NATIONAL CENTER FOR SCIENTIFIC RESEARCH
"DEMOKRITOS"

INSTITUTE OF BIOLOGY

2001
ANNUAL REPORT

TABLE OF CONTENTS

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:

- foster high quality research in the areas of Biochemistry, Biophysics, Cell and Molecular Biology, Genetics, Environmental Biology, Biomedicine and Biotechnology, and promote collaborations between Greek and foreign research institutions
- develop novel technologies in specialised areas of applied biology with the goal of finding solutions to important problems related to health and the environment
- transfer technology know-how to Greek and foreign industries and other national and international organisations
- participate in educational activities and produce highly-qualified personnel, mainly at the graduate and postdoctoral levels

Research activities at the IB are carried out in the context of three inter-related 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 he following pages.

In addition the IB provides specific services to researchers and the medical community of the country through the existence of two Service Units,

- **The Experimental Animal Colony and**
- **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 to cover at least part of the needs of the hospital surgical units for human transplants.

During 2001, 136 persons in total contributed to the R&D and service activities of the Institute. Amongst these are included permanent staff scientists, (Researchers and Specialized Scientists), Technical Staff, and other collaborators and administrative employees, as well as research associates, postdoctoral fellows, graduate students and undergraduate students.

The Institute's research activities are supported by research funds provided by the Greek government, the European Union, non-profit organizations and industrial organisations in Greece and abroad.

During this year a general three year planning of research activities of the Institute was accomplished. As a result of the extensive discussions between faculty members of the Institute three tightly interconnected targets of development were determined, as follows:

1. **Cellular Function and Methods for Combatting Pathological Conditions**
2. **Structure of Bioactive Molecules**
3. **Biotechnology and Natural Products**

In the meantime, the NCSR "Demokritos" also completed a three-year development planning that included a inter-institutional collaborations of high caliber. The goal was the continuation of cutting-edge research, the development of high technology services and new technologies in demand by third parties, and the continuation of provision of high quality education to new scientists. The Institute of Biology participates in 3 of the 10 inter-institutional collaborations and activities, that were defined through this planning process as follows:

1. **Bioactive Molecules, Natural Products and Biotechnology**
2. **Technologies of Drug and Diagnostic Tool Development**
3. **Control of Environmental Pollution**

The Institute of Biology is co-ordinating the first activity.

The definition of the three development targets for the Institute defines for the most part the main directions of the Institute of Biology for the immediate future. The defined targets are in accordance with the directions by the Supervising Ministry (Ministry of Development), which mandates the development of substantial inter-connections between institutional research and productivity by the user sector of the country. At the same time, the defined targets secure the continuity of competitive research activities by faculty members of the Institute in areas of research that advance in big strides. It remains to be determined whether the user sector responsible for the country's productivity will respond to the request by the government to substantially link with the Research Institutions and participate in their development efforts.

During 2001, the Institute of Biology welcomed to its faculty ranks a new Assistant Researcher, Dr. Luc Swevers, citizen of Belgium, PhD from the Catholic University of Leuven and Postoctoral Associate, initially at the University of Calgary, Canada and, subsequently at the Institute of Biology of the NCSR "Demokritos". His research interests focus on insect molecular biology and more specifically on the understanding of the role of the steroid hormone 20-hydroxy-ecdysone, its receptor as well as other "orphan" nuclear receptors, in terminal cell differentiation and insect development. We extend our best wishes to Dr. Swevers for a creative and productive career. In addition, we congratulate Drs. Maria Havredaki, Maria Pelekanou and Athina Tzinia for their promotion to Full, Associate, and Assistant Researher, respectively.

In July 2001, the Institute of Biology organized a two-week long, extremely successful "Summer School", whose aim was the systematic update of senior undergraduates and fresh science graduates from different Universities of the country on the current trends in Biomedical research. In this course, new developments in biomedical sciences and several of the research activities of the Institute were discussed. The general title of the course was "Days of Biology", and included three subjects, "Molecular Mechanisms of Regulation of Cellular Functions", " Structural Biology: Structure / Function relationships" and "Genomics and Post-Genomics". These were discussed by members of the research faculty of the Institute as well as by several invited specialists from Greece, Europe and Canada. Approximately 80 students and university graduates from Greece and other EEC countries attended the course, visited the Institute's laboratories and were informed about our research activities by the researchers in charge.

