NATIONAL CENTER FOR SCIENTIFIC RESEARCH
"DEMOKRITOS"

INSTITUTE OF BIOLOGY

2002
ANNUAL REPORT

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INTRODUCTION

The Institute of Biology (IB), one of the eight Institutes of the National Centre for Scientific Research (NCSR) "Demokritos", was established in 1962. Its mission is to:

- To foster high quality research in specialized areas of Biochemistry, Biophysics, Cellular Biology, Molecular Genetics, Environmental Biology, Biotechnology and Biomedicine.
- To train new scientists at the graduate and postdoctoral level.
- To promote the collaboration with Greek, European and other international research organizations.
- To apply the scientific findings toward the protection and improvement of public health and to solve problems of economic importance.
- To transfer new technological know-how to Greek Public Services and Organizations and to Greek, European and international industries.

The research activities at the IB are carried out in the context of three Research Programmes:

A. Biochemistry, Cell and Molecular Biology

B. Environmental Biology

C. Structural Biology

The various research projects encompassed by the three programmes are presented in the following pages of this report. The ongoing research projects aim at the achievement of the goals of the development plan of the Institute, which targets the following three fundamental areas of biosciences:

1. Cellular Function and Methods for Combating Pathological Conditions

2. Structure and Function of Bioactive Molecules

3. Biotechnology and Natural Products

These broad areas of research also determine the directions, which the activities of the Institute aim at. These directions satisfy the requirement for the building of strong links between the research area and the industrial sector while, at the same time, they ensure the continuation of competitive research by the personnel of the Institute in areas that are in the forefront of biosciences.

In addition, the IB has two Service Units that provide services to the public and private sector. These are:

- **The Experimental Animal Colony**
- **The Tissue Transplant Bank**

The mission of the first unit is to support the research activities of the NCSR “Demokritos” and other Research and Educational Institutions of the country, and that of the second unit is to cover at least part of the needs of the hospital surgical units for human transplants. The activities of the two Service Units are presented in detail in the relevant sections of the Annual Report.

In 2002, 137 persons in total participated in the R&D and service activities of the Institute. Amongst them are permanent staff members (Researchers, Specialized Scientists, Research Technicians, Assistants and Administrative Employees) as well as collaborating scientists, postdoctoral collaborators, graduate students and undergraduate students.

The Institute’s research activities are supported by funds obtained from the budget of the Centre, which is provided by the Greek government and from competitive research programs that are financed by the European Union, the General Secretariat of Research and Technology (GSRT) and other public and private funding agencies in Greece and abroad.

After a relatively long delay, calls for different competitive funding programs within the context of the 3rd (European) Community Support Framework were activated by the GSRT in 2002. These programs aimed at (1) the support of research programs of strategic importance, (2) the strengthening of scientific collaborations with a number of non-European countries, such as the USA, Canada, Australia, New

Zealand, Japan, North Korea, Taiwan, Malaysia and Singapore, with which no possibility of support existed until recently, and (3) the interconnection between Research and Industry and the support of initiatives that target the commercialization of research results. The Researchers of the Institute responded to these calls and it is anticipated that during 2003 new sources of support will become available for their research activities.

As in the previous year, during the month of July, the IB organized, for a period of two weeks, its Summer School "Days of Biology". The School offered lectures, focusing on the new developments in biomedical sciences as well as information on the research activities that take place at the Institute, to senior undergraduates and recent graduates of the country's Higher Educational Institutions. Speakers were recruited from the Institute of Biology as well as from other research centres and universities from Greece and abroad. Besides the attendance of lectures, the students had the opportunity to visit the laboratories of the Institute, engage in discussions with the personnel and become informed about the opportunities for graduate studies in Greece and abroad. For information on employment opportunities, a discussion was organized with participation of speakers from the academic world and the private sector. The 80 students who attended the complete program of the "Days of Biology" and completed the evaluation forms expressed very complimentary comments about it.

In December 2002, Dr. Nikos Grammatikakis, Assistant Professor at the Medical School of the University of Boston with a Ph.D. in Molecular Biology from the Department of Medicine of the University of Texas, Galveston and previous postdoctoral appointments at the Department of Anatomy and Cellular Biology of Tufts University in the USA, the Department of Microbiology and Immunology of Queens University in Canada and the Department of Physiology of Tufts, joined the ranks of the IB. Dr. Grammatikakis was selected by a selection committee, which examined candidates for a research position in the field of "Molecular Biology of Cellular Signaling". The research interests of Dr. Grammatikakis are concentrated on the role of chaperone proteins in the regulation of the function of proteins that are implicated in multiple signaling pathways in the cell. To Dr. Grammatikakis, who was elected to the available position as an Associate Research Scientist, we wish a creative and productive career at the IB and fruitful collaborations with other researchers of the Institute who conduct related studies on the mechanisms of cellular signaling.

Our recruitment effort for an additional faculty position in the field of "Functional Genomics and Cell Function", that also took place in December 2002, was unsuccessful because of lack of suitable candidates. The selection process for the vacant position will be re-activated in 2003.

The situation with the recruitment of new faculty members must, unfortunately, be contrasted to the retirements of senior research personnel and other staff from the Institute that took place during 2002. After a long and productive tenure at the IB, our colleagues Spiros Loukas, Bassilis Mazomenos, Thanasis Manoukas, Chariklia Stassinopoulou and Maria Havredaki (research scientists), Dimitris Kopanelis and Vassilis Papadopoulos (technicians) and Panagiotis Douvaras (adminitrator) retired from the Institute. The Institute expresses its appreciation for the multiple services that were rendered to it by our retiring colleagues over the years and wishes them success in their future endeavors, a long life and every personal happiness. In the midst of these personnel losses, we are happy that, based on a recent decision by the Centre's Board of Directors for the automatic replacement of scientific personnel positions which become vacant due to retirement, the Institute will have the opportunity in the immediate future to initiate new searches for the filling of 5 new faculty positions in research fields that are compatible with the development targets of the Institute.

It must also be noted that, after successful assessment by an external committee, our colleague, Dr Dimitris Kletsas, was promoted to the rank of Associate Research Scientist. The Institute extends its warm congratulations to Dr Kletsas and wishes him success in the continuation of his career.

Finally, in January 2003, the 5th Annual Retreat of the Institute took place at a hotel in the town of Vrahati in the Peloponnese. During the two days of the retreat, all members of the Institute had the opportunity to become updated about the research activities and new findings of the Institute's research groups, discuss in depth other topics of general interest to the Institute, and enjoy a few moments of peace in a hospitable environment away from the daily routine.

In closing this introduction and with the opportunity of the upcoming completion of my service as Director of the Institute in June 2003, I wish to express my sincere appreciation to all the personnel of the

Institute for the efforts they made toward the realization of the Institute's strategic goals, not only during this year but also during the whole period of my directorship, from the time of my appointment in June 1998 until now. I believe that our Institute is now significantly different than the one whose leadership I assumed in 1998 and that solid foundations for its future development are now in place. The transformation of the Institute to its present form necessitated much effort by all its personnel, the undertaking of multiple new initiatives and the implementation of sometimes difficult but always necessary decisions and measures on my part, which were essential for the realization of the aspired transformation. I hope that these efforts will continue undiminished in the future and wish our staff continuing success in all their activities.

Professor Kostas Iatrou
Director

P E R S O N N E L

DIRECTOR

Iatrou Kostas	Professor of Biochemistry and Molecular Biology

SCIENTIFIC STAFF

Research Scientists (Group Leaders)

Iatrou Kostas	Professor of Biochemistry and Molecular Biology
Havredaki Maria	Biologist
Manoukas Athanassios	Nutritionist
Mazomenos Vassilios	Chemical Ecologist
Sekeri Kalliope	Biochemist
Stassinopoulou Chariklia	Chemist
Tsilibary Effie	Biologist
Tsiropoulos George	Entomologist

Associate Research Scientists

Almirantis Yannis	Chemist
Georgoussi Zafiroula-Iro	Biochemist
Loukas Spyros	Biochemist
Pelecanou Maria	Pharmacist
Sophianopoulou Vassiliki	Biologist
Vlassi Metaxia	Physicist-Chrystallographer

Assistant Research Scientists

Kletsas Dimitris	Biologist
Labropoulou Vassiliki	Biochemist
Piperakis Stelios	Biologist
Prombona Anastassia	Biologist
Stamatakis Konstantinos	Biologist
Voutsinas Gerassimos	Biologist
Swevers Luc	Biologist
Tzinia Athina	Biochemist

Senior Research Specialists

Skarlou-Alexiou Vassiliki	Agronomist

Technical Specialists

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

RESEARCH TECHNICIANS

Avgeris Socrates
Doulgeridis George
Kakkos Stilianos
Kalokiri-Stilianidi Kalliope
Kopanelis Dimitrios
Koutroumani Marina
Pantazi-Mazomenou Anastassia
Prassas Theodoros
Sevaslidou Eleni
Tsolomiti-Gourgou Areti
Zafiropoulos Ioannis

ADMINISTRATIVE STAFF

Douvaras Panagiotis	Accountant
Kostakou Athanassia	Secretary

COLLABORATING RESEARCH SCIENTISTS

Collaborating Research Scientist	**LLaboratory**
Ignatiadou Lydia (Dr. Hydrobiologist)	Iatrou K.
Papageorgiou George (Dr. Biochemist)	Stamatakis K.
Papageorgiou Spyros (Dr. Physicist)	Almirantis I.
Sideris Eleftherios (Dr. Geneticist)	Sophianopoulou V.
Stathakos Dimitri (Dr. Biochemist)	Kletsas D.
Sourlingas Thomae (Dr. Biologist)	Sekeri K.

POSTDOCTORAL FELLOWS

Fellow	**Supervisor**
Andronopoulou Evi	Iatrou K.
Atlamazoglou Vassilios	Loukas S.
Arauzo – Hermandez Pedro	Mazomenos V.
Balatsos Nikolaos	Georgoussi I
Benaki Dimitra	Stassinopoulou C.
Dedos Skarlatos	Iatrou K.
Douris Vassilios	Iatrou K.
Drossopoulou Garifallia	Tsilibari E.
Georgakopoulos Ioannis	Mazomenos V.
Giannoulaki Eleni	Havredaki M.
Kafasla Panagiota	Sophianopoulou V.
Kefala Georgia	Vlassi M.
Kiriakopoulou Christina	Havredaki M.
Kitsiou Paraskevi	Tsilibary E.
Palaiomilitou Maria	Vlassi M.
Pratsinis Haris	Kletsas D.
Tsapali Dimitra	Sekeri K.
Zervolea Irene	Kletsas D.

GRADUATE STUDENTS

Student	Supervisor
Apostolidou Anastassia	Voutsinas G.
Argyrou Eleftheria	Sophianopoulou V.
Bouzarelou Dimitra	Rotation
Economou Konstantinos	Tsilibari E.
Erpapazoglou Zoi	Sophianopoulou V.
Georgomanolis Theodoros	Iatrou K.
Giannouli Christina	Kletsas D.
Handris Panagiotis	Kletsas D.
Kaldis Athanassios-Dimitrios	Prombona A.
Karakatsanis Ioannis	Kletsas D.
Konti Maria	Rotation
Kovaiou Ourania	Sekeri K.
Kypreou Aikaterini	Sekeri K.
Lallas George	Havredaki M.
Leontiadis Leonidas	Georgoussi I.
Massas Ioannis	Skarlou V. – **PhD obtained in 2002**
Mazarakou Georgia	Georgoussi I.
Morou Evaghelia	Georgoussi I.
Nikolaou Christoforos	Almirantis Y.
Sdralia Konstantia	Iatrou K.
Sideridou Maria	Sophianopoulou V.
Thomadaki Ellinida	Havredaki M.
Talamagas Anargiros	Tsilbari E.
Tartas Athanassios	Vlassi M.
Tavoularis Stefanos	Sophianopoulou V.- **PhD obtained in 2001**
Tsagaraki Ioanna	Rotation
Tzanopoulou |Stamatia	Rotation
Vieneratos Panagiotis	Rotation

GRADUATE RESEARCH ASSOCIATES

Fellow	Supervisor
Bouzarelou Dimitra	Sophianopoulou V.
Hatzis Michalis	Tsiropoulos G.
Tsilimigaki Smaragdi	Piperakis S.

