epithelial cells do not form

areas locally lifted from the substrates in their basolateral surface, when examined by SEM, thus lack of podocalyxin should be partly involved in the flattening of foot processes in late stages of diabetic nephropathy in vivo. This hypothesis was confirmed by the use of immunocytochemical experiments, in which the presence of podocalysin in kidney sections from control and streptozotocin (STZ)-diabetic rats was tested. Seven months after induction of diabetes, there was ~ 55% less podocalyxin in glimerular epithelia cells in glomeruli from sections of diabetic kidneys, compared to the control. Furthermore, in cultured glomerular epithelial cells, it took at least 6 months of culturing in the presence of increased glucose concentrations to suppress podocalyxin expression. In the case of restoring glucose levels in the culture medium to normal levels (5 mM), the expression of podocalyxin by glomerular epithelial cells is not restored for up to four weeks culture in the presence of normal glucose concentrations.

3. It was demonstrated for the first time that, kidney-specific protein GPBP (Goodpasture Antigen Binding Protein), which acts as an extracellular kinase (and phosphorylates the NC1 domain of collagen IV, thus facilitating intra- and inter-molecular associations and polymerization of collagen IV) is expressed in substantial amounts by cultured glomerular epithelial cells, whereas the presence of substrates of intact glomerular basement membrane as well as α3 NC1 (the substrate which undergoes phosphorylation by GPBP) reduce the exression of GPBP. This effect is probably due to the fact that in this case, NC1 is already polymerized and no further phosphorylation is necessary.
4. Cultured, insulin-secreting β-pancreatic cells undergo stryctural and functional changes in the presence of increased glucose concentrations. In the latter case, insulin secretion was moderately reduced, as was cell spreading on laminin substrates. In certain instances, the expression of α6 integrin was decreased in the presence of 30 mM glucose in the culture media.

2003 Publications

Kitsiou, P, Tzinia, A, Stettler-Stevenson, W, Michael, AF, Fan, W. Zhou, B., Tsilibary, EC (2003). Glucose-induced phenotypic modulation in glomerular epithelial cells. *Am. J. Physiol. Renal Physiol*. 284, 671-679.

Tsilibary, EC. (2003) Microvacular basement membranes in diabetes. Journal of Pathol. 200:537-46.

Economou CG, Kitsiou, PV, Tzinia, AK, Panagopoulou, Marinos E, Kershaw, DB, Effie C. Tsilibary EC. Regulation of Podocalyxin Expression by Basement Membrane in Human Glomerular Epithelial Cells. Effects of Podocalyxin-Matrix Interactions on Podocyte Morphology. *J Cell Sci* (In Press)

2003 Presentations at International Scientific Conferences

Economou, CG, Kitsiou P, Tzinia A, Kershaw D, Tsilibary EC: The role of matrix in podocalyxin-mediated foot process formation by cultured, immortalized human podocytes. 15th Meeting of the European Renal Cell Study Group, Venice, Italy, March 13-16, 2003.

E.C. Tsilibary, P.V. Kitsiou, C.V. Phenekos, E. Frangoulopoulos "Glucose induced changes of cell shape and integrin expression in murine, insulin-secreting pancreatic β-cells" IDF/EASD, August 25-28, Paris, FR, Diabetologia, abst. Volume 2003.

Impact Factors (for 2 publications): 9,587

Citations in Total 2001- 2003 (without self -citations): 263

Tsilibari E. (Tzinia's publications are icluded): 229
Tzinia A.: 34

Research Group: Environmental Mutagenesis-Carcinogenesis

Research Staff
Gerassimos Voutsinas, Assistant Research Scientist
Anastassia Apostolidou, Graduate Student
Dimitra Anastasiou, Collaborating Graduate Student
Athina Goudopoulou, Collaborating Graduate Student
Natalia Spyrou, Collaborating Graduate Student
Galenos Fanourakis, Collaborating Graduate Student
Sofia Melachrinou, Undergraduate Student
Olga Paparidou, Undergraduate Student
Stefanos Papaspiridakos, Undergraduate Student
Alexandra Stavropoulou, Undergraduate Student
Sokratis Avgeris, Research Technician

Research Interests

1. Genetic and epigenetic alterations in human genes involved in cellular metabolism, cell cycle, DNA repair and apoptosis
2. Involvement of apoptotic pathways in carcinogenesis and drug

2003 Findings

Microsatellite instability in Greek FAP patients: In 22 adenomas and 12 adenocarcinomas (34 samples) from 10 patients 10/22 and 6/12 mutations were found in *K-ras* codon 12, as well as 3/22 and 7/12 mutations in *p53* exons 5-8. The most important finding of the work is the detection of microsatellite instability in genetic loci BAT26, D5S346 and TβRII in two unrelated adenocarcinomas, which is a typical feature of the Hereditary Non-Polyposis Colorectal Cancer and not FAP. [N. Michalopoulos et al., European Journal of Surgical Oncology 29 (2003) 38-43]

***c-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. DNA extracted from the same samples was screened for mutations in *Fas* exon 9 by SSCP and sequencing. The effect of Fas, FasL and c-FLIP on clinical outcome was assessed by univariate and multivariate analysis. Positive immunostaining was detected for Fas, FasL and c-FLIP 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 UC samples analyzed showed evidence of mutations by PCR-SSCP and direct sequencing. Survival analysis demonstrated that although both FasL and c-FLIP expression adversely affected survival, only c-FLIP remained significant in multivariate analysis. The frequent expression and co-expression of Fas, FasL and c-FLIP in urothelial carcinomas implicates c-FLIP as an inhibitor of the Fas-FasL-induced death pathway in these tumors. Moreover, c-FLIP emerges as a contributor in bladder cancer progression, conveying independent prognostic information in the presence of classical prognosticators. [P. Korkolopoulou et al., Urology, in press]

Structural changes and expression pattern of the Fas (APO-1/CD95) gene in urinary bladder cancer: Structural changes in exon 9 of the gene coding for the cytoplasmic domain involved in receptor-mediated signal transduction were found in 5/53 urinary bladder tumor samples. Additionally, co-expression of Fas ligand and receptor in the same samples was detected by immunohistochemistry. [A. Saetta et al., submitted]

Mutation detection and expression pattern analysis in p53 and Fas (APO-1/CD95) genes in thyroid cancer: Mutation detection in exons 5-8 of *p53* gene and in exon 9 of *Fas* gene was

carried out in 38 thyroid cancer samples. Expression analysis of the two genes was carried out using immunohistochemistry and RT-PCR. No mutation was found in *Fas* gene, while a *p53* mutation was detected in one anaplastic thyroid tumor sample. Fas receptor was found to be expressed in all samples, but predominantly in those of the papillary type, while no nuclear localization of p53 protein was detected in any of the samples. At the mRNA level, tmFas and sFas transcripts were found at a ratio always greater than one. [G. Fanourakis et al., in preparation]

***B-RAF* gene is a mutational target in human thyroid cancer:** The RAS-RAF-MEK-ERK signal transduction pathway is an important mediator of extracellular signals that regulate cell proliferation, differentiation and apoptosis. Recently, activating mutations in the BRAF serine/threonine kinase were found in 66% of human melanomas, as well as in a number of other cancers. In this work, we have studied the occurrence and frequency of *BRAF* mutations in the different thyroid cancer subtypes. Mutations in *BRAF* exons 11 and 15 were evaluated in 13 thyroid cancer cell lines and 91 thyroid cancer samples of different subtypes. We have identified 20 mutations in 52 papillary thyroid carcinoma samples (38.4%) and 1 mutation in 3 anaplastic thyroid carcinomas (33%), which were all $BRAF^{V599E}$. On the contrary, no mutations were detected in 3 follicular, 5 Hurthle cell, 19 medullary and 2 mixed carcinomas, as well as in 7 follicular adenomas. While we could not establish a correlation between *BRAF* mutations and histopathological parameters, we have noticed that *BRAF* mutations occurred in papillary thyroid carcinomas at a significantly higher frequency in male patients than in females. [G. Fanourakis et al., Proceedings of the 55th Meeting of the Hellenic Society of Biochemistry and Molecular Biology (2003) 168-172.]