Finally, in January 2001, the 4th Scientific Retreat of the Institute was organized in Vrahati a picturesque resort town of the Peloponnese. During the two days of the workshop, all faculty members had the opportunity to become updated about research activities and developments by the Institute's research teams, discuss in depth other topics of general interest to the Institute, and of course, relax in a hospitable environment away from the everyday routine. The "George Akoyunoglou" award that was established in 2001, was also awarded for the first time to an excelling graduate student of the Institute, during the Retreat. The first student to receive the award was Eleftheria Argyrou, supervised by Dr. Vassiliki Sophianopoulou. Warm congratulations are extended to Ms. Argyrou for her achievements and her supervisor, Dr. Sophianopoulou.

In closing this introduction, I wish to extend my sincere gratitute to all faculty members of the Institute for their efforts to realize their goals during 2001 and, in particular their participation in the effort for the establishment of the three year development plan. I hope that in the next year our faculty will continue their efforts, and will also develop new research activities, which will allow them to fulfil more effectively the mission of creating new scientific knowledge for the benefit of our society.

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
Manoukas Athanassios	Nutritionist
Mazomenos Vassilios	Biologist
Sekeri Kalliope	Biochemist
Stassinopoulou Chariklia	Chemist
Tsilibary Effie	Biologist
Tsiropoulos George	Entomologist

Associate Research Scientists

Almirantis Yannis	Chemist
Georgoussi Zafiroula-Iro	Biologist
Havredaki Maria	Biologist
Loukas Spyros	Biochemist
Sophianopoulou Vassiliki	Biologist
Vlassi Metaxia	Physicist-Chrystallographer
Zervas George	Agronomist

Assistant Research Scientists

Kletsas Dimitris	Biologist
Labropoulou Vassiliki	Biochemist
Pelecanou Maria	Pharmacist
Piperakis Stelios	Biologist
Prombona Anastassia	Biologist
Stamatakis Konstantinos	Biologist
Voutsinas Gerassimos	Biologist
Swevers Luc	Biologist

Research Associates

Tzinia Athina	Biochemist

Senior Research Specialists

Skarlou-Alexiou Vassiliki	Agronomist

Technical Specialists

Stefanou Dimitra	Agronomist
Vavouraki Helen	Radiopharmacist

Research Specialists

Konstantopoulou Maria	Biologist

RESEARCH TECHNICIANS

Avgeris Socrates
Kalokiri-Stilianidi Kalliope
Kopanelis Dimitrios
Koutroumani Marina
Pantazi-Mazomenou Anastassia
Papadopoulos Vassilios
Prassas Theodoros
Sevaslidou Eleni
Tsolomiti-Gourgou Areti
Zafiropoulos Ioannis

ADMINISTRATIVE STAFF

Douvaras Panagiotis	Accountant
Kostakou Athanassia	Secretary

COLLABORATING RESEARCH SCIENTISTS

Collaborating Research Scientist	Laboratory
Ignatiadou Lydia (Dr. Hydrobiologist)	Iatrou K.
Fletser Mary (Dr. Chemist)	Mazomenos V.
Papageorgiou George (Dr. Biochemist)	Stamatakis K.
Papageorgiou Spyros (Dr. Physicist)	Almirantis I.
Sideris Eleftherios (Dr. Geneticist)	Sophianopoulou V.
Stathakos Dimitri (Dr. Biochemist)	Kletsas D.

POSTDOCTORAL FELLOWS

Fellow	Supervisor
Andreadaki Fotini	Iatrou K.
Dedos Skarlatos	Iatrou K.
Douris Vassilios	Iatrou K.
Georgakopoulos Ioannis	Mazomenos V.
Giannoulaki Eleni	Havredaki M.
Haveles Kostas	Sophianopoulou V.
Kiriakopoulou Christina	Havredaki M.
Kitsiou Paraskevi	Tsilibary E.
Kravariti Eleftheria	Iatrou K.
Lioupis Alexis	Iatrou K., Georgoussi I.
Petrakou Eftihia	Voutsinas G.
Pratsinis Haris	Kletsas D.
Smirli Despina	Vlassi M.
Sourlingas Thomae	Sekeri K.
Visvardis Evaghelos-Efstathios	Sophianopoulou V.
Zervolea Irene	Kletsas D.