COLLABORATING GRADUATE STUDENTS

Student (University)	Supervisor
Alexandratou Eleni (Technical Univ. of Athens)	Loukas S.
Ciofi Silvia (Univ. of Siena, Italy)	Iatrou K.
Fanourakis Galenos (Univ. of Athens)	Voutsinas G.
Fragopoulos Evaggelos (Univ. of Rome)	Tsilibari E.
Goudopoulou Athina (Univ. of Athens)	Voutsinas G.
Kosmopoulou Aggeliki (Univ. of Ioannina	Vlassi M.
Ladas Nektarios (Univ. of Athens)	Stamatakis K.
Maniou Vassiliki (Univ. of Athens)	Stamatakis K.
Metaxatou Aghelina (Univ. of Eagean)	Iatrou K.
Michalopoulos Nicholaos (Univ. of Athens)	Voutsinas G.

Moutzouris Dimitrios (Univ.of Athens)	Tsilibari E.
Nikolopoulos George (IRRP, NCSR "D")	Vlassi M.
Plearchopoulou Kiriaki (Univ. of Athens)	Voutsinas G.
Siskos Elias (Univ. of Cardiff)	Mazomenos V.
Tzanopoulou Stamatia (Univ. of Athens)	Pelekanou M.
Zacharioudakis Stilianos (Univ. of Athens)	Tsiropoulos G.

UNDERGRADUATE STUDENTS

Student (University)	**Supervisor**
Anagnostakis Nikos (Univ. of Athens)	Piperakis S.
Antoniadis Petros (Univ. of Athens)	Iatrou K.
Barkoulas Michalis (Univ. of Athens)	Prombona A.
Beloukas Apostolos (Univ. of Athens	Prombona A.
Billini Maria (Univ. of Athens)	Sophianopoulou V.
Christopoulos George (Univ. of Athens)	Piperakis S.
Economopoulou Ifigenia (Univ. of Reading, UK)	Iatrou K.
Giakountis Antonios (Univ. of Thessaly)	Prombona A.
Gikopoulos Triantafillos (Univ. of Wales, UK)	Prombona A.
Heliou Maria (Univ. of Patras)	Tsilibari E.
Kalariti Niki (Univ. of Athens)	Labropoulou V.
Kanavetas Panagiotis (Univ. of Athens)	Piperakis S.
Karambelas Theodore (Univ. of Aberdeen)	Kletsas D.
Karanastassi Georgia (Univ. of Athens)	Piperakis S.
Makris Leonidas (Univ. of Athens)	Sekeri. K.
Maridaki Kiriaki (Univ. of Athens)	Piperakis S.
Melachrinou Sofia (Univ. of Athens)	Voutsinas G.
Milioti Natalia (Univ. of Cardiff, UK)	Voutsinas G.
Mirsaliotis Antonios (Univ. of Cardiff)	Iatrou K. – Sophianopoulou V.
Nana Evaggelia (Univ. of Reading)	Voutsinas G.
Nikitopoulou Ioanna (Univ. of Athens)	Tsilibari E.
Papaspiridakos Stefanos (Univ. of Athens)	Voutsinas G.
Stavropoulou Alexandra (Univ. of Queen Mary, UK)	Voutsinas G.
Thanos Nikolaos (Univ. of Athens)	Georgoussi I.
Tsekrekou Maria (Technical Univ. of Athens)	Voutsinas G.
Vaggelatos Ioannis (Univ. of Athens)	Sophianopoulou V.
Vlachaki Chrissanthi (Charokopeio Univ.)	Tsilibari E.
Xedous Marios (Univ. of Athens)	Georgoussi I.

BIOCHEMISTRY, CELL AND MOLECULAR BIOLOGY

RESEARCH GROUP: Signal Transduction Mechanisms –Molecular Pharmacoloy

Research Staff

Iro Georgoussi, Associate Research Scientist
Nikolaos Balatsos, Postodoctoral Fellow
Georgia Mazarakou, Graduate Student
Evaghelia Morou, Graduate Student
Leonidas Leodiadis, Graduate Student
Nikolaos Thanos, Undergraduate 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 couples receptors in which opioid receptors belong and b) in the identification of the distinct signaling circuits that occur in the proximity or not of the receptor which lead in changes of certain transcription or mitogenic factors. Understanding the mechanisms of signal sorting and integration between different signaling pathways will aid in the design and development of novel therapeutic agents.

2002 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 we developed a minigene encoding this domain of the opioid receptor in an attempt to develop potential activators or inhibitors of this receptor with the G protein signaling cascade. Expression of this minigene in intact HEK 293 cells, stably transfected to express the μ or δ-opioid receptors, alters the levels of cAMP accumulation for opioid receptors (μ,δ). Experiments designed to observe the levels of the internalization upon chronic treatment of the opioid receptors in the presence of the minigene are under investigation.

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 exposure to morphine, or to the μ-opioid peptide DAMGO, of COS-7 cells transiently transfected with the μ-opioid receptor lead to phosphorylation of STAT5A/B members of the family of Signal Transducers and Activation of Transcription. In order to examine which protein kinases are responsible for morphine or DAMGO induced STAT5A phosphorylation selective inhibitors for various kinases are used. Our data suggest that the morphine phosphorylation of STAT5A is due to a Src kinase, whereas DAMGO induced STAT5A phosphorylation involves a MEK kinase.

Analyses of opioid receptor signaling pathways "beyond" G proteins and identification of modular interactive protein domains. As an alternative approach to assess novel interacting partners of G protein coupled receptors, particularly the opioid receptors, we have generated S-glutathione (GST) fusion proteins of the third intracellular loop and the carboxyl-terminal regions of the delta and mu opioid receptors to use them as probes in pull down assays. Indeed we demonstrate for the first time the ability of the c-terminal tails of the opioid receptors to behave as individualized signaling domains responsible to interact with novel interacting partners, the role of which in the opioid receptor signaling cascade is under investigation. *Collaborations:* Department of Pharmacology Vanderbilt University School of Medicine, Nashville, TN, USA.

Development of HighTroughput Screens (HTP): Efforts are being made to develop HTP screening systems for ligands that bind to the human serotonic receptor (h5-HT4_aR) and the μ and δ opioid receptor using insect cell-based assays that express these receptors. In this regard, transient transfections of Bm5 cells with expression constructs for the human 5-HT_{4A} serotonin and/or the opioid receptors demonstrate high expression levels and functional coupling of the receptors to the endogenous G protein machinery (by measuring increases in cAMP levels following agonist stimulation). In parallel, reporter systems for the serotonin and opioid receptors were also constructed. These consist of a basal reporter cassette (Green Fluorescence Protein (GFP) ORF downstream of a basal promoter derived from the silkmoth actin gene) containing several copies of the conserved CRE and TRE elements in its upstream region. To test the functionality of the reporter system, cells

cotransfected with the expression and reporter constructs are monitored for GFP fluorescence (before and) after administration of receptor agonists.

2002 Publications

Vassilaki T. Georgoussi Z. and Thermou K. Somatostatin receptors (sst2) are coupled to Go and modulate GTPase activity in the rabbit retina. J. Neurochem. (2002) In Press.

2002 Presentations at International Scientific Conferences

G. Mazarakou, D. Stravopodis and Z. Georgoussi (2002) Activation of the μ-opioid receptor leads to phosphorylation of Signal Transducers and Activators of Transcription 5 (STAT5**)** , 33^{d} International Narcotic Research Conference (INRC) July 9-14, 2002, Monterey, USA, August 2002

E. Morou, A. Prombona, and Z. Georgoussi (2002) In vivo expression of peptides encoding intracellular opioid receptor portions in intact cells alter G protein signalling. 33^{d} International Narcotic Research Conference (INRC) July 9-14, 2002, Monterey, USA August 2002

RESEARCH GROUP: Biochemistry of Proteins and Peptides

Research Staff

Spyros Loukas, Associate Research Scientist
Vassilios Atlamazoglou, Collaborating Graduate Student
Eleni Alexandratou, Collaborating Graduate Student

Research Interests

The biochemical and molecular mechanisms of the opioid receptors. Identification and synthesis of new specific opioid peptides. Synthesis of new fluorescent probes for the *in vitro* and *in vivo* diagnosis of malignant tumors. Application of fluorescence (spectroscopy and microscopy) and image analysis methods for the diagnosis of several types of cancer. Development, synthesis and studies of the action of new specific photosensitizers for photodynamic therapy (PDT) of tumors. Low power laser effects at the single cell level using confocal microscopy. Oxidative stress.

2002 Findings

Studies of the mechanisms of photodynamically induced oxidative stress at the single cell level and in real time are completed. The methodology that was used includes:

a) phthalocyanines to induce oxidative stress intracellularly. Phthalocyanines were synthesized from our laboratory. These molecules are second generation photosensitizers which are studied for the treatment of cancer.
b) confocal laser scanning microscopy for inducement of oxidative stress through the objective lens of the microscope at the single cell level and for further observation of the evoked intracellular changes at the same area of interest and in real time.
c) vital fluorescent probes for the observation of intracellular changes in real time
d) image analysis and processing techniques for the quantification of the observed changes.

First of all the subcellular localization of the photosensitizer was studied. It is of special importance, since it determines the localization of the primary target. In colocalization experiments using Mito Tracker Green fluorescent probe, it was calculated that phthalocyanine is mainly localized in the mitochondria region.

Furthermore, generation of reactive oxygen species, mitochondrial membrane potential $\Delta\Psi_m$, intracellular pH_i and changes in intracellular calcium [Ca^{2+}] were observed and quantified.

Reactive oxygen species production was confirmed as a result of phthalocyanine photoactivation.

Mitochondrial membrane potential ($\Delta\Psi_m$) decreases, from the resting level (~140 mV), immediately after the irradiation (~90mV), suggesting a serious depolarization of the mitochondria membrane. A gradually recovery of $\Delta\Psi_m$ to about the physiological level, after 15 min, suggest that mitochondria responded strongly to the stimuli but were not damaged

The intracellular pH_i decreases from the resting level (7.45) 0.4 pH units, after the oxidative stress stimuli. The recovery of this acidification is a very slow process. Dissipation of pH gradient was also recorded as a result of oxidative stress.

Finally, oxidative stress is accompanied by a sharp increase of intracellular Ca^{2+} from its resting level. Intracellular calcium returns to its basal level after about 4 min. The increase is mainly in the nuclear region and is due to intracellular as well as extracellular sources.