2003 Publications

Michalopoulos, N., Saetta A., Lazaris A., Voutsinas G. and Davaris P.S. (2003). Detection of genetic abnormalities in carcinomas from Greek patients with FAP. European Journal of Surgical Oncology 29, 38-43.

Puiu, L., Petrakou E., Apostolidou A., Athanassiadou A., Psiouri L., Papachatzopoulou A., Gorgoulis V., Tzoracoeleftherakis E., Maniatis G.M. and Voutsinas G. (2003). Lack of Fas (APO-1/CD95) gene structural alterations or transcript variant ratio changes in breast cancer. Cancer Letters 194, 91-97.

Korkolopoulou, P., Goudopoulou A., Voutsinas G., Thomas-Tsagli E., Patsouris E., Saetta A.A. (2003) c-FLIP expression in bladder urothelial carcinomas: Its role in resistance to Fas-mediated apoptosis and clinicopathological correlations. Urology, in press.

Impact Factors (for 3 publications): 5,819

Ciatations 2001- 2003 (without self-citations): 35

B. ENVIRONMENTAL BIOLOGY

Research Group: DNA Repair Systems and Cancer

Research Staff
Stelios Piperakis, Associate Research Scientist
Smaragdi Tsilimighaki, Graduate Research Associate
Nikolaos Anagnostakis, Undergraduate Student
Panagiotis Kanavetas, Undergraduate Student
Georgia Karanastassi, Undergraduate Student
Nadia Kontogianni, Undergraduate Student
Kyriaki Maridaki, 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.

2003 Findings

1. We have now published two papers from the analyses of the results of the programme “pesticides effects on humans”.
2. The results of study "stress and DNA damage-repair" were published this year.
3. The results of study "DNA damage-repair and effects of solar seasonal variations" were published this year.
4. We published the results of our study “DNA damage-repair in schizophrenic patients”
5. Results on a study (in cooperation with the University of Thessaly) of Greek primary school children dietary habits were published.
6. Results on a study (in cooperation with the University of Thessaly) about the Greek primary school children’s comprehension of sun exposure were published.
7. The study “effects of common variable immunodeficiency in DNA repair” is near completion.
8. The study “Effects of antioxidants (vitamins C, E) in human lymphocytes” is near completion.
9. The study “diabetes mellitus - DNA damage and repair” is near completion.
10. The study “Effects of alcohol and hydrogen peroxide in breast cancer patients” is progressing.
11. The study “Lung cancer patients, DNA damage and repair” is progressing.
12. The study «Obstractive sleep apnoea, DNA damage-repair has just started.
13. The study “European network on children’s susceptibility and exposure to environmental genotoxicants” has just started.

2003 Publications

S.M. Piperakis, E. Petrakou, S.Tsilimigaki, M. Sagnou, E. Monogiudis, G. Haniotakis, H. Karkaseli and E. Sarikaki, 2003. Biomonitoring with the comet assay of Greek greenhouse workers exposed to pesticides. Environm. Molec. Mutagenesis v.41 p. 104-110.

E. Dimitroglou, M. Zafiropoulou, N.Messini-Nikolaki, S.Doudounakis, S. Tsilimigaki and S. M. Piperakis, 2003. DNA damage in a human population affected by chronic psychogenic stress. Int. J. Hyg. Environ. Health, v.206, p. 39-44.

S. Tsilimigaki, N. Messini-Nikolaki, M. Kanariou, S.M. Piperakis, 2003. A study on the effects of seasonal solar radiation on exposed populations. Mutagenesis, v. 18, p.139-143.

S. Pastor, A. Creus, T. Parron, A. Cebulska-Wasilewska, C. Siffel, S. Piperakis, R. Markos, 2003. Biomonitoring of four European populations occupationally exposed to pesticides: use of micronuclei as biomarkers. Mutagenesis, v. 18, p. 249-58.

S.M. Piperakis, V. Papadimitriou, M.M. Piperakis, P. Zisis. 2003. **Understanding Greek Primary School Children's Comprehension of Sun Exposure. Journal of Science Education and Technology, v.12, p. 135-141.**

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. In press.

S.M. Piperakis , A. Sotiriou, E. Georgiou, A. Thanou, M. Zafiropoulou, 2004. **Understanding nutrition : a study of Greek primary school children dietary habits, before and after classroom nutrition intervention. Journal of Science Education and Technology.** In press.

V. Papadimitriou, S.M. Piperakis, 2004. An investigation into the Greek secondary school graduates' knowledge and awareness of healthy diet and nutrition. Journal of Science Education. In press.

2003 Presentations at International Scientific Conferences

4th PAEMS International Meeting on child health and environmental mutagenesis, Cairo, Egypt. March 2003. "DNA repair, apoptosis and necrosis in children and young and older adults".

Impact Factors (for 5 publications): 6,932

Citations 2001- 2003 (without self-citations): 19

Research Group: Radionuclide Transfer in the Soil-Plant System

Research Staff
Vassiliki Skarlou, Senior Research Specialist
Ioannis Massas, Postdoctoral Fellow
Marina Koutroumani, Research Technician
Theodoros Prassas, Research Technician

Research Interests

Soil pollution and radionuclide transfer from soil to annual crops and evergreen trees.

Soil parameters influencing radionuclide availability to plants.

Soil classification on the basis of transfer factors of radionuclides from soil to reference plants.

Fertigation for improved crop production and environmental protection (use of ^{15}N labeled fertilizers).

The behavior of heavy metals in soils.

2003 Findings

In the framework of investigating the main soil properties influencing radionuclide availability to plants, the main conclusions are:

1. In the framework of an IAEA Coordinated Research Program the classification of soil types according to ^{134}Cs uptake by reference plants was completed.
2. In all studied soil systems, the constant ratio between ^{134}Cs transfer factor for corn and the leafy crops observed in previous years, was also confirmed.
3. ^{134}Cs transfer factors for all studied crops were again higher in the volcanic – marginal soils than in the representative agricultural soils of the country.

2003 Presentations at International Scientific Conferences

V. Skarlou, I. Massas, C. Haidouti and Y. Papatheohari. 2003. Time dependent ^{134}Cs transfer factors for crops grown on deviating Greek soil types. 3rd FAO/IAEA/IUR Research Co-ordination Meeting of the Co-ordinated Research Project on the "Classification of Soil Systems on the basis of Transfer Factors of Radionuclides form Soil to Reference Plants". Chania Crete, 22-26 September.

Pavlou G., C. Ehaliotis, A. kardimaki and I. Massas. 2003. Effect of ^{15}N labelled N-sources (KNO3 and Urea) on pepper yield and N fertilizer utilization under fertigation practices.. I.A.E.A. project "Fertigation for improved crop production and environmental protection". Busteni, Romania 22-25 September.

Citations 2001- 2003 (without self-citations): 10

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Research Group: Insect Ecophysiology

Research Staff
George Tsiropoulos, Research Scientist
Mihalis Hatzis, Graduate Research Associate
Stilianos Zacharioudakis, Collaborating Graduate Student

Research Interests

Development of plant growth and protection technology using trapping systems containing food and sex attractants, antimetabolites and photosensitization substances, as well as, the use of agrochemicals acceptable for biological cultures. Technology development for the production of certified organic tobacco.

2003 Findings

Experimental research was continued for a 4th consecutive year, developing the necessary biotechnology for the production of organic tobacco. Problems regarding plant protection and tobacco field fertilization solved successfully and the final official certificate was awarded by DEO.

More specifically, it is pointed out the successful development and application of an innovative aquaculture system for the development of tobacco seedlings, using special containers filled with an appropriate plant growth mixture of nutrients (Float System). Tobacco seeds encapsulated within a special mixture of nutrients and micronutrients, were placed in floating multiple receptacles, allowing the root system to develop freely in the nutrient solution. The system produced high quality seedlings for field transplantation, reducing dramatically the need for re-transplantations. Moreover, the substitution of soil seedling development beds with the "Float System", nullified the need to use toxic soil disinfestation chemicals, such as methyl bromide, the use of which will be banned from 1-1-2005, according to a European Union Directive.

Also, the neurophysiological studies of Bactrocera oleae's chemical communication chemoreception organs with Electroantennographic methods were continued, in cooperation with Prof. G. Theophilides at the University of Thessaloniki, Biology Department, lab of animal physiology.