GRADUATE STUDENTS

Student	Supervisor
Apostolidou Anastassia	Voutsinas G.
Argyrou Eleftheria	Sophianopoulou V.
Economou Kostas	Tsilibary E.

Erpapazoglou Zoi	Sophianopoulou V.
Georgomanolis Theodoros	Iatrou K.
Giannouli Christina	Kletsas D.
Handris Panagiotis	Kletsas D.
Kaldis Athanassios-Dimitrios	Prombona A.
Karamessinis Panayotis	Tsilibary E.
Kypreou Aikaterini	Sekeri K.
Koveou Ourania	Sekeri K.
Lallas George	Havredaki M.
Leontiadis Leonidas	Rotation
Massas Ioannis	Skarlou V.
Mazarakou Georgia	Georgoussi I.
Morou Evaghelia	Georgoussi I.
Nikolaou Christoforos	Almirantis Y.
Tavoularis Stefanos	Sophianopoulou V.
Thomadaki Ellinida	Havredaki M.
Sdralia Konstantia	Iatrou K.
Sideridou Maria	Sophianopoulou V.
Talamagas Anargiros	Tsilibary E.
Tartas Athanassios	Vlassi M.
Tsapali Dimitra	Sekeri K.- **PhD obtained in 2001**

GRADUATE RESEARCH ASSOCIATES

Fellow	**Supervisor**
Bouzarelou Dimitra	Sophianopoulou V.
Douvara Despina	Mazomenos V.
Dovletoglou Evaghelia	Georgoussi I.
Hatzis Michalis	Tsiropoulos G.
Kefala Georgia	Vlassi M.
Kesanopoulos Kostas	Prombona A.
Konstantopoulou Maria	Stamatakis K.
Koussidis Panagiotis	Prombona A.
Laskaris Evaghelos	Tsilibary E.
Seferi Maria	Vlassi M.
Xenou-Kokoletsi Magdalini	Mazomenos V.
Zouganelis George	Tsilibary E.

COLLABORATING GRADUATE STUDENTS

Student (University)	**Supervisor**
Alexandratou Eleni (Univ. of Athens)	Loukas S.
Atlamazoglou Vassilios (Univ. of Athens)	Loukas S.
Fanourakis Galenos (Univ. of Athens)	Voutsinas G.
Maniou Vassiliki (Univ. of Athens)	Stamatakis K.
Metaxatou Aghelina (Univ. of Eagean)	Iatrou K.
Michalopoulos N. (Univ. of Athens)	Voutsinas G.
Morteza Movahedy Feizal (Univ.of Teherani, Iran)	Mazomenos V.
Nikolopoulos George (IRRP, NCSR “D”)	Vlassi M.
Plearchopoulou Kiriaki (Univ. of Athens)	Voutsinas G.
Siskos Elias (Univ. of Cardiff)	Mazomenos V.
Tellier Aurelien (ENITA de Bordeaux, France)	Mazomenos V.
Tzanopoulou Stamatia (Univ. of Athens)	Pelekanou M.

UNDERGRADUATE STUDENTS

Student (University)	Supervisor
Athanassopoulos Panagiotis (Agricultural Univ. of Athens)	Prombona A.
Barkoulas Michalis (Univ. of Athens)	Prombona A.
Billini Maria (Univ. of Athens)	Sophianopoulou V.
Christopoulos George (Univ. of Athens)	Piperakis S.
Dimaras Ioannis (Univ. of Athens)	Piperakis S.
Dimitroglou Evanthia (Univ. of Athens)	Piperakis S.
Economopoulou Ifigenia (Univ. of Reading, UK)	Iatrou K.
Economou Gregorios (Univ. of Athens)	Voutsinas G.
Giannakopoulou Foteini (Agricultutal Univ. of Athens)	Skarlou V.
Gioti Anastasia (Univ. of Athens)	Sofianopoulou V.
Kalariti Niki (Univ. of Athens)	Labropoulou V.
Kanavetas Panagiotis (Univ. of Athens)	Piperakis S.
Kandri Niki (Univ. of Athens)	Piperakis S.
Kiriakopoulos Andreas (Univ. of Athens)	Georgoussi I.
Kontarakis Zacharias (Univ. of Crete)	Iatrou K.
Leptourgidou Flora (Univ. of Crete)	Mazomenos V.
Maniati Maria (Univ. of London)	Labropoulou V.
Maridaki Kiriaki (Univ. of Athens)	Piperakis S.
Papadopoulou Dimitra (Univ. of Athens)	Mazomenos V.
Papassaikas P. (Univ. of Athens)	Voutsinas G.
Politi Eleni (Univ. of Athens)	Iatrou K.
Psimadas Dimitrios (Univ. of Athens)	Piperakis S.
Psiouris Nikolaos (Univ. of Crete)	Piperakis S.
Tatsis Miltiadis (Agricultural Univ. Athens)	Skarlou V.
Thanos Nikolaos (Univ. of Athens)	Georgoussi I.
Tsilimigkaki Smaragdi (Univ. of Athens)	Piperakis S.
Valogiannis Spyros (Agricultural Univ. of Athens)	Skarlou V.
Vogiatzi Tereza-Andriani (Univ. of Crete)	Sophianopoulou V.
Xedous Marios (Univ. of Athens)	Georgoussi I.