2002 Publications

E. Alexandratou, D. Yova, P. Handris, D. Kletsas and S. Loukas Human fibroblasts alterations induced by low power laser irradiation at the single cell level using confocal microscopy, Photochem Photobiol. Sci., 1: 547-552, 2002

RESEARCH GROUP: Growth Factors and the Regulation of Tissue Homeostasis-Cellular Aging

Research Staff

Dimitris Kletsas, Assistant Research Scientist
Dimitris Stathakos, Collaborating Research Scientist
Haris Pratsinis, Postdoctoral Fellow
Irini Zervolea, Postdoctoral Fellow
Panagiotis Handris, Graduate Student
Christina Giannouli, Graduate Student
Ioannis Karakatsanis, Graduate Student
Theodoros Karabelas, 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, extracellular matrix production and apoptosis, as well as their evolutionary conservation is under investigation. Alternative mechanisms of cell proliferation and differentiation, such as autocrine regulation and the effect of mechanical forces, are also studied. Furthermore, the structural and functional characteristics of the senescent (non-proliferating) cell – in contradistinction to the cancer cell – are also investigated, aiming at the elucidation of the mechanisms underlying ageing and longevity, as well as malignant transformation.

2002 Findings

During 2002 we have continued our studies on the action of the multifunctional growth factor TGF-β on human fibroblasts. We have already 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, such as $p15^{INK4B}$. 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.

The importance of the extracellular environment on the action of TGF- has also been shown. In particular, we have found that while human lung fibroblasts are stimulated by TGF-β on conventional plastic substrata, they are strongly inhibited in 3-D gels of polymerized collagen. This inhibition seems to be mediated by the activation of integrins, as well as of ERKs (members of the MAPK superfamily).

We are also studying the effect of mechanical forces on osteoblastic proliferation and differentiation. In particular, we have found that these forces can stimulate cellular proliferation and the expression and activation of c-Fos and c-Jun proteins – members of the AP-1 transcriptional complex – through the MAP and Rho kinases. Furthermore, they also induce the transcription factor Cbfa1, a crucial marker of osteoblastic differentiation.

We have also continued our investigations on the structure and function of the senescent (non-proliferating) cell, in contradistinction to the cancer 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.

The reverse relation between stress and longevity is well known. By studying patients suffering form Cushing's syndrome - characterized by highly elevated glucocorticoid levels – we have shown that their skin fibroblasts exhibit a significant extension of their in vitro lifespan. Furthermore, they express an anabolic phenotype, marked by an increase of their proliferative capacity and collagen synthesis. These findings are in accordance to the theory of hormesis, claiming that low-intensity stresses can be beneficial to the tissue homeostasis.

Furthermore, in collaboration with other groups, we have studied the expression and interplay of oncogenes and tumor-suppressor genes at the tissue level and their possible role in tumor development. Finally, we have developed specific cell assay systems and have investigated the cytostatic/cytotoxic and would healing activity of natural and new synthetic compounds.

2002 Publications

Kletsas, D., Basdra, E.K., Papavassiliou, A.G. (2002) Effect of protein kinase inhibitors on the stretch-elicited c-Fos and c-Jun up-regulation in human PDL osteoblast-like cells. J. Cell. Physiol. 190, 313-321.

Ziros, P.G., Rojas Gil, A.P., Georgakopoulos, T., Habeos, I., Kletsas, D., Basdra, E.K., Papavassiliou, A.G. (2002). The bone-specific transcriptional regulator Cbfa1 is a target of mechanical signals in osteoblastic cells. J. Biol. Chem. 277, 23934-23941.

Gonos, E.S., Agrafiotis, .D, Dontas, A.S., Efthimiopoulos, S., Galaris, D., Karamanos, N.K., Kletsas, D., Kolettas, E., Panayotou, G., Pratsinis, H., Sekeri-Pataryas, K.E., Simoes, D., Sourlingas, T.G., Stathakos, D., Stratigos, A.J., Tavernarakis, N., Trougakos, I.P., Tsiganos, C.P., Vynios, D.H. (2002). Ageing research in Greece. Exp. Gerontol. 37, 735-747.

Alexandratou, El., Yova, D., Handris, P., Kletsas, D., Loukas, S. (2002). Human fibroblast alterations induced by low power laser irradiation at the single cell level using confocal microscopy. Photochem. Photobiol. Sci. 1, 547-552.

Gorgoulis, V.G., Zacharatos P., Mariatos, G., Kotsinas, A., Bouda, M., Kletsas, D., Asimacopoulos, P.J., Agnantis, N., Kittas, C., Papavassiliou, A.G. (2002). Transcription factor E2F-1 acts as a growth-promoting factor and is associated with adverse prognosis in non-small cell lung carcinomas. J. Pathol. 198, 142-156.

Pratsinis, H., Tsagarakis, S., Zervolea, Ir., Giannakopoulos, F., Stathakos, D., Thalassinos, N., Kletsas, D. (2002) Chronic in vivo exposure to glucocorticoids prolongs cellular lifespan: The case of Cushing's syndrome patients' fibroblasts. Exp. Gerontol. 37, 1237-1246.

Gazouli, M., Kokotas S., Zoumpourlis, V, Zacharatos, P., Mariatos, G., Kletsas, D., Kittas, C., Gorgoulis, V. (2002). The complement inhibitor CD59, and the lymphocyte function-associated antigen-3 (LFA-3, CD58) genes possess functional binding sites for the p53 tumor suppressor protein. Anticancer Res. (in press).

Mitropoulou, T., Tzanakakis, G.N., Kletsas, D., Kalofonos, C., Karamanos, N.K. (2002). Letrozol as a potent inhibitor of cell proliferation and expression of metalloproteinases (MMP-2 and MMP-9) by human breast cancer cells. Int. J. Cancer (in press).

Papazafiri, P., Kletsas, D. (2002). Developmental and age-related alterations of calcium homeostasis in human fibroblasts. Exp. Gerontol. (in press).

Gorgoulis VG, Zacharatos P, Kotsinas A, Kletsas D, Mariatos G, Zoumpourlis V, Kittas C, Ryan KM and Papavassiliou AG "The p53 pathway activates ICAM-1 (CD54) expression in an NF-κB-independent manner" EMBO J (2003) (in press).

2002 Presentations at International Scientific Conferences

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RESEARCH GROUP: Pathobiology of the Exracellular Matrix

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Dimitrios Moutzouris, Collaborating Graduate Student
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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 neuroblatoma 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. Recent approaches include the study of integrin-related mechansisms ahich sither lead to cell apoptosis or prevent this process in insulin-secreting β-pancreatic cells, osteoblasts, etc. The aim is to unravel signaling pathways regulating gene expression via interactions with matrix components, such as 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), 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.

2002 Findings

The presence of the αβ peptide (1-40), an extracellular product of APP, which is accumulated in Alzheimer's disease, induces an increased expression of the α2 integrin subunit in SK-N-SH human neuroblastoma cells. A study of interactions between the αβ peptide and neuroblastoma cells indicated that these cells bind to the peptide, mainly using the α2β1 and, to a lesser extent, α3β1 integrins. Furthermore, the presence of peptide αβ resulted in increased expression of metalloproteinases MMP-2 (up to 1.5X increase) and mainly MMP-9 (up to 20X increase).

The expression of the matrix anti-adhesive sialoprotein podocalyxin, by renal glomerular epithelial cells (podocytes) is enhanced by the presence of anti-α3 integrin antibodies (the presence of these antibodies in the culture medium mimicks α3 ligands, such as laminin). Furthermore, the use of confocal micrscopy and SEM/TEM indicated that, the presence of laminin and intact basement membrane substrates iduces the differentiated expression of podocalyxin in areas of the basal cell surface which are not in contact with the substrate, and form the specialized foot processes. Therefor, podocalyxin expression specifically in these areas, results in inhibition of binding to the matrix, which we previously described, thus participating in the differentiated morphology of the basal podocyte surface. For the optimal differentiation of podocytes, the presence of intact basement membrane is required.

2002 Publications

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RESEARCH GROUP: Nuclear Proteins and Chromatin Function

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Research Interests

Studies regarding changes in the constitution of chromatin related to histone variant expression and histone acetylation using a number of cellular systems in order to obtain information which will relate the histone constitution of chromatin to its biological functional state. The systems which have been used are the in vitro ageing cell systems of human diploid fibroblasts and long term T-lymhocyte cell cultures, as well as peripheral blood lymphocytes from normal individuals and patients with bipolar disorder.

2002 Findings

During the year 2002 we have continued the study of the influence of inhibitors of histone deacetylases in *in vitro* ageing cell systems of fibroblasts and T lymphocytes in order to investigate the interaction between the acetylation level of chromatin with the expression of the linker histone H1o gene as well as the premature ageing of fibroblasts and the apoptosis of lymphocytes as a function of the age of the cells. The results obtained using a much larger number of samples have verified the first findings from the pilot study of the previous year. It has been shown that the effects caused by the presence of a histone deacetylase inhibitor, i.e., acetylation of histone H4 and expression of histone H1o increase with the age of the cells.

On the other hand experiments are under way which are related to the elucidation of the role of the histone H1o in the activation of the DNA fragmentation factor (DFF40). We have conducted a number of immunoprecipitations of apoptotic chromatin with antibodies against DFF40 and H1o in order to isolate a DFF4-H1o complex and compare the activation of DFF40 by histone H1o with that obtained by H1.

Another series of experiments, which is also under way, refers to the study of the retinoic acid response element (RARE) of the histone H1o gene. The first preliminary results which were obtained, using as an experimental system the erytholeukemic cells, U937, showed that the expression of the gene is not induced by the presence of retinoic acid. Furthermore in the presence of the inhibitor of histone deacetylases, trichostatin A, which in other cells works synergistically with retinoic acid in the induction of genes that possess a RAR element, in the case of U937 it has the opposite effect, i.e., in the presence of both agents, gene induction is reduced. This work is in progress in order to investigate the functional significance of RARE's presence in the H1o gene promoter region.

2002 Publications

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Sourlingas, T.G., Kypreou, K.P., Kovaiou, O., K.E.Sekeri-Pataryas. Histones: apoptosis and ageing. Immunology and Ageing in Europe (ImAginE) (2002). Third Conference on basic biology and clinical impact of immunosenescence, Palermo, 2002

RESEARCH GROUP: Molecular Genetics of insects and Biotechnology

Research Staff

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Theodoros Georgomanolis, Graduate Student
Eleftheria Argyrou, Collaborating Graduate Student
Silvia Ciolfi, Collaborating Graduate Student
Aghelina Metaxatou, Collaborating Graduate Student
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Antonios Mirsaliotis, Undergraduate Student
Petros Antoniades, Undergraduate Student
Marina Sagnou, Technical Specialist
Dimitrios Kopanelis, Research Technician

Research Interests

Insect oogenesis: a paradigm for long-term differentiation programs induced by steroid hormones
Molecular biology and genetic engineering of insect viruses:

a) Viruses expressing insect host-incapacitating proteins
b) Disabled baculoviruses as vectors for genetic transformation
c) Engineered baculoviruses as vectors for human gene therapy

Functional Genomics:

d) Insect cell line protein expression system
e) High-throughput screening systems for biologically active substances
f) Protein-protein interactions by FRET-technology

2002 Findings

RNA interference (RNAi) is a rapidly emerging technique that is useful for the determination of the function of genes in organisms that are not easily amenable to genetic analysis. We continued our experiments regarding the applicability of RNAi to tissue culture cells and ovaries from the silkworm, *Bombyx mori*. After transfection of double-stranded RNA (dsRNA) into tissue culture cells, a dose-dependent in decrease in gene activity could be observed. Decreases in gene activity were monitored at the level of mRNA expression (Northern blot), protein expression (Western blot) and by functional assays (activation of relevant reporter constructs).