2003 Publications

K.Liaropoulos, G.Zervas, V.Mavraganis, T.Broumas, G.Tsiropoulos and V.Tsirogianis.2003. Comparative Field Studies of Various Traps and Attractants for the Mediterranean Fruit Fly, Ceratitis capitata. Entomologia Hellenica.*Accepted*.

K.Liaropoulos, R.L Zuparko, G.Tsiropoulos, B.Mavraganis and K.S.Hagen.2003. Key to the Hymenopteran Parasitoids Associated with the Olive Fly, Bactrocera oleae. Biocontrol. *Accepted*.

Citations 2001- 2003 (without self-citations): 1

Research Group: Chemical Ecology and Natural Products

Research Staff

Vassilios Mazomenos, Collaborating Research Scientist
Panagiotis Mylonas, Postdoctoral Fellow
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
- Study of insect chemical communication
- Development of pheromone formulation technologies, and biotechnological methods for pest control.
- Study of insect - host relationships
- Study of the molecular mechanisms involved in insect chemical communication

2003 Findings

- The study of the entomotoxic effect of the fungi *Mucor hiemalis* and *Penicillium chrysogenum*, in adult dipterans was carried out. The toxicity levels of the metabolites that are produced by the fungi were evaluated on adults of *Bactrocera oleae* and *Ceratitis capitata* in oral and contact bioassays. The chemical characterization of toxin structures was approached with the utilisation of chemical methods such as: High Pressure Liquid Chromatography (HPLC), Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS). The structure of the *M. hiemalis* toxins is very closed to be identified.

- The kairomonal effect of *Prays oleae*, and *Palpita unionalis* sex pheromone components as well as olive green leaf volatiles, in different concentrations, was investigated on the searching activity of the egg-parasitoid, *Trichogramma oleae*.

- 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). This pheromone formulation will be applied also for the last year of experiments of project

- 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 a second year in pilot plots for the control of Lepidoptera olive pests.

2003 Publications

Konstantopoulou M. and D. Raptopoulos (2003). Alcohol dehydrogenase allele frequencies in *Bactrocera oleae*: effect of ethanol addition in larval diet. J. Appl. Entom. 127, 243-247.

Swevers, L., Kravariti, L., Ciofli, S., Xenou-Kokoletsi, M., Ragousis, M., Smagghe, G., Nakagawa, Y., Mazomenos, B. and Iatrou K. (2003). A cell-based high-throughput screening

system for detecting ecdysteroid agonists and antagonists in plant extracts and libraries of synthetic compounds. *FASEB J* 17 (14): U218-U243.

Athanasiou, C.G., N.G. Kavallieratos, and B.E. Mazomenos**.** Effect of trap type, trap color, trapping location, and pheromone dispenser on male captures of *Palpita unionalis* (Lepidoptera: Pyralidae). *J. Econ. Entomol*. (in press).

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).
Mazomenos, B.E., Ch. A. Athanasiou, N. Kavalieratos and P. Milonas. 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*. (in press).

2003 Presentations at International Scientific Conferences

M. Konstantopoulou, P. Araujo and B. Mazomenos. Toxic metabolites to *Bactrocera oleae* from *Mucor hiemalis* SMU-21 isolate. 1st European meeting of the IOBC/WPRS Study Group "*Integrated Control in Olives*" pp 9, Maich-Chania Crete, Hellas, May 29-31, 2003.

E. Siskos, F. Krokos, M. Jervis, and B. Mazomenos. Insecticidal activity of *Citrus aurantium* chemicals against *Bactrocera oleae*. 1st European meeting of the IOBC/WPRS Study Group "*Integrated Control in Olives*" pp 37, Maich-Chania Crete, Hellas, May 29-31, 2003.

B. Mazomenos and A. Pantazi-Mazomenos. Mating disruption main component in an IPM system to control *Prays oleae* (Bern). 1st European meeting of the IOBC/WPRS Study Group "*Integrated Control in Olives*" pp 52, Maich-Chania Crete, Hellas, May 29-31, 2003.

C. Athanassiou, N. Kavallieratos and B. Mazomenos. Population dynamics of *Palpita unionalis* (Hübner) [Lepidoptera: Pyralidae] in central and northen Greece. 1st European meeting of the IOBC/WPRS Study Group "*Integrated Control in Olives*" pp 54, Maich-Chania Crete, Hellas, May 29-31, 2003.

C. Athanassiou, N. Kavallieratos and B. Mazomenos. Effect of trap type, trap color, trapping location and pheromone dispensers on male captures of *Palpita unionalis* (Bübner) [Lepidoptera: Pyralidae]. 1st European meeting of the IOBC/WPRS Study Group "*Integrated Control in Olives*" pp 55, Maich-Chania Crete, Hellas, May 29-31, 2003.

E. Agamy, A. Bento, B. Hafez, S. Hassan, E. Hegazi, A. Herz, T. Zardak, M. Ksantini, M. Konstantopoulou, B. Mazomenos, F. Nasr, J. Pereira, L. Torres, and A. Youssef. Trephelio-an international research project for sustainable control of Lepidopterous pests in olive groves. 1st European meeting of the IOBC/WPRS Study Group "*Integrated Control in Olives*" pp 71, Maich-Chania Crete, Hellas, May 29-31, 2003

Impact Factors (for 2 publications): 9,171

Citations 2001- 2003 (without self -citations): 73

C. STRUCTURAL BIOLOGY

Research Group: NMR Studies of Biomolecules and Parmaceuticals

Research Staff
Maria Pelekanou, Associate Research Scientist
Dimitra Benaki, Postdoctoral Fellow
Aggeliki Panagiotopoulou, 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

2003 Findings

In 2003 the activity of our team in the field of Alzheimer's disease continued with main focus on the study with NMR and CD of the solution structure of β-amyloid peptide (Aβ) as well as of its interactions with compounds known to prevent its aggregation. NMR and CD experimental data from the aqueous solution of plain Aβ(1-40) have been collected and are being processed in combination with theoretical modeling approaches (collaboration with Metaxia Vlassi). The product of the interaction of Aβ with Congo red has been isolated and is in the process of characterization with NMR and mass spectral analysis. A series of small molecules including thioflavin T, oleuropein, rifampicin, melatonin, and 4'-iododoxorubicin, known to prevent Aβ aggregation, have been investigated with NMR for binding to Aβ in D_2O and DMSO-d_6 solutions. In the case of thioflavin T and oleuropein, interaction with Aβ was verified in aqueous solution.

In parallel, in the effort to develop a radiodiagnostic for *in vivo* imaging of Alzheimer's amyloid plaques, a series of complexes of oxorhenium and oxotechnetium have been synthesized based on the structure of the dye thioflavin T which selectively dyes amyloid plaques in slices of brain tissue. Affinity of the synthesized ligands and complexes for amyloid plaques is being tested on human brain tissue slices from an Alzheimer's patient employing fluorescence microscopy.

Finally, the solution structure of humanin, a peptide with neuroprotective activity against Alzheimer's disease-related neurotoxicity, was elucidated with NMR and CD spectroscopies and theoretical modeling (collaboration with Metaxia Vlassi).

In the field of radiopharmaceuticals, investigation of the ligating properties of rhenium and technetium at oxidation states +5, +3 and +1 generated a number of novel complex structures. The affinity of theses complexes for specific target-tissues, namely, the 5-HT_{1A} receptors, the amyloid plaques of Alzheimer's disease, and malignant melanoma, was defined by the proper selection of ligand systems. Finally, progress has been made in the NMR study of the structure of a number of derivatives of the neuropeptide bombesin, as well as of their oxorhenium complexes. Receptors for bombesin are overexpressed in a number of cancer tissues, so this study is part of the effort to develop radiopharmaceuticals specific for tumors imaging and radiotherapy.