OTHER TECHNICAL STAFF

Staff	Supervisor
Aligizaki-Zorba Aikaterini	Stamatakis K.
Anagnostopoulou Margarita	Tsilibary E.

BIOCHEMISTRY, CELL AND MOLECULAR BIOLOGY

RESEARCH GROUP: Signal Transduction Mechanisms –Molecular Pharmacoloy

Research Staff

Iro Georgoussi, Associate Research Scientist
Alexandros Lioupis, Postdoctoral Fellow
Georgia Mazarakou, Graduate Student
Evaghelia Morou, Graduate Student
Evaghelia Dovletoglou, Graduate Research Associate
Nikolaos Thanos, Undergraduate Student
Andreas Kiriakopoulos, Undergraduate Student
Marios Xidous, Undergraduate Student

Research Interests

The overall objective of our research interests is to apply to drug development the mechanisms which mediate the diverse effects of signal transduction of G protein coupled receptors (GPCRs) in order to understand the molecular and cellular basis of various types of adaptations that underlie the long lived aspects of addiction, and define the pathways and downstream components that connect opioid receptors with other neurotransmitter, or tyrosine coupled receptors that lead to changes of certain transcription or mitogenic factors

2001 Findings

***Structural and functional determinants 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-opioid receptor, and after application of a range of different delineated assay end points we demonstrate that the minigene encoding the third intracellular loop, alters the levels of cAMP accumulation, blocks IP formation and activates Gi/Go proteins as assessed by DAMGO induced stimulation of [^{35}S]GTPγS binding.

Cellular signaling of opioid receptors leading to alterations in gene expression-New alternative signaling mechanisms "beyond" G proteins. Another area of our research activity covers the molecular signaling circuits that lead opioid receptors to tolerance and dependence. In this regard, we observed for the first time that acute or chronic exposure to morphine, or to μ- opioid peptide DAMGO, of COS-7 cells transiently transfected with the μ-opioid receptor lead to phosphorylation of STAT5A and STAT5B members of the family of Signal Transducers and Activation of Transcription, but not those of STAT3. The mechanisms behind this signal are under investigation.

Development of HighTroughput Screens (HTP): Efforts are being made to develop a system for HTP screens for ligands that bind to the human serotonic receptor (h5-HT$4_{a)}$ using cell-based assays that express this receptor. In this regard, transient transfections in Bm5 cells (which is normally devoid of endogenous serotonin receptors) with the human 5-HT$_{4A}$ serotonin receptor demonstrated both its efficient expression (by specific ligand binding assays) and its functional coupling to the endogenous G protein machinery (by measuring increases in cAMP levels following agonist stimulation). In parallel, a reporter system for the human 5-HT$_{4A}$ serotonin has also been constructed. This consists of a reporter cassette (Green Fluorescence Protein, GFP) with several copies of the conserved CRE (CREB Response Element) binding site for CREB in the upstream region of a basal silkworm actin promoter element that is transcribed at extremely low levels in the absence of CREB-mediated binding and activation. To test the functionality of the reporter system, cells transfected with the expression and reporter constructs are monitored for GFP fluorescence (before and) after administration of serotonin.