So far, however, experiments have been carried out using long dsRNAs (0.7 kb–2.5 kb). Although the long dsRNAs can be introduced into tissue culture cells by regular transfection techniques, their introduction in animal tissues (as the ovary) *in vitro* is predicted to be more problematic because of the impenetrability of the basal membrane of many tissues to large nucleic acid fragments. Recently, however, a technique was developed to “dice” large dsRNAs into small fragments (21-25 bp) using the *E. coli* RNase III enzyme. The small RNA fragments (known as “small interfering RNAs” or siRNAs) retain the full potency of gene silencing but are predicted to penetrate tissues more efficiently than the large fragments. To that end, the coding region of the RNase III gene of *E. coli* was isolated by PCR cloning and subsequently expressed in bacteria for RNase III enzyme production. The generation of siRNAs by RNase III and their application to specific gene silencing is currently in progress.

Lepidopteran cells have considerable potency to be developed as new cell-based high-throughput screening (HTS) systems because of their cheap maintenance, their adaptability to growth in serum-free medium and the high activity of lepidopteran expression vectors. Progress was made regarding the development/applicability of HTS systems for insect nuclear receptors as well as for a mammalian G protein-coupled receptors (GPCRs).

A fully functional HTS system, based on silkmoth tissue culture cells, was used to screen a chemical library of dibenzoylhydrazine compounds for ecdysone mimetic or antagonistic activity (collaboration with Dr. Nakagawa, Kyoto University, Japan). Out of 174 compounds, two compounds were identified that showed very high activity that was comparable to that of the best commercially available compounds. The identified compounds will subsequently be evaluated further for their activity in tissue-based biological assays and in toxicity assays on whole insects (collaboration with Dr. Guy Smagghe, University of Ghent, Belgium).

The development of a screening system for compounds with serotonin-like activity was initially based on the employment of two genetic elements, *i.e.* an expression construct for the human serotonin receptor 4_{SA} ($hHT4_{SA}R$) and a reporter construct that contains a cAMP-responsive element (CRE) upstream of a basal promoter (collaboration with Dr. I. Georgoussi). Introduction of the serotonin receptor-construct into tissue culture cells results in the appearance of serotonin-binding activity in membrane extracts and in an increase of intracellular cAMP in response to serotonin administration. However, activation of the reporter gene was triggered by the serotonin receptor in the absence of ligand, indicating that the serotonin receptor is constitutively active in the insect cells. Coexpression of the universal coupling Gα protein, $G_{\alpha 16}$, or the cAMP-inducible CRE-binding (CREB) protein could not rescue the ligand-inducibility of the CRE-reporter. However, it was observed that $G_{\alpha 16}$ greatly enhanced the transcriptional activity that is constitutively triggered by the serotonin receptor. Thus, an assay could be developed aimed at the isolation of compounds that interfere with the coupling of the serotonin receptor to the $G_{\alpha 16}$ protein.

Baculoviruses are insect-specific viruses that have been developed as efficient vectors for protein production and gene transduction in gene therapy. Generally, baculovirus production is carried out in insect cell media that are supplemented with 10 % fetal bovine serum (FBS) for optimal growth. Since FBS is considered as a potential carrier of harmful pathogens, production of baculoviruses in serum-free medium is considered as an important improvement to the production process. However, insect cells that are cultured in serum-free media behave poorly with respect to baculovirus production. To improve the capacity of insect cell lines in baculoviral productivity, cell lines were engineered for the constitutive expression of the “promoting protein” (PP), a protein derived from the silkmoth fat body that was shown to increase the infectivity of baculoviruses. Silkmoth-derived tissue culture cells that express the PP protein show a 100 to 1,000-fold enhancement in baculovirus production in serum-free media. The engineered cell line therefore can have important applications in the generation of baculoviral vectors for gene therapy as well as for recombinant protein production in serum-free media.

2002 Publications

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O. Gotsis-Skretas, A. Metaxatos and L. Ignatiades 2002. Ooccurrence of species of the toxic dinoflagellate genus A*lexandrium* in the Eastern Mediterranean and the Black Sea. 2nd International Conference on Oceanography of the Eastern Mediterranean and the Black Sea. October 14-18, 2002, Ankara, Turkey.

RESEARCH GROUP: Theoretical and Developmental Biology

Research Staff

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Christoforos Nikolaou, Graduate Student

Research Interests

A. Mathematical properties of DNA sequences: **(a)** randomness and order, **(b)** long and short range correlations, **(c)** linguistic aspects.
B. Modeling of developmental events: **(a)** early development, main body axes formation, **(b)** left-right asymmetries, **(c)** limb development.
C. Reaction-Diffusion systems: **(a)** spontaneous symmetry breaking, **(b)** minimal requirements of pattern formation, **(c)** applications in biology.

2002 Findings

During this year, we have focused our research activity on the quantification of the deviations from randomness at the "short" length scale of nucleotide n-duplets (n=2,3,..). This is the length-scale, most influenced by the modalities of the protein-coding grammar and syntax. We have developed an algorithm quantifying the degree of non-randomness (and thus the expectation for a sequence to be coding or non-coding), measuring the differences in the frequencies of occurrence of mutually symmetric trinucleotides. The resulting quantity (Codon Asymmetry Measure, CAM), distinguishes systematically coding from non-coding segments. Moreover, the corresponding value distributions are species independent. Thus, our algorithm does not need training set of known functionality sequences for its standardization, when applied on genomes of different organisms.

We are also working in the development of a method for the assessment of the (computationally derived) artificial annotation of genomic sequences, with encouraging first results. This method is based on a combination of the presented algorithm with a measure of the clustering of similar nucleotides in genomic sequences (Modified Standard Deviation, MSD) developed earlier by our group.

2002 Publications

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RESEARCH GROUP: mRNA 3′- end Formation

Research Staff

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Research Interests

The research interest focuses into the regulation of mRNA 3'-end formation and its crucial role in normal cell growth, development and transformation. The basic effort can be divided into two overlapping areas of (i) cellular processes including differentiation, cell division and cell death related to functional and structural alterations of the enzyme of polyadenylation (PAP) and (ii) molecular mechanisms which loosely reflect molecules influencing the response of a cell at the post-transcriptional level of gene expression.

2002 Findings

Chemotherapeutic agents (rIFNα, 5FU, Taxol, Etoposide, Cordycepin) with different mechanisms of function mediated both dephosphorylation and inactivation of PAP. Moreover a cell type modulated differential response of cells was revealed [1, 2, 3]. The findings yield information on a possible correlation between the enzyme responses to the drug and the different apoptotic pathways. Cancer cells when uncoupled from the apoptotic process can survive and become drug resistant. In case, a link restoration between apoptosis and cancer cell is attained, PAP may become a new useful marker to assess drug effectiveness.

RESEARCH GROUP: Environmental Mutagenesis-Carcinogenesis

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Sofia Melachrinou, Undergraduate Student
Stefanos Papaspiridakos, Undergraduate Student
Natalia Milioti, Undergraduate Studen t
Maria Tsekrekou, Undergraduate Student
Evaggelia Nana, Undergraduate Student
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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 resistance
3. Protein-protein interactions

2002 Findings

Structural changes and analysis of polymorphic transcripts of the Fas (APO-1/CD95) gene in breast cancer: In 90 breast tumor samples, no structural changes were detected in exons 9 and 6 of the Fas gene, which code for the cytoplasmic domain involved in receptor-mediated signal transduction and the transmembrane domain, respectively. Additionally, there was no difference in mRNA expression ratio of transmembrane tmFas versus soluble sFas between normal and tumor tissue of the breast. Therefore, inhibition of the Fas pathway found to occur in breast cancer is not due to structural changes in exons 9 and 6 of the gene, while elevated amounts of the soluble receptor found in breast cancer patients are not produced by the tumor cells, but most likely by a systemic response of the immune system against the breast tumor.

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.

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.

Study on the role of Stat proteins in the apoptotic process: In this work we have focused on the functional role of a hybrid receptor consisting of the erythropoietin receptor (EpoR) and the domain of the interferon-γ receptor (IFN-γR), which is involved in Stat1 phosphorylation. We have shown that expression of the hybrid molecule in stably transfected cells is capable to drive these cells to apoptosis, whereas IFN-γR seems to activate in parallel other pathways which inhibit the apoptotic activity elicited by this domain of the protein responsible for Stat1 phosphorylation.

α-Synuclein (α-syn) and ubiquitin C-terminal hydrolase-L1 (UCHL1) expression in Parkinson's disease (PD): In an EBV-transformed cell line (mC1) that we established from B-cells coming from a PD patient bearing the Ala53Thr α-syn mutation, we have found monoallelic

expression of the normal allele. *UCHL1* gene expression was found to be slightly higher in PD cell line mC1 than in normal B-cell line mV2.

2002 Publications

Michalopoulos, N., Saetta A., Lazaris A., Voutsinas G. and Davaris P.S. (2002). Detection of genetic abnormalities in carcinomas from Greek patients with FAP. European Journal of Surgical Oncology, in press.

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RESEARCH GROUP: Microbial Molecular Genetics and Radiation Genetics

Research Staff

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Stefanos Tavoularis, Graduate Student
Demitra Bouzarelou, Graduate Research Associate
Maria Billini, Undergraduate Student
Ioannis Vaggelatos, Undergraduate Student
Antonios Mirsaliotis, 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 parasitic protozoan, bacteria, plants and humans using *A. nidulans* as a novel model system.
Study of Expansin-like proteins.

2002 Findings

We studied the expression of all purine transporter genes (*uapA*, *uapC* and *AzgA*) of *Aspergillus nidulans*, during conidiospore germination and the development of young mycelia. Our studies have shown that all purine transporter genes are transiently induced during conidiospore germination and that this developmental induction is independent of the regulatory factors AreA and UaY.

We performed analysis analysis in strains carrying induced DNA alterations in order to study structure–function relationships of the major proline transporter PrnB of *Aspergillus nidulans.* We found two mutations within TMS6 (transmembrane segment) of PrnB that directly affect proline binding and transport.

We have cloned a gene that encodes a putative protein required for localization of amino acid permeases in the membrane of ER in *Aspergillus nidulans*.

We expressed and studied putative nucleobase transporters from *Leishmania major* and *Arabidopsis thaliana* in *Aspergillus nidulans.* Our results shown that functional expression of foreign nucleobase transporters in *Aspergillus nidulans* depends on the % of GC content of these genes.

We have cloned a gene that encodes a putative Expansin-like protein in *Aspergillus nidulans*.
Expression and molecular characterization of the human ascorbate transporter (SVCT2) in *Aspergillus nidulans*.

2002 Publications

S. N. Tavoularis, U. H. Tazebay, G. Diallinas, M. Sideridou, A. Rosa, C. Scazzocchio and V. Sophianopoulou (2002). Mutational analysis of the major proline transporter (PrnB) of *Aspergillus nidulans*. Mol. Membr. Biol. (*in press*).

A. Konsta, E-E. Visvardis, K.S. Haveles, A.G. Georgakilas and E.G. Sideris (2002). "Detecting radiation induced DNA damage: from changes in dielectric properties to programmed cell death" J. Non-Cryst. Solids 305:295-302.

A. Tsoulou, C.A. Kalfas and E. G. Sideris (2002). Probing irradiated DNA with the perturbed angular correlation method. Radiat. Res 156:181-185.