2003 Publications

Papachristou, M., Pirmettis, I., Siatra-Papastaikoudi, Th., Pelecanou, M., Tsoukalas, Ch., Raptopoulou, C. P., Terzis, A., Chiotellis, E., Papadopoulos, M. (2003). Synthesis and characterization of oxorhenium and oxotechnetium complexes with a novel tetradentate N_3O bifunctional agent. Eur. J. Inorg. Chem. 3826-3830

Patsis, G., Pirmettis, I., Tsoukalas, C., Pelecanou, M., Raptopoulou, C., Terzis, A., Papadopoulos, M., Chiotellis, E. (2003). Synthesis and characterization of binuclear μ-oxorhenium mixed ligand complexes containing tridentate (SNSEt) and monodentate (SR) ligands. Inorg. Chim. Acta 342, 272-278

Tsoukalas, C., Pirmettis, I., Patsis, G., Pelecanou, M., Bodo, K., Raptopoulou, C., Terzis, A., Papadopoulos, M., Chiotellis, E. (2003). Novel oxorhenium and oxotechnetium $MO[NS][S]_2$ complexes in the development of 5-HT_{1A} receptor imaging agents. J. Inorg. Biochem. 93**,** 2960-2967

Papachristou, M., Pirmettis, I. C., Tsoukalas, C., Papagiannopoulou, D., Raptopoulou, C., Terzis, A., Stassinopoulou, C. I., Chiotellis, E., Pelecanou, M., Papadopoulos, M. (2003). Synthesis and characterization of oxotechnetium (^{99}Tc and ^{99m}Tc) and oxorhenium complexes from the 2,2'-bipyridine (NN)/Thiol (S) mixed-ligand system. Inorg. Chem. 42, 5778-5784

Papagianopoulou, D., Pirmettis, I. C., Pelecanou, M., Tsoukalas, Ch, Raptopoulou, C. P., Terzis, A., Chiotellis, E., Papadopoulos, M. (2003). Synthesis and structural characterization of a novel Re[P][NN][S][SO] mixed ligand rhenium(III) complex. Inorg. Chim. Acta 346, 270-274

Benaki, D. C., Mikros, E., Hamodrakas, S. J. (2003). Conformational analysis of peptide analogues of silkmoth chorion protein segments using CD, NMR and molecular modelling. J. Peptide Sci., *in press*

2003 Presentations at International Scientific Conferences

Pirmettis, G. Patsis, C. Tsoukalas, S. Tzanopoulou, M. Pelecanou, M. Papadopoulos (2003). Tricarbonyl complexes of Re and Tc with a bifunctional tridentate NNO ligand carrying the 2-phenylbenzothiazole moiety. 15th International Symposium on Radiopharmaceutical Chemistry, August 10-14, 2003, Sydney, Australia.

C. Tsoukalas, I. Pirmettis, G. Patsis, M. Pelecanou, A. Papadopoulos, C. P. Raptopoulou, A. Terzis, M. Papadopoulos (2003). (2-Benzimidazolylmethylthio) acetic acid: a novel NSO ligand for the *fac*-$[M(CO)_3]^{3+}$ core (M = Re, ^{99m}Tc). 15th International Symposium on Radiopharmaceutical Chemistry, August 10-14, 2003, Sydney, Australia.

I. Pirmettis, C. Tsoukalas, G. Patsis, M. Pelecanou, A. Papadopoulos, D. Dokić, D. Janković, M. Papadopoulos (2003). A novel NSO ligand for the *fac*-$[M(CO)_3]^{+}$ core (M= Re, ^{99m}Tc). Congress of Nuclear Medicine - Serbia and Montenegro, September 24-27, 2003, Vrnjačka Banja, Serbia.

E. Mikros, K. Stathopoulou, D. Benaki, L. Leondiadis, N. Ferderigos, M. Pelecanou (2003). Binding studies of small molecules to amyloid peptide *β*-AP(1-28) using 1D NOESY experiments. Small Molecule NMR Conference 2003, September 15-17, 2003, Verona, Italy.

D. Benaki, K. Stathopoulou, A. Evangelou, Ch. Zikos, N. Ferderigos, E. Livaniou, M. Vlassi, M. Pelecanou, E. Mikros (2003). NMR structural studies of humanin, a peptide against Alzheimer's disease-related neurotoxicity. Euresco Conferences "NMR in Molecular Biology", September 5-10, 2003, Obernai, France.

Impact Factors(for 5 publications): 9,938

Citations 2001- 2003 (without self-citations): 60

Research Group: Protein Crystallography

Research Staff
Metaxia Vlassi, Associate Research Scientist
Maria Palaiomilitou, Postodoctoral Fellow
Athanassios Tartas, Graduate Student

Research Interests

Our current research activities are focused on structural studies of proteins of medical interest based on a combination of biophysical methods (x-ray Crystallography, Circular dichroism (CD)) and Biocomputing (3D Modelling). Our aim is to: 1) study the TPR mediated protein-protein interaction mechanism which is involved in many important biological functions, 2) investigate the structure/function relationship of a peptide with neuroprotective activity against Altzheimer's desease and 3) elucidate the structure/thermostability relationship of a thermostable chitinase from *Streptomyces thermoviolaceus*.

2003 Findings

1) In order to study the TPR mediated protein-protein interactions:
   - We performed structural and thermostability studies on two deletion mutants of Ssn6 (S4T & S400) containing different number of TPRs, using circular dichroism spectroscopy (CD).
   - We produced antibodies against the Tup1 interaction domain of Ssn6 using purified ScB (see previous annual reports).
   - We performed *in vitro* experiments to test the ability of S4T and S400 to interact with deletion mutants of Tup1 comprising its Ssn6 interaction domain. The complex formation was checked by Western Blots using antibodies against Ssn6 and Tup1. We found that only S4T is able to interact with the Tup1 deletion mutants we used for the experiments. Therefore, we designed new Ssn6 deletion mutants of the type of S400 based on tertiary structure predictions. The new variants are going to be cloned in different expression vectors than the ones used so far. The cloning experiments are in progress by our collaborator D. Tzamarias (IMBB/ITE, Heraklion, Crete). The objective is to identify structurally stable deletion mutants of both Ssn6 and Tup1 which will be able to interact and crystallize in order to determine their 3D structure by x-ray crystallography.
2) In the framework of our collaboration with M. Pelecanou (I.B., NCSR "Demokritos") in the field of Alzheimer disease, we studied the peptide humanine which shows a neuroprodective activity against the disease: we produced a theoretical 3D model of the humanine structure and studied its secondary structure using circular dichroism spectroscopy (CD). The 3D model was combined with NMR experiments performed by M. Pelecanou's group. The above study is financed by the Institute of Biology in the framework of an intrainstitutional collaboration with M. Pelecanou.
3) We produced a 3D model of a thermostable chitinase (Chi40) from *Streptomyces thermoviolaceus*. The 3D model will be used to estimate the thermodynamic parameters of the wild type as well as of designed deletion mutants of the protein. Comparison of the theoretic with experimentally derived thermodynamic parameters, using differential scanning calorimetry (DSC) (from G. Nounesis group, IRRP), is expected to indicate those domains of the protein responsible for its thermostability. This work is a collaborative work with the

groups of K. Vorgias (Univ. of Athens) and G. Nounesis (IRRP, NCSR "Demokritos").

2003 Publications

Mounaji, K., Vlassi M., Erraiss, N-E., Wegnez, M., Serrano, A., Soukri, A (2003). In vitro effect of metal ions on the activity of two amphibian glyceraldehyde-3-phosphate dehydrogenases: Potential metal binding sites. Comp Biochem Physiol B (Biochem Mol Biol). 135(2), 241-54

Leonidas D, Chavali G, Oikonomakos N, Chrysina E, Kosmopoulou M, Vlassi M, Frankling C, Acharya KR (2003). High-resolution crystal structures of ribonuclease A complexed with adenylic and uridylic nucleotide inhibitors. Implications for structure-based design of ribonucleolytic inhibitors. Protein Sci. 12(11), 2559-74.

2003 Presentations at International Scientific Conferences

A. Tartas, M. Palaiomylitou**,** G. Kefala, M. Vlassi (2003) Structural studies on the SSN6-TUP1 transcriptional repressor complex: evidence for a conformational change. 7th International School on the Crystallography of Biological Macromolecules, May 10-14, 2003, Como, Italy.