2001 Publications

Morou E., Prombona A. and Georgoussi Z. (2001) "Expression of the third intracellular loop of the δ-opioid receptor alters G protein signaling" Protein Modules in cellular signaling, L.Heilmeyer and P. Friedrich (Eds) IOS Press, NATO series A: Life Sciences Vol.318, p.114-122

Morou E. and Georgoussi Z. (2001) "Expression of the third intracellular loop of the δ-opioid receptor alters opioid receptors' signaling" Klin. Pharmacol. Pharmakokin Rev.- Int. Ed. In press

2001 Presentations at International Scientific Conferences

Morou E., Gazouli M., Mazarakou G., Arvanitakis L., and Georgoussi Z., "Mutational analysis of conserved residues of the delta opioid receptor responsible for receptor activation and function" International Narcotic Research Conference, Helsinki, Finland, 14-19 July 2001

Mazarakou G., Merkouris M., Stravopodis D., and Georgoussi Z., "Acute and chronic exposure of the μ- opioid receptor to morphine induces CREB and STAT5A/5B phosphorylation" FEBS / EMBO Advanced Lecture Course: "Molecular Mechanisms in Signal Transduction" Spetses, Greece, 19-30 August 2001.

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.

2001 Findings

The study of low power laser (near IR) effects at the single cell level is completed.

Studies of the mechanisms of photodynamically induced oxidative stress at the single cell level and in real time were performed. 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.

In particular, 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.

Furthermore, this study is concentrated on the mechanisms of signal transmission, after oxidative stress, from intracellular region to nuclei which result in release of Ca^{2+} inside the nuclei.

2001 Publications

Kampa M, Loukas S, Tsapis A, Castanas E (2001). Receptorphin: A conserved peptide derived from the sequence of the opioid receptor, with opioid displacement activity and potent antiproliferative actions in tumor cells. *BioMed Central Pharmacology*, 1-9.

Atlamazoglou V, Yova D, Kavantzas N, and Loukas S (2001). Microscopical Examination of the Localisation Patterns of Two Novel Rhodamine Derivatives in Normal and Neoplastic Colonic Mucosa. Lasers Med Sci., 16, 253-259.

Atlamazoglou V, Yova D, Kavantzas N, and Loukas S (2001). Texture analysis of fluorescence microscopic images of colonic tissue sections. Med. and Biol. Eng. Comp., 39, 145-151.

Mikros E, Benaki D, Humpfer E, Spraul M, Loukas S, Stassinopoulou C, Pelekanou M (2001). High-Resolution NMR Spectroscopy of the β-Amyloid (1-28) Fibril Typical for Alzheimer's Disease. Angew Chem. Int. Ed., 40, 3603-3605.

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

Research Staff

Dimitris Kletsas, Assistant Research Scientist
Dimitri Stathakos, Collaborating Research Scientist
Haris Pratsinis, Postdoctoral Fellow
Irini Zervolea, Postdoctoral Fellow
Panagiotis Handris, Graduate Student
Christina Giannouli, Graduate 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 particularly studied. Furthermore, the structural and functional characteristics of the senescent (non-proliferating) cell – as opposite to the cancer cell – are also investigated, aiming at the elucidation of the mechanisms of ageing and longevity, as well as those of malignant transformation.

2001 Findings

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, the activation by TGF-β of the SMAD and MAPK signaling pathways, as well as their cross-talk, is under investigation. Furthermore, we are also studying the expression of cell cycle regulators, with emphasis to cyclin-dependent kinase inhibitors (e.g. $p21^{WAF1}$).

In parallel, we are studying the effect the extracellular environment on the action of TGF-β. In particular, we have found that extracellular matrix proteins, such as collagen, can drastically modulate TGF-β action. Currently, the cross-talk of integrin-mediated and TGF-β -activated signaling pathways is under investigation. In addition we are studying also the effect of mechanical forces on human cells. In particular, we have found that these forces can stimulate the expression and activation of c-Fos and c-Jun proteins – members of the AP-1 transcriptional complex – through the MAP and Rho kinases, as well as of specialized transcription factors, i.e. CBFA1.

We have also continued the investigation of the structure and function of the senescent (non-proliferating) cell, in opposition to the cancer cell. We are currently studying morphological and functional changes of the nucleus of the senescent cell: we have characterised the expression of structural and regulatory components of the nuclear lamina – especially the expression of LAP2 isoforms (thymopoeitins) – as well as, by using real time microscopy, defects in the last mitoses approaching senescence. In addition, developmental and age-related alterations in crucial regulators of cellular homeostasis, such as intracellular calcium concentrations, have also been investigated.