A. Tsoulou, C. A. Kalfas and E .G. Sideris (2002). Changes in DNA flexibility after irradiation with γ rays and neutrons studied with the perturbed angular correlation method. Radiat. Res. (*in press*).

2002 Presentations at International Scientific Conferences

K. Stamatakis, E. Argyrou and V. Sophianopoulou (2002). Isolation and characterization of a Na^+/H^+ antiporter gene from *Synechococcus* sp. PCC 7942. Abstract (n^o 125) of the 5th European Workshop on the Molecular Biology of Cyanobacteria, June 9-12, page 125. Stockholm, Sweden

M. Sideridou, St. Tavoularis and V. Sophianopoulou (2002). Structure-Function analysis of the major proline transporter of *Aspergillus nidulans*. Abstract of the 28th FEBS Meeting, 20-25 October, PS3-021. Istanbul, Turkey

RESEARCH GROUP: Biogenesis and Function of the Photosynthetic Membrane

Research Staff

Anastassia Prombona, Assistant Research Scientist
Athanasios-Dimitrios Kaldis, Graduate Student
Michalis Barkoulas, Undergraduate Student
Apostolos Beloukas, Undergraduate Student
Antonios Giakountis, Undergraduate Student
Triantafillos Gikopoulos, Undergraduate Student
Sokrates Avgeris, Research Technician

Research Interests

Investigation of the biological clock function in plants. Molecular mechanisms of its synchronization / entrainment with the varying light-conditions of the environment. Elucidation of the involvement of PvLHY, putative transcription factor and component of the central oscillator, in the regulation of clock outputs (light-harvesting genes) in bean. Study of *PvLHY* gene regulatory elements and of PvLHY protein function in order to understand the dual role of the factor as positive and negative regulator in rhythmic transcription.

2002 Findings

The ongoing research regards the study of PvLHY, a putative transcription factor and component of the central oscillator in bean. The first project scopes the isolation of the *PvLHY* gene promoter. We achieved the cloning of a 700 bp long genomic fragment that includes the 5' UTR of *PvLHY* gene. The design of new primers from this region will enable us the chromosome walking more upstream. Moreover, in pilot experiments bean protoplasts were transformed with the green fluorescent protein sGFP-TYG. This will be used as the reporter gene in the study of the *PvLHY* promoter activity. The second project aims the study of the PvLHY protein function. Thus, towards the production of polyclonal antibodies, we cloned a 150 amino acids long hydrophilic peptide in an *E. coli* pGEX expression vector and tested the conditions for optimal production of the fusion protein GST-PvLHYpeptide. The next steps include the purification of the peptide and the immunization of the rabbits.

In a different project, we screened two subtracted cDNA libraries that were constructed from two opposite phases of the circadian rhythm of *Lhcb* (light-harvesting protein complex of photosystem II). Two kinds of cDNAs were identified, one that exhibits similarity to a kinase and a second that is similar to a plastidic complex protein. The transcriptional analysis of the corresponding genes is under investigation.

2002 Presentations at International Scientific Conferences

A.D. Kaldis and A. Prombona (2002). Interaction between the light-induced acute response and the following circadian cycle in *Phaseolus vulgaris*. 13th Congress of the Federation of European Societies of Plant Physiology, September 2-6, 2002, Hersonissos, Heraklion, Crete, Book of Abstracts, p. 339

Prombona A., A.D. Kaldis, K. Kesanopoulos and P. Kousidis (2002). Identification of novel genes involved in the regulation of light-dependent circadian rhythms in *Phaseolus vulgaris*. 13th Congress of the Federation of European Societies of Plant Physiology, September 2-6, 2002, Hersonissos, Heraklion, Crete, Book of Abstracts, p. 343

RESEARCH GROUP: Biophysics and Biotechnology of Membranes

Research Staff

Kostas Stamatakis, Assistant Research Scientist
George Papageorgiou, Collaborating Research Scientist
Vassiliki Maniou, Collaborating Graduate Student
Nektarios Ladas, Collaborating 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.

2002 Findings

Cells of fresh water cyanobacterium *Synechococcus* sp. PCC 7942 import NaCl passively and export Na^+ actively, primarily *via* Na^+/H^+ antiporter. In the research project of 2001 a gene encoding a putative Na^+/H^+ antiporter from *Synechococcus* sp. PCC 7942 has been cloned. This cloning was performed using nested–PCR and screening of a genomic cosmid DNA library of *Synechococcus* sp. PCC 7942.

2002 Publications

George C. Papageorgiou. Photosynthesis Research in Greece: A Historical Snapshot (1960-2001). Photosynth Research (in press 2003).

2002 Presentations at International Scientific Conferences

K. Stamatakis, E. Argyrou, V. Sophianopoulou (2002). Isolation and characterization of a Na^+/ H^+ antiporter gene from Synechococcus sp PCC 7942. 5th European Workshop on the Molecular Biology of Cyanobacteria, June 9-12, 2002, Stockholm, Sweden.

ENVIRONMENTAL BIOLOGY

RESEARCH GROUP: DNA Repair Systems and Cancer

Research Staff

Stelios Piperakis, Assistant Research Scientist
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Georgia Karanastassi, 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.

2002 Findings

We completed the experimental analyses of the results of the programme “pesticides effects on humans”. We have now published the first results.

The study of the DNA repair systems of human lymphocytes in which we have used inhibitors in several pathways of the repair capacity in order to find its relationship to necrosis and apoptosis has been completed and the results have been published this year.

The study "stress and DNA damage-repair" has been completed. The results have been published this year.

The study "DNA damage-repair and effects of solar seasonal variations" has finished. The results have been published this year.

The study “effects of alcohol in human lymphocytes” was completed.

The study “effects of common variable immunodeficiency in DNA repair” is in progress.

The study “Effects of antioxidants (vitamins C, E) in human lymphocytes” is in progress.

The study “diabetes mellitus - DNA damage and repair” is in progress.

The study “Effects of alcohol and hydrogen peroxide in breast cancer patients” is in the beginning.

The study “Lung cancer patients, DNA damage and repair” is in the beginning.

2002 Publications

V. A. Tronov, E.M. Konstantinov, E. Petrakou and S.M. Piperakis (2002). Nicotinamide protects resting lymphocytes exposed to hydrogene peroxide from necrosis but not from apoptosis. Cell Biol. Toxicol. v.18, p.359-367.

S.M. Piperakis, E. Petrakou, E. Monogiudis, G. Haniotakis, H. Karkaseli, E. Sarikaki, S.Tsilimigaki (2002). Pesticides effects on humans. Environm. Molec. Mutagenesis. In press.

E. Dimitroglou, M. Zafiropoulou, N.Messini-Nikolaki, S. Doudounakis, S.Tsilimigaki and S. M. Piperakis (2002). DNA damage and repair capacity in a human population affected by chronic psychogenic stress. Int. J. Hyg.Environ. Health. In press.

S. Tsilimigaki, N. Messini-Nikolaki, M. Kanariou, S.M. Piperakis (2002). A study on the effects of seasonal solar radiation on exposed populations. Mutagenesis. In press.

2002 Presentations at International Scientific Conferences

Pastor S, Creus A, Parron T, Cebulska-Wasileuska A, Siffel C, Piperakis SM, Marcos R. Micronuclei biomonitoring of four European populations occupationally exposed to pesticides. 32st European Environmental Mutagen Society, meeting Warsow, Poland, September 2002.

RESEARCH GROUP: Chemical Ecology and Natural Products

Research Staff

Vassilios Mazomenos, Research Scientist
Vassiliki Labropoulou, Assistant Research Scientist
Pedro Hernandez – Arauzo, Postodoctoral Fellow
Ioannis Georgakopoulos, Postodoctoral Fellow
Maria Konstantopoulou, Technical Specialist
Dimitra Stefanou, Technical Specialist
Elias Siskos, Collaborating Graduate Student
Niki Kalariti, Undergraduate Student
Anastassia Pantazi-Mazomenou, Research Technician

Research Interests

Study of insect chemical communication
Development of pheromone formulation technologies, and biotechnological methods for pest control.
Study of insect host relationships
Screening of plants and microorganisms for the isolation of biological active chemicals of agricultural and pharmaceutical interest.
Study of the molecular mechanisms involved in insect chemical communication (pheromone binding proteins PBPs and general odorant binding proteins GOBPs).

We are currently studding the molecular mechanisms involved in insect chemical communication and generally in olfaction. Our future plans include the survey of OBP interacting proteins that might be involved in OBP transport from the antennas to the neural cells. We are going to search such interacting proteins in cDNA library of *Anopheles gambiae* using the newly isolated cDNAs sequences from *Anopheles gambiae* that have been found to have high sequence homology with other OBP proteins.

Another aspect of our research is the development of an *in vitro* assay for searching molecules that could have possible antibacterial properties and the study of antibacterial resistance for certain antibiotics.

2002 Findings

The isolation and identification of the toxic (to insects) metabolites produced by the fungi species *Mucor hiemalis* isolate (MU-11) and *Penicillium crysogenum* isolate (PC-14), is under investigation. One highly toxic component has been isolated and purified by HPLC from *M. hiemalis* extracts. UV; FTIR; HPLC-MS and NMR spectra have been obtained, the assignment of the chemical structure is under investigation. SPE and HPLC chromatographic procedures are used for the isolation of the toxic metabolites produced by *Penicillium crysogenun*.

In the contents of TRIPHELIO (Cont No ICA4-CT2001-10004) project, aiming to developed an IPM for lepidopterous olive pests, based on natural enemies and pheromones a study have been initiated to investigate the kairomonal effect of *Prays oleae*, and *Palpita unionalis* sex pheromone components, frass and scale extracts. Z-7-14:Ald main pheromone component of *P. oleae* substantially decrease parasitoids searching activity.

Monitoring systems based on pheromones 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.

We have also examined the expression of PBP/OBP proteins in the antenna of *Sesamia nonagriodes* by immunohistochemistry. In a attempt to search PBP/OBPs sequences in *Sesamia nonagriodes* genome we identified and cloned sequences that their PCR products cross-hybridize with OBP sequences from other lepidoptera species. Theses sequences seems to have homology with the sequences of PBP/OBP proteins from other species but cloning of the genes was not possible.

In a collaboration project with Agronomic Institute of Chania (MAICH) we cloned and expressed one of the two subunits of bacterial DNA gyrase, namely GyrA and the two subunits of the topoisomerase type IV (ParE and ParC). These proteins are the most important antibacterial targets and

the expressed proteins can be used *in vitro* to examine substances for possible antibacterial activity. The study is under development and efforts have been made to express the GyrB subunit in order to test the expressed proteins for the functional activity of the enzyme. This will done using plasmid DNA and electrophoresis to check for supercoiled or relaxed DNA.

Finally, the cytotoxic effects of Cinoxanin, and Ciprofloxacin, with probable antibiotic properties were examined in bacterial strains bearing antibiotic resistance. These strains will also be checked for possible mutation in topoisomerase II (GyrA andGyrB) genes.

2002 Publications

R. Albajes, M. Konstantopoulou, O. Etchepare, M. Eizaguirre, B. Frérot, A. Sans, F. Krokos, A. Améline, B. Mazomenos (2002). Mating disruption of the corn *borer Sesamia nonagrioides* (Lepidoptera: Noctuidae) using sprayable formulations of pheromone. Crop Protection. 21**,** 217-225.