Pyrpassopoulos S., Ladopoulou A., Vlassi M., Papanikolau Y., Vorgias C.E., Yannoukakos D., Nounesis G. (2003) Thermodynamic Stability of the BRCT Tandem Repeats of Human Tumour Suppressor Gene Product BRCA1 – Characterisation of the Partly Unfolded Intermediate. 14th General Meeting of the Breast Cancer Linkage Consortium (BCLC) and the International Collaborative Group on Familial Breast and Ovarian Cancer (ICG-FBOC), June 2003, Madrid, Spain.

D. Benaki, K. Stathopoulou, A. Evangelou, Ch. Zikos, N. Ferderigos, E. Livaniou, M. Vlassi, M. Pelecanou, E. Mikros (2003). NMR structural studies of humanin, a peptide against Alzheimer's disease-related neurotoxicity. Euresco Conference "NMR in Molecular Biology", September 5-10, 2003, Obernai, France.

Impact Factors (for 2 publications): 4,303

Citations 2001- 2003 (without self-citations): 60

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 2003

The numbers of the various types of grafts, which were produced and delivered to Hospitals, during 2003, are listed in the following table.

GRAFTS	DELIVERY
Cancellous Bone	569
Cortical bone	14
Mixed bone	11
Dura mater	116
Cartilage	4
Cranium bone	8

2003 Publications

Vavouraki H., Dereka X., Vrotsos I., Marcopoulou C. Ability of a bovine bone graft alone or enriched with PDGF-BB or rhBMP-2 to promote periodontal ligament (PDL) cells proliferation. Cell and Tissue Banking, V4, p. 17-23, 2003.

Papadopoulou C., Dereka X., Vavouraki H., Vrotsos I. In Vitro Evaluation of the Mitogenic Effect of Platelet-Derived Growth Factor-BB on Human Periodontal Ligament Cells Cultured with Various Bone Allografts. Journal of Periodontology, 74 (4): 451-457, 2003.

Marcopoulou C., Vrotsos I.,Vavouraki H., Dereka X. Human Periodontal Ligament Cell Responses to Recombinant Human Bone Morphogenetic Protein-2 with and without Bone Allografts. Journal of Periodontology,74 (7) : 982-989, 2003.

Marcopoulou C., Vavouraki H., Dereka x., Vrotsos I. Proliferative effect of TGF-b1, PDGF-BB and rhBMP-2 in gingival fibroblasts and PDL cells. Journal of the International Academy of Periodontology, 5/3: 63-70, 2003.

EXPERIMENTAL ANIMAL COLONY

Research Staff

Effie-Fotini Tsilibary, Research Scientist
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 2003, the Animal Facility made available the following animals:

Users	***Rats***	***Mice***	***Rabbits***
Institute of Biology	7	48	1
Institute of Radioisotopes & Radiodiagnostics	30	236	2
University of Athens – Dpt Biology	266	8	
BAXTER SA	151		
"ELPEN" Pharmaceuticals	115		
ALCHIMICA SA		12	
Hellenique Institute Pasteur		35	
Doctors (6)	238		
Total of animals provided	**807**	**339**	**3**

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 an 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.

2003 Findings

During the year 2003, 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.

2003 Findings

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

EDUCATIONAL ACTIVITIES

EDUCATION

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

a. Training of young scientists at the postdoctoral level
b. Pre-graduate and graduate thesis work
c. Courses at the graduate level

During the year 2003, 16 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 2003, four of our graduate students finished their thesis work and became PhDs.

Moreover, 31 students from the University are carrying out their pre-graduate project thesis work at the Institute. Additionally, 7 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 2003 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: K. Sekeri, M. Voutsinas, V. Lampropoulou, and coordinator: V. Sophianopoulou].

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

- *Mutagenesis: cellular and environmental factors* (**Dr. G. Voutsinas**, University of Patras, Medical School)
- *The role of G proteins in health and disease* (**Dr. Iro Georgoussi,** Department of Biology, University of Athens)
- *Insect molecular biology and insect pest control* (**Prof. K. Iatrou**, Department of Biology, University of Athens)
- *Cell cultures – Tissue cultures* (**Dr. D. Kletsas**, Department of Biology, University of Athens).
- *Cell Cycle Checkpoints and Consequences for Physiological Cell Function* (**Dr. K. Sekeri** and **Dr. Th. Sourlinga** 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)
- *Matrix-mediated Signal transduction: The role of growth factor, proteoglycan, and integrin receptors* (**Dr. E. Tsilibari**, Department of Biology, University of Athens)

- *Matrix-mediated signal transduction: The role of proteoglycans, growth factor and integrin receptors*
- *Teaching in the framework of the postgraduate courses: "Bioinformatics" and "Clinical Bichemistry & Molecular Diagnostics" (***Dr. M. Vlassi**, Department of Biology, University of Athens)
- *General Biology* (**Dr. S.Piperakis**, School of Humanities, University of Thessalia)

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 (2003) 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 2003

GRADUATE STUDENT	TITLE OF DOCTORAL THESIS	ADVISOR (in Institute of Biology)	UNIVERSITY
Ellinida Thomadaki	Regulation of apoptosis during poyadenylation of mRNAs	Maria Chavredaki (retired)	Medical School, University of Athens
George Lallas	Strategies for chemoterapeutic agents and development of cellular resistance: post-translational modifications and apoptosis	Maria Chavredaki (retired)	Medical School, University of Athens
Aghelina Metaxatou	"Study of the ecology of the prominent bivalves in the Northern Euboikos Gulf with emphasis to the species *Callista chione* (L. 1753)."	Lydia Ignatiades	Marine Science Dpt, University of Aegean
Konstantinos Economou	"Regulation of podocalyxin gene expression and functional interplay with basement membranes in glomerular epithelial cells"	Fotini – Effie Tsilibary	Medical School, University of Athens

SEMINAR PROGRAMME 2003

DATE	SPEAKER	TITLE
17/2/03	**V. Douris** Institute of Biology, NCSR "Demokritos	Overexpression of recombinant polyDNA virus proteins in insect cells
24/2/03	**H. Pratsinis** Institute of Biology, NCSR "Demokritos"	Stress, ageing and tissue homeostasis: the case of fibroblasts from patients with Cushing syndrome
3/3/03	**E. Andronopoulou** Institute of Biology, NCSR "Demokritos"	Purification – biochemical characterization of chitinases and isolation – cloning of a chitinolytic enzyme gene fragment from the hyperthermophilic, anaerobic archaeon Thermococcus chitonophagus
17/3/03	**P. Kafasla** Institute of Biology, NCSR "Demokritos"	Involvement of the translational factor elF4G in pre-mRNA processing
26/3/03	**D. Bouzarelou** Institute of Biology, NCSR "Demokritos"	Characterization of fungal genes homologous to expansin genes of higher plants
26/3/03	**S. Tzanopoulou** Institute of Biology, NCSR "Demokritos"	Complexes of Rhenium and Technetium for radiodiagnostic applications
31/3/03	**M. Palaiomylitou** Institute of Biology, NCSR "Demokritos"	Ice Nucleation Protein : Relation between protein structure and ice nucleation activity
2/4/03	**A. Tartas** Institute of Biology, NCSR "Demokritos"	Biochemical and structural studies on the N-terminal of Ssn6 protein and its complexes with the N-terminal of Tup1 protein
7/4/03	**N. Balatsos** Institute Of Biology, NCSR "Demokritos"	Inhibition of PARN activity by the 80kDa subunit of CBC (nuclear cap-binding complex)
9/4/03	**I. Karakatsanis** Institute of Biology, NCSR "Demokritos"	The effect of TGF-beta on proliferation of mammalian fibroblasts.
14/4/03	**G. Drossopoulou** Institute of Biology, NCSR "Demokritos"	Sequential long and short – range Shh and Bmp signalling in vertebrate limb patterning
30/4/03	**Ch. Nikolaou** Institute of Biology, NCSR "Demokritos"	Skews in the use of n-tuplets as indices in the distinction between coding and non-coding genomic sequences
24/5/03	**L. Leodiadis** Institute of Biology, NCSR "Demokritos"	Functional interactions of proteins with G protein coupled receptors
21/5/03	**G. Pawelec** University of Tübingen	The Immune Risk Phenotype in Human Ageing
21/5/03	**A. Apostolidou** Institute of Biology, NCSR "Demokritos"	Identification of hemocyte proteins of Manduca sexta that interact with PDV gene products from Cotesia congregata
3/6/03	**Th. Sourlinga**	Changes in the linker histone H1 constitution of