The project concerning the localisation of Greek centenarians and the registration of the data concerning their life-style and health parameters in an electronic data base, aiming at the identification of parameters of healthy ageing and longevity, was completed during 2001.

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, and also the evolutionary conservation of the presence and action of growth factors. Finally, we have developed specific cell assay systems and have investigated the cytostatic/cytotoxic and would healing activity of natural and new synthetic compounds.

2001 Publications

Tsagarakis, S., Tsigos, C., Vassiliou, V., Tsiotra, P., Pratsinis, H., Kletsas, D., Trivizas, P., Nikou, A., Mavromatis, T., Sotsiou, F., Raptis, S., Thalassinos, N.: Food-dependent androgen and cortisol secretion by a GIP-receptor expressive adenocortical adenoma leading to hirsutism and subclinical Cushing's syndrome: In vitro and in vivo studies. *J. Clin. Endocrinol. Metabol.* 86 (2001) 583-589.

Ottaviani, E., Barbieri, D., Malagoli, D., Kletsas, D.: "Involvement of PI-3-Kinase, PKA and PKC in PDGF- and TGF-beta-mediated prevention of 2-Deoxy-D-Ribose-induced apoptosis in the insect cell line IPLB-LDFB" *Cell Biol. Int.* 25 (2001) 171-177.

Gorgoulis, V.G., Zacharatos, P., Mariatos, G., Liloglou, T., Kokotas, S., Kastrinakis, N., Kotsinas, A., Athanasiou, A., Foukas, P., Zoumpourlis, V., Kletsas, D., Ikonomopoulos, J., Asimakopoulos, P.J., Kittas, C., Field, J.K.: "Deregulated expression of c-mos in non-small cell lung carcinomas: relationship with p53 status, genomic instability, and tumor kinetics" *Cancer Res.* 61 (2001) 538-549.

Tsoli, E., Gorgoulis, V.G., Zacharatos, P., Kotsinas, A., Mariatos, G., Kasrtrinakis, N.G., Kokotas, S., Kanavaros, P., Asimacopoulos, P., Field, J.K., Kletsas, D., Kittas, C.: "Low Levels of p27 in Association With Deregulated p53-pRb Protein Status Enhance Tumor Proliferation and Chromosomal Instability in Non-Small Cell Lung Carcinomas" *Mol. Med*. 7 (2001) 418-429.

Ottaviani, E., Franchini, A., Kletsas, D.: "PDGF and TGF-b in invertebrate immune and neuroendocrine interactions: another sign of conservation in evolution" *Comp. Biochem. Physiol. C Toxicol Pharmacol.* 129 (2001) 295-306.

Kletsas, D., Basdra, E.K., Papavassiliou, A.G.: "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.* (2002) (in press)

Pratsinis, H., Konstantinidou, A., Liaouri, A., Kletsas, D., and Davaris, P. (2002). "*In vitro* studies of cell proliferation and cell death for the evaluation of natural products and synthetic compounds" Epith. Klin. Pharm. Pharmakokin. Int. Ed. (in press).

2001 Presentations at International Scientific Conferences

Pratsinis H., Ir. Zervolea, D. Kletsas "Studies on the signal transduction of TGF-β in normal human fibroblasts". Jacques Monod Conference "Signaling and control of transcription", 9-13 Ιουνίου 2001, Aussois, France.

Giannouli Ch., H. Pratsinis, Ir. Zervolea, D. Kletsas "Differential effect of TGF-β on the proliferation of human fibroblasts" FEBS-EMBO Advanced Lecture Course "Molecular mechanisms in signal transduction" 19-30 August 2001, Spetses, Greece

Zervolea Ir., H. Pratsinis, D. Kletsas "Autocrine regulation of tissue formation by human fibroblasts". 11th Annual European Tissue Repair Society Conference, 5-8 September 2001, Cardiff, UK.

Kletsas D., E.K. Basdra, P. Ziros, A.G. Papavasiliou "Molecular mechanisms of mechano-inductive osteogenesis" 11th Annual European Tissue Repair Society Conference, 5-8 September 2001, Cardiff, UK.