F. Krokos, A. Ameline, J. Bau, A. Sans, M. Konstantopoulou1ou, B Frerot, A. Guerero, M. Eizaguirre, C. Malosse, O. Etcherare, R. Albajes & B.E. Mazomenos (2002). Comparative studies of female sex pheromone components and male response of the corn stalk borer (*Sesamia nonagrioides)* in three different populations. J. Chem Ecol. 28, 1463-1472.

M.T. Fletcher, B.E. Mazomenos, J.H. Georgakopoulos, M.A. Konstantopoulou, B.J. Wood, J.De Voss, and W. Kitching (2002). Sex pheromone biosynthesis in the female olive fruit-fly. Double labelling from [^{18}O]-dioxygen into 1,7-dioxaspiro[5,5] undecane. Chem. Commun., 1302-1303.

M.A. Konstantopoulou, F.D. Krokos and B.E. Mazomenos (2002). Chemical stimuli from corn plants affecting host selection and oviposition behaviour of the cornstalk borer *Sesamia nonagrioides* (Lef*).* J. Econ. Entomol. 95, 1289-1293.

Konstantopoulou M. and D. Raptopoulos (2002). Alcohol dehydrogenase allele frequencies in Bactrocera oleae: effect of ethanol addition in larval diet. J. Appl. Entom. (in press).

2002 Presentations at International Scientific Conferences

Araujo P. W., Konstantopoulou M.A., Georgakopoulos J.H and B.E. Mazomenos**.** Isolation of toxic metabolites to fruit flies adults from *Mucor hiemalis* (SMU-21). Envir. Sc. Pollut. Res. (special issue 3/2002) 8th FECS Conference on Chemistry and the Environment. Athens, Greece, 31 Aug. to 4 Sept. 2002

Siskos, E.P. F.D. Krokos, B.E. Mazomenos. Chemicals with insecticidal properties isolated from *Citrus aurantium* peel extracts. Envir. Sc. Pollut. Res. (special issue 3/2002) 8th FECS Conference on Chemistry and the Environment. Athens, Greece, 31 Aug. to 4 Sept. 2002.

Koutalidis, S., F. Krokos, P.A. Siskos, B.E. Mazomenos. Preliminary results on levels of polychlorinated biphenyls in Greek cow's milk. Envir. Sc. Pollut. Res. (special issue 3/2002) 8th FECS Conference on Chemistry and the Environment. Athens, Greece, 31 Aug. to 4 Sept. 2002

Mazomenos. B.E. and A. Pantazi-Mazomenos Studies of female sex-pheromones components and male response of *Sesamia nonagrioides* in three different populations. IOBC wprs Pheromone Working Group Meeting - Sicily, September 22-27, 2002**.**

Mazomenos B.E. C.G. Athanasiou, and N. Kavallieratos. Identification of Alkadienes, as sex pheromone components of the Almond seed wasp *Eurytoma Amygdali*. VIIth European Congress of Entomology, Thessaloniki, Creece. October. 7-13, 2002

Konstantopoulou.M. A. & B.E. Mazomenos**.** Pathogenicity of *Mucor hiemalis* on *Bactrocera oleae* and *Ceratitis capitata*. VIIth European Congress of Entomology, Thessaloniki, Creece. October. 7-13, 2002**.**

Fletcher M.T., J.J. De Voss**,** B.E. Mazomenos & W. Kitching. Spiroketal Biosynthesis in selected *Bactrocera oleae* Species of Fruit Flies. VIIth European Congress of Entomology, Thessaloniki, Creece. October. 7-13, 2002.

Siskos, E.P., F. D. Krokos & B.E. Mazomenos**.** Screening of *Citrus aurantium* extracts for insecticidal activity against *Bactrocera oleae* adults. VIIth European Congress of Entomology, Thessaloniki, Creece. October. 7-13, 2002.

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.

2002 Findings

Research and experimentation for the development of organic tobacco technology was continued for a third year in the area of Xanthi. Technological problems related to organic tobacco pest protection and fertilization were successful solved and, finally, the official "organic tobacco certificate" was issued by DHO. Thus, the production of organic tobacco is now a reality.

Moreover, the neurophysiological study of the chemical communication organs of Bactrocera oleae, using electroantennogram technology, was continued, in cooperation with Prof. G. Theophilidis of the animal physiology lab, Biology Department, University of Thessaloniki.

2002 Presentations at International Scientific Conferences

S.S. Zacharioudakis, G.J. Tsiropoulos and L.H. Margaritis (2002). Chemical communication by olive fruit fly, Bactrocera oleae. 6th International Symposium on Fruit Flies of Economic Importance, 6-10 May 2002, Stellenbosch, South Africa.

RESEARCH GROUP: Nutritional and Biochemical Ecology

Research Staff

Athanassios Manoukas, Research Scientist
Anastassia Pantazi-Mazomenou, Research Technician

Research Interests

Nutritional Ecology and Biochemistry with emphasis on insects, agricultural production and environmental protection.

2002 Findings

The olive fruit is an important part of the Mediterranean diet and a source of tocopherols and other antioxidants. There is a great interest in the tocopherol content of foods because of the inceasing acceptance of vitamin E as a major memrane bound antioxidant and the increasing number of biological studies linking vitamin E status to lower risk of certain health problems. In addition the olive fruit is the exclusive food of the olive fruit fly larvae in nature.The tocopherol content of certain table varieties and types of the olive fruit was detemined. It was found that the tocopherol content was different among the varieties and types of samples in each variety. The a-tocopherol equivalent (vitamine E) of four main Greek varieties sold in bulk was determined and found to be the follwing (in μg/g lipids): Kalamon-small 200, Calamon-large 178, Conservolia-green 152, Conservolia–black 200, Chalkidiki–green 102, Chalkidiki–black 144 and Throumbolia 211.

The larval diet used today for mass rearing of the Mediterranean fruit fly is of low efficiency mainly because the nutritional and dietary reuirements are not known. The content of the most important essential minerals (anions and cations) in the diet and pupae was measured and their utilization was calculated. It was found that mineral utilization was very low and statistically different. The results showed that the diet contained high and unbalanced quantities of minerals with respect to their requirements. In addition the composition, chemical analysis and efficiency of certain improved diets was determined in relation to those used today.

2002 Presentations at International Scientific Conferences

Manoukas, A. G., 2002. Composition and Efficiency of a larval diet containing alfalfa insead of yeast for rearing Mediterranean fruit fly *Ceratitis capitata*, (Diptera: Tephritidae). 6th International Symposium on fruit flies of economic importance. Stellenbosch, South Africa, 6-10 May, 2002. Abstarcts p 6

Manoukas, A. G., 2002. Performance and utilization of a larval diet containing alfalfa with three egg densities for rearing the Meditarranean fruit fly. VII Eurpopean Congress of Entomology, October 7-13, 2002,Thessaloniki, Greece. Abstracts p. 86.

RESEARCH GROUP: Radionuclide Transfer in the Soil-Plant System

Research Staff

Vassiliki Skarlou, Senior Research Specialist
Ioannis Massas, Graduate Student
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.

2002 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 attempt to classify the soil types according to ^{134}Cs uptake by reference plants is continuing.
2. In all studied soil systems, the constant ratio between ^{134}Cs transfer factor for corn and the leafy crops observed in previous years, is 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.
4. Irrigation level did not significantly influence the ^{134}Cs transfer factors of sunflower plants.

2002 Publications

Massas I., V. Skarlou and C. Haidouti. 2002. ^{134}Cs uptake in relation to soil properties and time. Journal of Environmental Radioactivity. 59, 245-255.

Frissel, M.J., D.L. Deb, M. Fathony, Y.M. Lin, A.S. Mollah, N.T. Ngo, I. Othman, W.L. Robison, V. Skarlou-Alexiou, S. Topcuoglu. 2002. Generic values for soil-to-plant transfer factors of radiocesium. Journal of Environmental Radioactivity. 58 (2-3), 113-128.

2002 Presentations at International Scientific Conferences

Polychronidis M. and I. Massas. 2002. Urea utilization by cotton leaves under different N rates, levels of irrigation and fertigation. I.A.E.A. project "Fertigation for improved crop production and environmental protection". Kalamata 4-8 November.

Pavlou G., C. Ehaliotis and I. Massas. 2002. 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". Kalamata 4-8 November.

STRUCTURAL BIOLOGY

RESEARCH GROUP: Protein Crystallography

Research Staff

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Georgia Kefala, Postodoctoral Fellow
Athanassios Tartas, Graduate Student
Aggeliki Kosmopoulou, Collaborating 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, 2) identify the functional role of the BRCT domain of BRCA1 protein that is related to hereditary breast/ovarian cancer, 3) investigate the role of the La/(SSB) protein in cellular responses linked with autoimmune diseases such as systemic lupus erythematosus and Sjogren's syndrome.

2002 Findings

1) In order to study the TPR mediated protein-protein interactions we have produced *in vitro* and purified the ScB/Tn108 protein complex that comprises the interaction domains of the Ssn6 (ScB) and Tup1 (Tn108) proteins. ScB contains the three N-terminal TPRs (TetratricoPeptide Repeats) of Ssn6 known to be involved in its interaction with Tup1. The ScB/Tn108 complex was further studied with circular dichroism (CD). Comparison of its far-UV CD spectrum with the spectra of ScB and Tn108 gives evidence for a conformational change occurring upon the complex formation.

In addition, we purified two other fragments of Ssn6 (S4T and S400) of different length than ScB containing, however, its Tup1 interaction domain. A new Tup1 fragment (Tn9) was also expressed in *E.coli* and its complexes with both ScB and S4T were produced *in vitro* and purified. CD experiments on both complexes are in progress. The objective is to identify structurally stable Ssn6/Tup1 complexes that are able to crystallize with the aim to determine their 3D structure by x-ray crystallography.

2) We performed a structure-based estimation of thermodynamic parameters of the BRCT domain of human BRCA1 (BRCT-tan) protein that is related to hereditary breast/ovarian cancer. The estimation of the thermodynamic parameters was based upon a model of the structure of a dimeric form of the molecule that we propose. Comparison of the theoretic parameters with those obtained experimentally by Differential Scanning Calorimetry (DSC) (from the group of G. Nounenis, IRRP), leads to the identification of a region of the BRCT-tan molecule that is essential for its thermodynamic stability. Interestingly, cancer related missense mutations of BRCT-tan are located in this region. [Pyrpassopoulos, S, Ladopoulou, A., Vlassi, M., Papanikolau, Y., Vorgias, C.E., Yannoukaos, D. and Nounesis, G. Thermodynamic stability of the BRCT tandem repeats of human BRCA1-characterisation of the partly folded intermediate. *To be submitted*].

3) We produced a 3D model of two alleles of the class II histocompatibility proteins (MHC-II) linked with autoimmune diseases such as systematic lupus erythematosus and Sjogrens's syndrome. Subsequently, we modeled their interaction with peptides derived from the aminoacid sequence of the La/(SSB) protein that is also related to the above autoimmune diseases. Prediction of T-cell epitopes of La/(SSB) for the two MHC-II molecules using biocomputing techniques is in progress. The objective is to produce peptide analogues of the La/(SSB) epitopes and use them in immunology experiments with the aim to investigate the role of the La/(SSB) protein in cell responses compatible with the above autoimmune diseases.

4) In order to understand the differential sensitivity to metals exhibited by two amphibian glyceraldehyde-3-phosphate dehydrogenases (GAPDHs), we produced three-dimensional models of *X. laevis* and *P. waltl* GAPDH structures based on GAPDHs of known three-dimensional structures. Putative metal binding sites were predicted based on the modelled structures and explained the differential sensitivity of the amphibian GAPDHs to metal ions compared to GAPDHs from other species. This work has been accepted for publication in Comp Biochem Physiol B (Biochem Mol Biol).