	Institute of Biology, NCSR "Demokritos"	chromatin in peripheral blood lymphocytes as a function of donor age
4/6/03	**A. Talamagas** Institute of Biology, NCSR "Demokritos"	Amyloid-β interacts with integrins and modulates the expression of integrins and collagenases in SK-N-SH neuroblastoma cell line
4/6/03	**E. Morou** Institute of Biology, NCSR "Demokritos"	Structural determinants of opioid receptor coupling with G proteins and effectors
9/6/03	**D. Benaki** Institute of Biology, NCSR "Demokritos"	Structural studies of β-AP(1-40) and β-AP(1-28) peptides with NMR and CD
11/6/03	**Th. Georgomanolis** Institute of Biology, NCSR "Demokritos"	Characterization of the BmSH3 protein of silkworm *Bombyx mori*
11/6/03	**G. Mazarakou** Institute of Biology, NCSR "Demokritos"	Mechanism of STAT5A transcriptional factor phosphorylation
18/6/03	**K. 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*
18/6/03	**E. Erpapazoglou** Institute of Biology, NCSR "Demokritos"	Study of nucleobase and amino acid transporters in the filamentous fungus *Aspergillus*
25/6/03	**A. Kypraiou** Institute of Biology, NCSR "Demokritos"	The role of histone H1o and histone H4 acetylation during ageing and apoptosis of T-lymphocytes
25/6/03	**Ch. Giannouli** Institute of Biology, NCSR "Demokritos**"**	A study of differential effects of TGF – B on human fibroblasts replication
1/7/03	**M. Konstantopoulou** Institute of Biology, NCSR "Demokritos"	Mycotoxins as biological control tools
29/9/03	**S. Papageorgiou** Institute of Biology, NCSR "Demokritos"	The mystery of Hox gene collinearity and its proposed solution
20/11/03	**Ch. Nikolaou** Institute of Biology, NCSR "Demokritos"	Regulatory transcriptional networks in the yeast *S. cerevisiae*
20/11/02	**Z. Erpapagoglou** Institute of Biology, NCSR "Demokritos"	Role of a peptide tagging system in degradation of proteins synthesed from damaged messenger RNA
27/6/03	**I. Karakatsanis** Institute of Biology, NCSR "Demokritos"	A beta-arrestin-dependent scaffold is associated with prolonged MAPK activation in pseudopodia during protease-activated receptor-2-induced chemotaxis
27/6/03	**P. Venieratos** Institute of Biology,	High Glusose Causes Apoptosis in Cultured Human Pancreatic Isles of Langerhans. A

	NCSR “Demokritos”	Potential Role for Regulations of Specific Bcl Family Genes Toward an Apoptotic Cell Death Program
4/12/03	**A. Kypraiou** Institute of Biology, NCSR “Demokritos”	Histone deacetylase 6 (HDAC 6) interacts with and deacetylates tubulin and microtubules in vivo
11/12/03	**M. Billini** Institute of Biology, NCSR “Demokritos”	Topological analysis of a plant vacuolar Na+/H+ antiporter reveals a luminal C terminus that regulates antiporter cation selectivity
18/12/03	**A. Tartas** Institute of Biology, NCSR “Demokritos”	Structure of Simian virus 40 at 3,8 Å resolution
18/12/03	**I.Tsagaraki** Institute of Biology, NCSR “Demokritos”	Apoptotic release of histones from nucleosomes

COLLECTIVE DATA

FINANCIAL REPORT 2003

1. Internal Funding from the Special Account Department

Income	**E u r o**
Carried over from 2002	133.483
Funding from NCSR “D”	25.000
Matching Funds	43.623
Income from services	8.233
Donations from companies	1.235
Transfer from other sources	3.426
TOTAL	**215.000**

Expenses	
Equipment	17.000
Supplies	42.000
Salaries	23.700
Travels	11.500
Supplies from “Demokritos”	9.000
Other expenses	17.500
Committed	10.000
TOTAL	**130.700**

2. Governmental Funding

	E u r o
Equipment maintenance	6.000
Travel	1.050
Daily compensation	700
Replacement parts	3.550
Animal chow	5.500
Liquid nitrogen, helium	6.000
Xeroxing and similar supplies	700
TOTAL	**23.500**

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 (6)	194.260	63.000	31.740	289.000
General Secretariat for Research & Technology (10)	159.741	-	-	159.741
KOTSIKA Foundation (1)	6.000	-	-	6.000
NOVONORDISK – HELLAS (1)	20.000	-	-	20.000
National Organisation for Atomic Energy (1)	-	5.000	-	5.000
SEKAP S.A. (1)	-	55.000	-	55.000
National Bank of Greece (1)	-	-	4.850	4.850
Other international funds (3)	28.165	-	-	28.165
TOTAL	**408.166**	**123.000**	**36.590**	**567.756**

COLLECTIVE DATA ON PRODUCTIVITY OF SCIENTIFIC PROGRAMMES

	PROGRAMME			
	A	**B**	**C**	**INSTITUTE**
Researchers and Senior Research Specialists	13	3	2	18
Technical Specialist	1	2	1	$_{\$}$5*
Collaborating Research Scientists	8	1	-	9
Postdoctoral Fellows	12	2	2	16
Graduate Students	24	-	2	26
Collaborating Graduate Students	8	2	-	10
Graduate Research Associates	-	1	-	1
Undergraduate Students	26	5	-	31
Research Technicians	5 #	3	-	12 @
Administrative Staff	-	-	-	3
Total Personnel	**97**#	**19**	**7**	**131**
Publications in Peer-Reviewed Journals	**19**+	**7**+	**7**	**34****
Publications (Average) in Peer-Reviewed Journals per Scientist	1.46	2.33	3.5	1.78
Cumulative Impact Factor in Peer-Reviewed Journals (number of publications)	**68.798 (19)**	**16.103 (7)**	**14.241 (7)**	**94.195 (34)**
Average Impact Factor in Peer-Reviewed Journals	3.620	2.300	2.034	2.770
Cumulative Impact factor per Scientist	5.292	5.368	7.120	4.958
Proceedings to International Conferences	**9**	**1**	**-**	**10**
Proceedings (Average) per Scientist	0.69	0.33	-	0.55
Total International Publications	**28**	**8**	**7**	**44**
International Publications (Average) per Scientist	2.15	2.66	3.5	2.31
Publications to Greek Journals, Books or Proceedings	28	3	5	16
Total Publications	**36**	**10**	**12**	**60**
Publications (Average) per Scientist	2.76	3.33	6	3.15
International Patents	**-**	**-**	**-**	**-**
Greek Patents	**-**	**-**	**-**	**-**
Presentations to International Conferences	**14**	**6**	**8**	**28**
Presentations (Average) per Scientist to International Conferences	1.07	2	4	1.55
Presentations to Greek Conferences	**31**	**16**	**2**	**49**
Presentations (Average) per Scientist to Greek Conferences	2.38	5.33	1	2.72
Total Presentations to Conferences	**45**	**22**	**10**	**77**
Presentations (Average) per Scientist to Conferences	3.46	7.33	5	4.27

** 1 Technical Specialist of Human Tissues Bank and 1 Technical Scientist of Laser Confocal Microscopy are included*

$_{\$}$1 Technical Specialist common in A and B programme is included

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

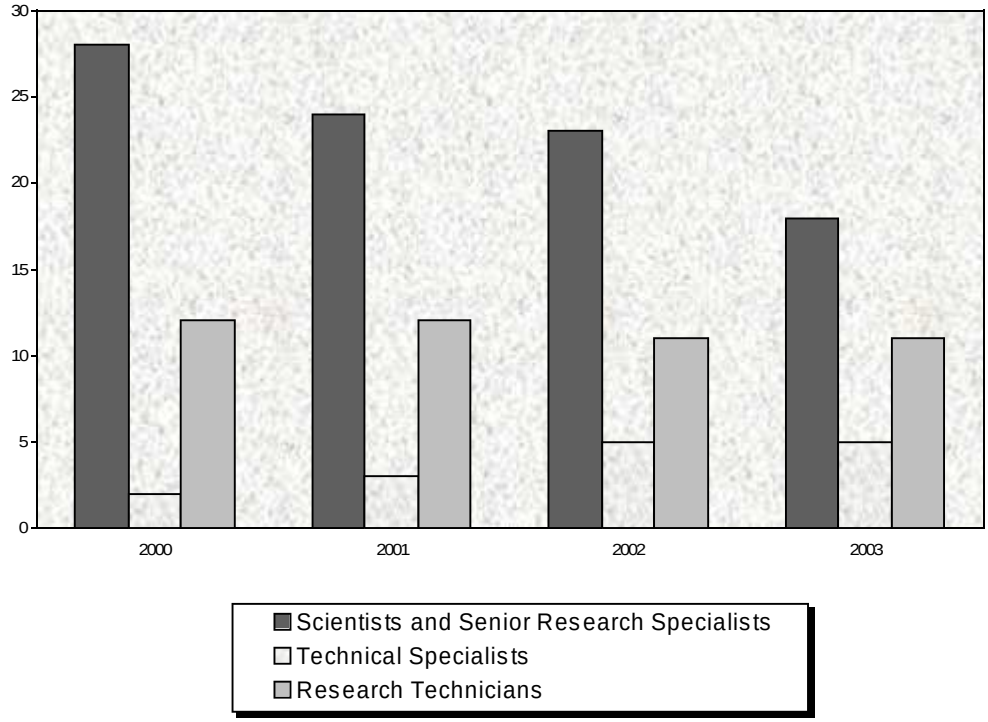
@ 2 Research Technicians who are occupied in Experimental Animal Colony and 1 Research Technician who is occupied in Human Tissue Bank are included

+1 publication common to A and B programme is included

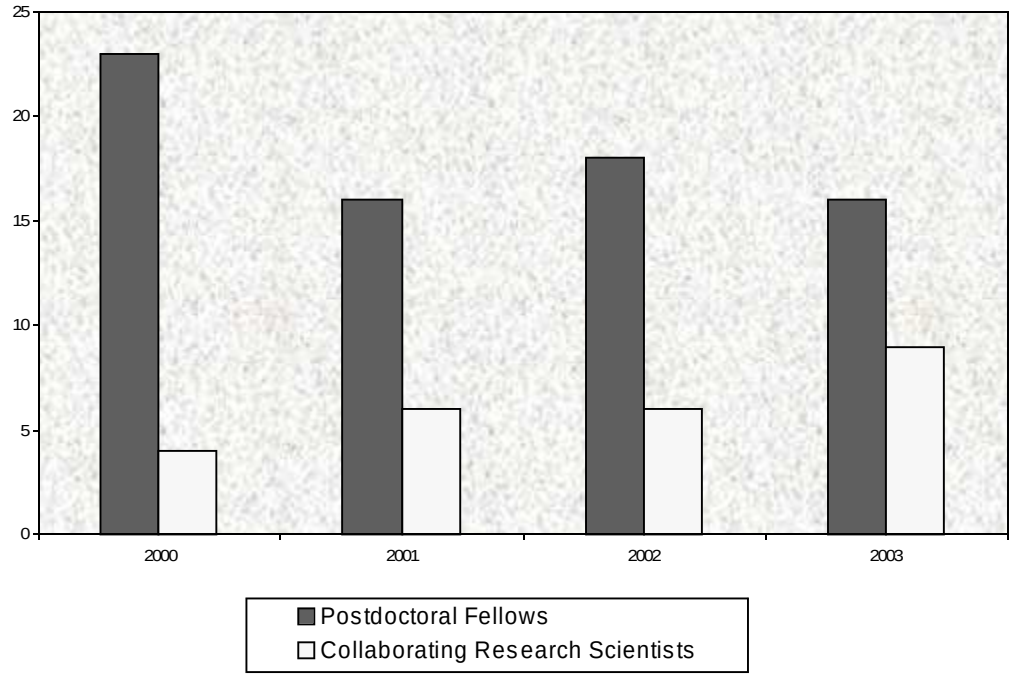
***2 publications of the Head of Human Tissue Bank are included*

CHANGES OF IB STAFF DURING 2000-2003

"TENURED EMPLOYEES"

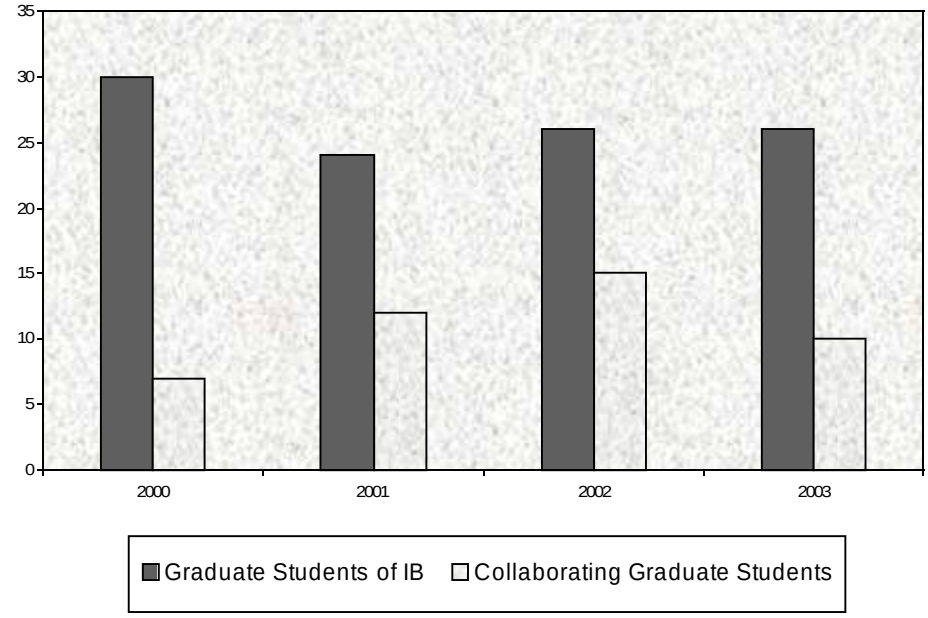


"POSTDOCTORAL FELLOWS AND COLLABORATING RESEARCH SCIENTISTS"

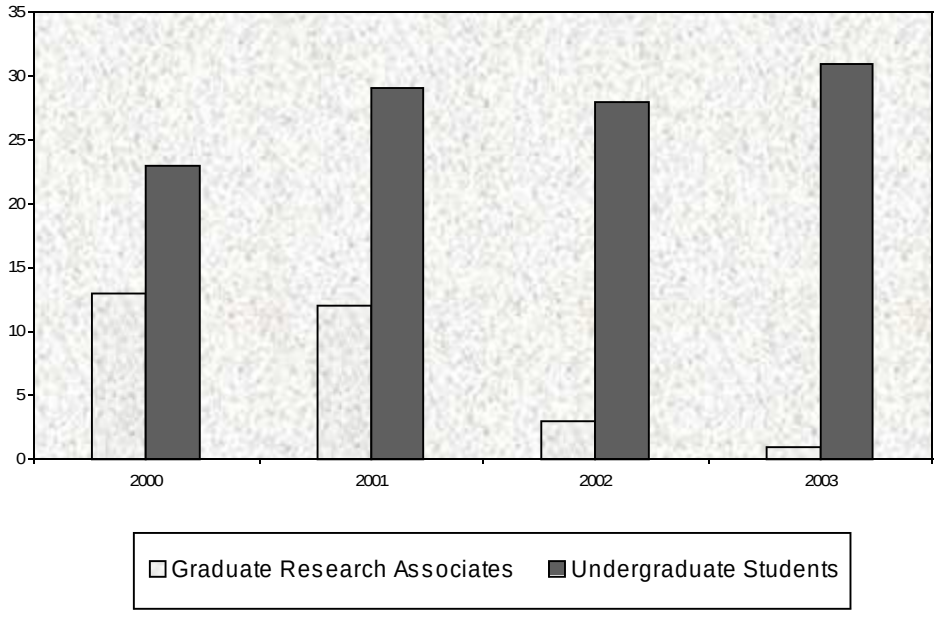


CHANGES OF IB STAFF DURING 2000-2003

"GRADUATE STUDENTS"