5) In a previous work we had proposed a three-dimensional model of a region of TIN antigen (TubuloInterstitial Nephritis antigen) predicted to be responsible for calcium binding. Calcium uptake experiments [from Prof. A. Charonis's group (National Academy of Greece and Univ. of Patras) from a

synthetic TIN peptide modified according to the model, confirmed our prediction (Manuscript in preparation).

2002 Publications

Mounaji, K. M. Vlassi, N-E., Erraiss, M. Wegnez, A. Serrano and A. Soukri (2002). 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*), In press.

RESEARCH GROUP: NMR Studies of Biomolecules and Parmaceuticals

Research Staff

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Maria Pelekanou, Associate Research Scientist
Dimitra Benaki, Postdoctoral Fellow
Aggeliki Panagiotopoulou , Technical Specialist
Stamatia Tzanopoulou, Collaborating Graduate Student
Kalliope Kalokiri-Stilianidi, Research Technician

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 pharmaceuticals

2002 Findings

In 2002 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) as well as in the presence of the azo dye Congo red, which is known to prevent the Aβ's aggregation, have been collected and they are being processed in combination with theoretical modeling approaches (collaboration with Metaxia Vlassi). In parallel, in the effort to develop a radiodiagnostic for *in vitro* imaging of 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. The system which will allow the evaluation of the affinity of these complexes for amyloid plaques in slices of brain tissue is being set up.

In the field of radiopharmaceuticals of rhenium and technetium, the detailed study of the chemistry of the widely used diaminedithiol (DADT) ligand system in the presence of oxorhenium core and of the structures of the complexes isolated was published (Publication #1). Research in the field of radiodiagnostics specific for serotonin 5-HT_{1A} receptor imaging continued with the synthesis and evaluation of new complexes that carry in their ligand the serotonin antagonis WAY-100635 (Publications # 2, 3). The study of two novel structures of oxorhenium ReO(V) (binuclear, μ-oxorhenium complex, Publication #4, as well as of Re(III) (mixed ligand complex with ligands bearing P, N, S, and O donor atoms, Publication #5) was completed. Our results on the investigation of the coordination sphere of oxorhenium and oxotechnetium with the bipyridine molecule, which is a model for the development of a radiodiagnostic for Alzheimer' s disease, were submitted (M. Papachristou, I. C. Pirmettis, Ch. Tsoukalas, C. Raptopoulou, A. Terzis, C. I. Stassinopoulou, E. Chiotellis, M. Pelecanou, M. Papadopoulos. Synthesis and Characterization of Novel Oxotechnetium (^{99}Tc and ^{99m}Tc) and Oxorhenium Complexes from the 2,2´-Bipyridine (NN)/Thiol (S) Mixed-Ligand System) to *Inorganic Chemistry* for publication. Finally, progress has been made on the NMR study of the structure of the complex of the neuropeptide bombesine with oxorhenium in the effort to develop a radiopharmceutical specific for tumors of the endocrinic system and of the lung.

2002 Publications

Chryssou, K., Pelecanou, M**.,** Pirmettis, I. C., Papadopoulos, M., Raptopoulou, C., Terzis, A., Chiotellis, E., Stassinopoulou, C. I. (2002). New oxorhenium(V) complexes from the widely used diaminedithiol (DADT) ligand system. Inorg. Chem. 41**,** 4653-4663

Papagiannopoulou, D., Pirmettis, I., Tsoukalas, Ch., Nikoladou, L., Drossopoulou, G., Dalla, C., Pelecanou, M., Papadopoulou-Daifotis, Z., Papadopoulos, M., Chiotellis, E. (2002). Oxotechnetium ^{99m}TcO[SN(R)S][S] complexes as potential 5-HT_{1A} receptor imaging agents. Nucl. Med. Biol. 29**,** 825-832

Tsoukalas, Ch., 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., in press

Patsis, G., Pirmettis, I., Tsoukalas, Ch., Pelecanou, M., Raptopoulou, C., Terzis, A., M. 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

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, *in press.*

2002 Presentations at International Scientific Conferences

M. Mallo, G. Patsis, I. Pirmettis, D. Papagiannopoulou, L. Nikoladou, Ch. Tsoukalas, M. Pelecanou, G. Drossopoulou, C. Dalla, Z. Papadopoulou-Daifotis, A. León, E. Manta, M. Papadopoulos, E. Chiotellis (2002). Oxotechnetium ^{99m}TcO[SNS][S] complexes as potential 5-HT$_{1A}$ receptor imaging agents. 6th International Symposium on Technetium in Chemistry and Nuclear Medicine, September 4-7, Bressanone, Italy

M. Papachristou, I. Pirmettis, T. Papastaikoudi, M. Pelecanou, Ch. Tsoukalas, C. Raptopoulou, A. Terzis, M. Papadopoulos, E. Chiotellis (2002). Synthesis and biodistribution of ReO, ^{99g}TcO and ^{99m}TcO complexes with a novel tetradentate NNOO bifunctional ligand. 6th International Symposium on Technetium in Chemistry and Nuclear Medicine, September 4-7, Bressanone, Italy

I. Pirmettis, D. Papagiannopoulou, Ch. Tsoukalas, M. Papachristou, G. Patsis, C. Raptopoulou, A. Terzis, C. I. Stassinopoulou, M. Pelecanou, M. Papadopoulos, E. Chiotellis (2002). Oxotechnetium and oxorhenium bipyridine complexes with tridentate [SNO] ligands. 6th International Symposium on Technetium in Chemistry and Nuclear Medicine, September 4-7, Bressanone, Italy

Ch. Tsoukalas, I. Pirmettis, D. Papagiannopoulou, G. Patsis, M. Pelecanou, A. Papadopoulos, C. P. Raptopoulou, A. Terzis, M. Papadopouloos, E. Chiotellis (2002). Synthesis and characterization of novel oxorhenium ReO[NN][SNO] complexes. 6th International Symposium on Technetium in Chemistry and Nuclear Medicine, September 4-7, Bressanone, Italy

D. Papagiannopoulou, I. Pirmettis, M. Pelecanou, C. Tsoukalas, M. Papachristou, G. Patsis, C. P. Raptopoulou, A. Terzis, M. Papadopoulos, E. Chiotellis (2002). Isolation of a novel Re[P][NN]] [S][SO] mixed ligand hexacoordinated rhenium(III) complex. 6th International Symposium on Technetium in Chemistry and Nuclear Medicine, September 2002, Bressanone 4-7, Italy

S. Tzanopoulou, C. Tsoukalas, M. Papachristou, I. Pirmettis, G. Patsis, A. Papadopoulos, C. P. Raptopoulou, A. Terzis, E. Chiotellis, C. I. Stassinopoulou, M. Papadopoulos, M. Pelecanou (2002). 2-Pyridin-2'-yl-benzothiazole oxorhenium complexes: Synthesis and characterization. 6th International Symposium on Technetium in Chemistry and Nuclear Medicine, September 4-7, Bressanone

S E R V I C E U N I T S

- ***HUMAN TISSUE BANK***
- ***EXPERIMENTAL ANIMAL COLONY***

HUMAN TISSUE BANK

Research Staff

Helen Vavouraki, Technical Specialist
Theodoros Prassas, 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 continue the development and evaluation. of bovine bone graft according to our established production method.

Service Unit Activities during 2002

The numbers of the various types of grafts, which were produced and delivered to Hospitals, during 2002, are listed in the following table.

GRAFTS	DELIVERY
Cancellous Bone	494
Cortical bone	4
Mixed bone	3
Dura mater	78
Cartilage	2
Cranium bone	4

2002 Publications

Papadopoulou C., Dereka X., Vavouraki H.,Vrotsos I. (2002). In vitro evaluation of the mitogenic effect of PDGF-BB on human PDL cells cultured with different bone grafts. Journal of Periodontology (in press).

Marcopoulou C., Vrotsos I., Vavouraki H., Dereka X. (2002). Effect of rhBMP-2 on human PDL osteoblastic differentiation with and without bone grafts. Journal of Periodontology (in press).

2002 Presentations at International Scientific Conferences

Marcopoulou C. E., Vavouraki H. N., Dereka X.E., VrotsosI.A., Mantzavinos Z.S. The osteogenic potential of “Demokritos” Bank bone allografts alone and enriched with rhBMP-2, on human PDL cells. In vitro study. 11th International Conference on Tissue Banking and EATB (European Association of Tissue Banks) Annual Meeting, October 23-26, 2002, Bratislava, Slovak Republic. Book of Abstracts, pg 75

Vavouraki H.N., Marcopoulou C.E., Dereka X.E., Vrotsos I.A. Effect of a bovine bone graft, alone or enriched with PDGF-BB or rhBMP-2, on human periodontal ligament (PDL) cells proliferation. A preliminary study. 11th International Conference on Tissue Banking and EATB (European Association of Tissue Banks) Annual Meeting, October 23-26, 2002, Bratislava, Slovak Republic. Book of Abstracts, pg 103

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 2002, the Animal Facility made available the following animals:

Users	***Rats***	***Mice***	***Rabbits***
Institute of Biology	3	179	6
Institute of Radioisotopes & Radiodiagnostics	150	288	
University of Athens – Dpt Biology	143	10	
TEI, Athens	30		
"ELPEN" Pharmaceuticals	332		
Protopapas M., MD	24		
Makris L., MD	2		
Fragoulis M, MD	10		
Total of animals provided	**694**	**477**	**6**

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.

Educational Activities

Methods related to animal breeding, handling and maintenance were demonstrated in collaboration with other Research and Educational Institutions, and practice releated to the facility was taught to students from Technical Institutions of the University of Athens.

EDUCATIONAL ACTIVITIES

EDUCATION

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

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

During the year 2002, 19 scientists were trained at the postdoctoral level at our Institute. Furthermore, 23 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 2002, two of our graduate students finished their thesis work and became PhDs.

Moreover, 28 students from the University are carrying out their pre-graduate project thesis work at the Institute. Additionally, 7 students from Universities abroad (U.K.), 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 2002 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 courses were given by scientists of the Biology Institute:

- **Cell to Cell Communication** [course lecturers: H. Georgoussi, D. Kletsas and E. Tsilibary (coordinator)].
- **Structural Biology and Theoretical Modelling** [course lecturers: Y. Almirantis, M. Vlassi, M. Pelecanou and H. Stassinopoulou (coordinator)].

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:

- *The role of G proteins in cellular signaling* (**Dr. Iro Georgoussi,** Department of Biology, University of Athens)
- *Cell cultures – Tissue cultures* (**Dr. D. Kletsas**, Department of Biology, University of Athens).
- *Matrix-mediated signal transduction: The role of proteoglycans, growth factor and integrin receptors* (**Dr. E. Tsilibari**, Department of Biology, University of Athens)
- *Development of Technology for organic tobacco production, frre of agrochemical residues* (**Dr. G. Tsiropoulos**, Department of Biology, University of Crete)
- *Preparation and supervision of computer practicals on bioinformatics and 3D modelling (***Dr. M. Vlassi**, Department of Biology, University of Athens)
- *General Biology* (**Dr. S.Piperakis**, School of Humanities, University of Thessalia)
- *From genomics to functional genimics and proteomics* (**Prof. K. Iatrou**, Department of Biology, University of Athens)

During July 2002 the second Biology Summer School of our Institute (Biology Days 2002) was held. This came to continue the long tradition of Summer Schools of the NRCPS "Demokritos" and has included talks from Institute researchers and of invited speakers coming from other Greek Institutions and abroad. Students also had the occasion to visit the laboratories of the Institute, discuss with the scientific staff on their research activities and on the possibilities of postgraduate research in its premises. A discussion with the participation of invited speakers from research centres, universities and the private sector on this subject has also been held in the framework of Biology Days 2002.