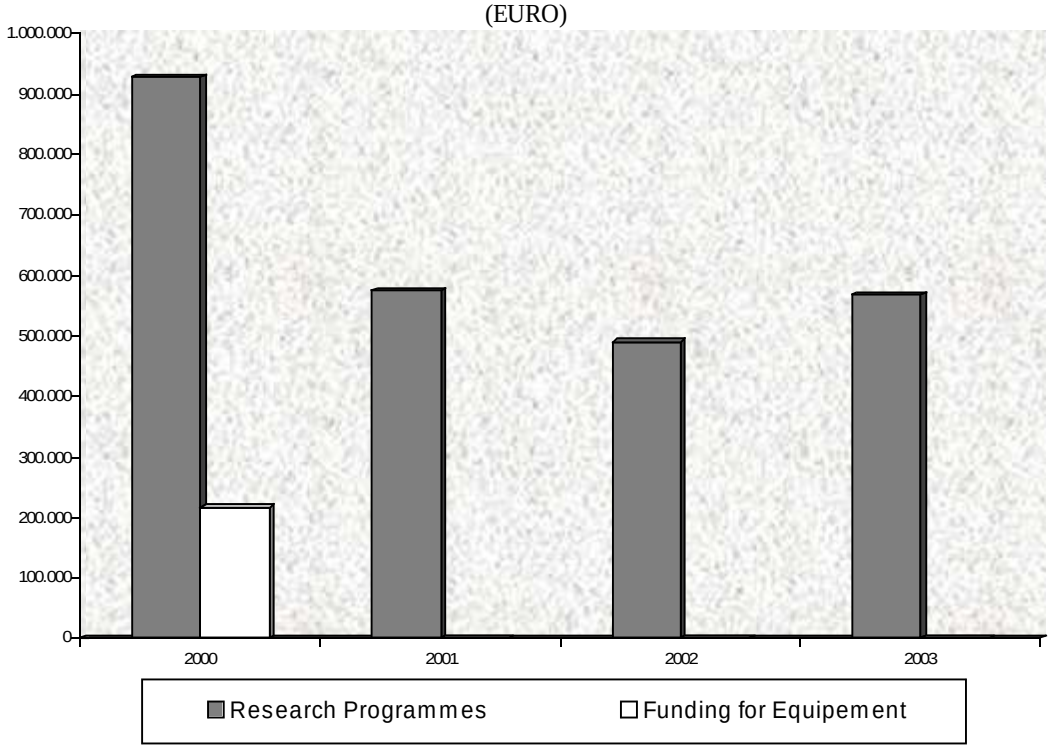


"GRADUATE RESEARCH ASSOCIATES AND UNDERGRADUATE STUDENTS "



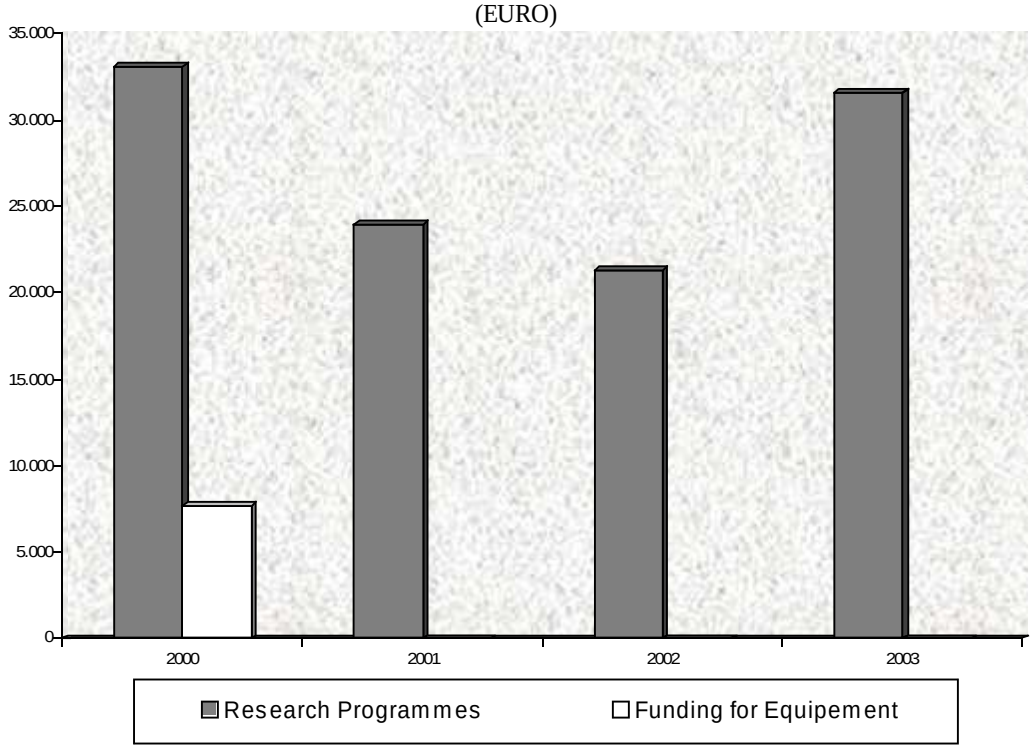
CUMULATIVE EXTERNAL FUNDING OF THE INSTITUTE DURING 2000-2003

(EURO)

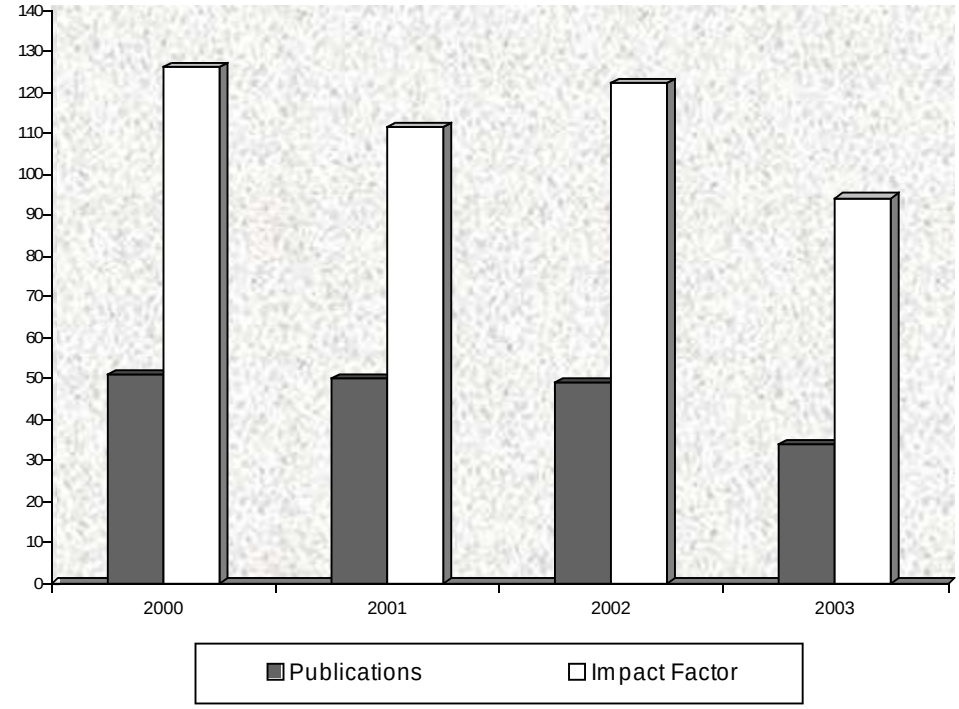


EXTERNAL FUNDING OF THE INSTITUTE PER SCIENTIST DURING 2000 - 2003

(EURO)



PUBLICATIONS IN PEER-REVIEWED JOURNALS AND CUMULATIVE IMPACT FACTOR DURING 2000-2003



PUBLICATIONS IN PEER-REVIEWED JOURNAL AND AVERAGE IMPACT FACTOR PER SCIENTIST DURING 2000-2003