Approximately eighty students attended all the sessions and provided written (anonymous) evaluation comments at the end. Despite the varying background of the participating students (Biology, Chemistry, Pharmacology, Medicine and Agricultural Sciences), the comments were overall positive and highly complimentary for the quality and the effort to include in the lectures the latest developments in every field. The effort for the presented material to be understood by students at a less advanced level of knowledge, without being oversimplified for attendants with more extensive background, was also acknowledged. Overall, the Summer School has been a positive experience not only for the students who attended it, but the entire Institute as a whole.

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 (2002) 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 2002

GRADUATE STUDENT	TITLE OF DOCTORAL THESIS	ADVISOR (in Institute of Biology)	UNIVERSITY
Stefanos Tavoularis	"Structure – function analysis of a main proline transporter *Aspenguillus nidulans*"	Vasiliki Sophianopoulou	Department of Biology, University of Athens
Ioannis Massas	"Soil factors influencing the availability in sunflowers and soyia bean plants"	Vassiliki Skarlou - Alexiou	Agricultural University of Athens

LECTURE CONTRIBUTIONS TO
THE 2002 SUMMER SCHOLL
OF THE NCSR "DEMOKRITOS"
(July 2002)

SPEAKER	TITLE
M. Vlassi Institute of Biology, NCSR "Demokritos"	Introduction to protein structure: Principles of X-ray Crystallography
N. Papandreou: Agricultural Univ. of Athens	Protein folding and three-dimensional structure prediction
A. Yonath Weitzmann Institute, Israel	- Structure is function: I. large biological assemblies can be subjected to structural studies - Structure is function: II. The ribosome as a target for antibiotics
A. Prombona Institute of Biology, NCSR "Demokritos"	Control of Plant Gene Expression by the Biological Clock
P. Hatzopoulos Agricultural Univ. of Athens	Plant Biotechnology: Achievements and Future Perspectives
E. Tsagris Univ. of Crete	Virus-Host Interactions in Plants
M. Tabler Univ. of Crete, IMMB	Mechanisms of Gene Silencing in Plants and Animals
G. Diallinas Univ. of Athens	Biological role and the importance of studying transmembrane transporters: The paradigm of nucleobase transporters.
V. Sophianopoulou Institute of Biology, NCSR "Demokritos"	Structure-Function analyses of amino acid transporters conserved from bacteria to humans
S. Frillingos Univ. of Ioannina	Molecular mapping of transmembrane nucleoside transporters: the value of cysteine scanning
C. Cazzochio Université de Paris-XI (Sud), France	Control of gene expression in a model microbial eukaryote: from classical genetics to chromatin structure
S. Hamodrakas: University of Athens	Prediction of the 3D structure of membrane proteins
G. Voutsinas Institute of Biology, NCSR "Demokritos"	An Introduction to Cancer Genetics
D. Kletsas Institute of Biology, NCSR "Demokritos"	Cell proliferation, cell senescence and carcinogenesis
D. Thanos BSRC "Alexander Fleming"	Regulation of gene expression in eucaryotes
L. T. Furcht University of Minnesota	- Dynamic role of extracellular matrices in modulating metastatic tumor cell behaviour (part I) - Dynamic role of extracellular matrices in modulating metastatic tumor cell behaviour (part II)
Ch. Zervas University of Cambridge, UK	Matrix-cytoskeleton interactions:Filling the gap
E. Tsilibari Institute of Biology, NCSR "Demokritos"	The role of integrin receptors in inflammation and wound healing: Regulation of matrix expression and matrix turnover

SEMINAR PROGRAMME

DATE	SPEAKER	TITLE
14/1/02	**Sp. Efthimiopoulos** Univ. of Athens	Metabolism, subcellular distribution and interactions of presenilim-1
15/1/02	**E. Thomadaki** Institute of Biology, NCSR "Demokritos"	Activation of differential cytochrome c release pathways induced after apoptosis with a etoposide
15/1/02	**G. Lallas** Institute of Biology, NCSR "Demokritos"	Bax translocation is crucial for sencitization of leukemic cells to etoposide
16/1/02	**A. Skorilas** NCRS "Demokritos"	Discovery and study of the physiological role of new proteins – molecular targets. Immunochemistry and development of new micotechnologies
21/1/02	**G. Mossialos** BSRC "Alexander Fleming"	Molecular mechanism of action of the oncoprotein LMP1
22/1/02	**A. Kypraiou** Institute of Biology, NCSR "Demokritos"	Ranscriptional repression by the Retinoblastona protein through the recruitment of a histone methyltransferase
22/1/02	**O. Kovaiou** Institute of Biology, NCSR "Demokritos"	A transcriptively active complex of APP with Fe65 and histone acetyltransferase Tip60
30/1/02	**A. Talamagas** Institute of Biology, NCSR "Demokritos"	Effects of the β-Amyloid and Carboxyl-terminal Fragment of Alzheimer's Amyloid Precursor Protein on the Production of the Tumor Necrosis Factor–α and Matrix Metalloproteinase 9 by Human Monocytic THP-1
30/1/02	**E. Argyrou** Institute Of Biology, NCSR "Demokritos"	Characterization of a concentrative type of adenosine transporter from Arabidopsis thaliana
1/2/02	**V. Papadopoulos** Georgetown University, USA	Peripheral benzodiazepin receptor: structure and function in health and disease
6/2/02	**A. Apostolidou** Institute of Biology, NCSR "Demokritos"	Suppression of the lepidoptera immune response from endoparasitoid hymenoptera.
13/2/02	**Th. Georgomanolis** Institute of Biology, NCSR "Demokritos"	Characterization of BmSH3, a Bombyx mori protein
20/2/02	**M. Sideridou** Institute of Biology, NCSR "Demokritos"	Function analysis of the main proline transporter PrnB of Aspergillus nidulans
28/2/02	**A. Garcia-Bellido** Universidad Autonoma Cantoblanco, Madrid, Spain	The control of size and shape in the Drosophila wing
6/3/02	**E. Morou** Institute of Biology, NCSR "Demokritos"	Structural determinants of opioid receptor- G protein interface
13/3/02	**Z. Erpapazoglou** Institute of Biology, NCSR "Demokritos"	Study of nucleobase transport systems from parasitic protozoa

20/3/03	**A. Tartas** Institute of Biology, NCSR "Demokritos"	Biochemical and structural studies of the N-terminus of SSN6 protein
27/3/02	**A. Kaldis** Institute of Biology, NCSR "Demokritos"	Investigation of the Biological Clock Function in *Phaseolus vulgaris*
3/4/02	**G. Lallas** Institute of Biology, NCSR "Demokritos"	Strategies of anticancer drugs and cell resistance: a post-transcriptional modifications and induction of apoptosis
17/4/02	**A. Talamagas** Institute of Biology, NCSR "Demokritos"	β-Amyloid interacts with integrins and modulates the expression of integrins and collagenases in SK-N-SH neuroblastoma cell line
15/5/02	**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*
29/5/02	**P. Handris** Institute of Biology, NCSR "Demokritos"	Structural and biochemical changes in the nucleus of replicative senescent human fibroblasts
30/5/02	**P. Hatzopoulos** Agricultural Univ. of Athens	Development and differentiation of *Arabidopsis thaliana* root hairs
5/6/02	**Ch. Giannouli** Institute of Biology, NCSR "Demokritos"	Study of the diverse effect of TGF-β on the proliferartion of human fibroblasts
12/6/02	**K. Economou** Institute of Biology, NCSR "Demokritos"	Regulation of the expression and functional studies of podocalyxin (PCLP)
19/6/02	**Ch. Nikolaou** Institute of Biology, NCSR "Demokritos"	Asymmetric patterns in the genetic text
3/7/02	**G. Mazarakou** Institute of Biology, NCSR "Demokritos"	Activation of μ-opioid receptor induces STAT5 (Signal Transducers and Activators of Transcription 5) phosphorylation
24/7/02	**A. Kypraiou** Institute of Biology, NCSR "Demokritos"	The role of histone H1o and acetylation of histone H4 during senescence and apoptosis of T lymphocytes
11/9/02	**V. Doukas**	Function of promyelocytic (PML)- complexomes in the deposition of histone-code on specific chromatin regions
18/9/02	**E. Thomadaki** Institute of Biology, NCSR "Demokritos"	Regulation of apoptosis at the polyadenylation stage of the mRNAs
3/10/02	**Th. Georgomanolis** Institute of Biology, NCSR "Demokritos"	Real-Time Monitoring of Intracellular mRNA Hybridization Inside Single Living Cells
10/10/02	**A. Apostolidou** Institute of Biology, NCSR "Demokritos"	Detailed Characterization of Polydnavirus Immunoevasive Proteins in an Endoparasitoid Wasp
10/10/02	**A. Tartas** Institute of Biology, NCSR "Demokritos"	Introduction to the bacterial ribosome structure

16/10/02	**N. Grammatikakis**	Chaperone-regulated kinase as an emerging novel concept in cell signaling: The Raf paradigm
17/10/02	**A. Talamagas** Institute of Biology, NCSR "Demokritos"	S-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death
24/10/02	**E. Morou** Institute of Biology, NCSR "Demokritos"	Regulation of opioid receptor trafficking and morphine tolerance by receptor oligomerization
24/10/02	**G. Mazarakou** Institute of Biology, NCSR "Demokritos"	Modulation of postendocytic sorting of G protein-coupled receptors
31/10/02	**Ch. Giannouli** Institute of Biology, NCSR "Demokritos"	Nucleocytoplasmic shuttling of Smads 2, 3, and 4 permits sensing of TGF- beta receptor activity
31/10/02	**L. Leodiadis** Institute of Biology, NCSR "Demokritos"	Regulator of G protein signaling Z1 (RGSZ1) interacts with Galpha i subunits and regulates Galpha i-mediated cell signaling
7/11/02	**G. Lallas** Institute of Biology, NCSR "Demokritos"	Loss of the Bcl-2 loop domain of phosphorylation increases the resistance of U937 to taxol – induced apoptosis and mitochondrial disfuction
7/11/02	**E. Thomadaki** Institute of Biology, NCSR "Demokritos"	Taxol-induced apoptosis on ovarial cancer cells (SCOV3) and breast cancer cells (MCF7), is independent of the caspase –3 and -9 activation
14/11/02	**A. Kypraiou** Institute of Biology, NCSR "Demokritos"	Human SIR2 deacetylates p53 and antagonizes PML/ p53 induced senescence
28/11/02	**Ch. Nikolaou** Institute of Biology, NCSR "Demokritos"	Cytosine Deamination plays a primary role in the evolution of mammalian isochores
14/3/02	**I. Karakatsanis** Institute of Biology, NCSR "Demokritos"	TGF-β autocrine signal trasduction regulates the motility of human cancer cells
12/12/02	**K. Sdralia** Institute of Biology, NCSR "Demokritos"	Analysis of gene function in somatic mammalian cells using small interfering RNAs
19/12/02	**Z. Erpapagoglou** Institute of Biology, NCSR "Demokritos"	Orientation of DNA replication establishes mating-type switching pattern in S. Pombe