RESEARCH GROUP: Pathobiology of the Exracellular Matrix

Research Staff

Fotini-Effie Tsilibary, Research Scientist
Athina Tzinia, Research Associate
Paraskevi Kitsiou, Postdoctoral Fellow
Panagiotis Karamessinis, Graduate Student
Kostas Economou, Graduate Student
Argiris Talamaghas, Graduate Student
George Zouganelis, Graduate Research Associate
Evaghelos Laskaris, Graduate Research Associate

Research Interests

The group's interests focus on integrin-mediated regulation of gene expression in different cell types, in normal and pathological conditions. In this context, integrin-mediated signaling pathways, and the expression of matrix-related macromolecules, which involves integrin receptors, are examined. The systems used for these approaches include cell culture systems, which simulate diabetic conditions, peripheral blood cells from diabetic human subjects, and cell culture systems, which simulate conditions of Alzheimer's disease. Furthermore, interactions between integrin subunits from different cell types and isoform chains of basement membrane collagen, as well as interactions of other surface molecules / receptors such as podocalyxin, with this type of collagen. The goal is a molecular understanding of mechanisms by which basement membranes membranes such as collagen IV and TIN antigen, regulate gene expression. Finally, new functions of TIN antigen, such as enzymatic activity are being investigated.

Functional studies, on basement membrane macromolecules and constituents involved in the degradative pathway, such as, enzymes and their inhibitors. Alterations in their expression appear to be of pathophysiological significance in certain diseases.

2001 Findings

Differential regulation of integrin-related collagenase /matrixin expression in different cell types. In human renal glomerular epithelial cells, α3β1 induces the expression of matrixin MMP-2 (up-regulation), whereas the expression of matrixin MMP-9 is not effected by any of the major integrin receptors of these cells, α3β1, α2β1, α5β1, or αvβ3. In renal proximal tubular epithelial cells and SK-N-SH neuroblastoma cells, α3β1 integrin induces the expression of MMP-2 (up-regulation), whereas αvβ3 results in down-regulation of MMP-9. Integrins α3β1 and αvβ3 mediate the binding of SK-N-SH cells to collagen IV; the presence of this collagen as a substrate modulates integrin expression by these cells and induces the expression and activation of matrixins MMP-2 and MMP-9 which are secreted by these cells.

Cell interaction and binding with basement membrane components is mediated by different integrins, depending on glucose concentration. In renal glomerular epithelial cells, integrins α3β1 and α5β1 mediate binding to collagen IV in normal glucose concentrations, whereas in the presence of high glucose the binding is preferentially mediated by αvβ3. In the case of proximal tubular epithelial cells, the binding to collagen IV and laminin is mediated by αvβ3 and α5β1 integrins in normal glucose concentrations, but in high glucose there is preferential binding of α2β1 integrin. The binding via different integrins, depending on glucose concentration, may serve for triggering different signaling pathways in each case, which modulate accordingly gene expression in "diabetic" conditions.

The expression of β1 integrin in monocytes isolated from the peripheral blood of diabetic patients is selectively decreased in the subpopulation of diabetic subjects who suffer from microangiopathy / nephropathy. We conclude that the decrease of β1 integrin isdue to gerentic factors, and possibly exists before the onset of complications. Statistical evaluation of the data strongly suggests that decreased expression of β1 integrin may serve as a predictor of diabetic microvascular complications, and more selectively, nephropathy.

Cell surface glycoprotein podocalyxin of renal glomerular epithelial cells has anti-adhesive effects on basement membrane substrates. Podocalyxin antagonizes the adhesive effect of β1 integrin subunit, as well as cell spreading on collagen IV substrata. The expression of this protein is induced by laminin and intact basement membrane, which also induce the formation of specialized foot processes by these cells. Podocalyxin therefore, plays an important role in induction of the differentiated morphology of these cells.

Interactions of human neuroblastoma cells with collagenic substrates.

Integrin-mediated interactions with collagen IV and its domains were examined in a human neuroblastoma cell line. It was demonstrated that binding of cells to solid-phase intact collagen IV and synthetic cell-binding peptide HEP-III is mainly mediated by β1, α3 and αvβ3 integrins Culture of neuroblastoma cells on collagen IV resulted in alteration of integrin expression, as well as in the induction of expression and activation of collagenases A and B, with a concomitant increase in the expression of their inhibitors TIMP-1, TIMP-2. Finally the expression of MMP-2 was significantly up regulated by anti-α3β1 antibodies, whereas ligation of anti-αvβ3 antibodies, resulted in a modest down regulation of MMP-2.

2001 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.

2001 Findings

The expression of the linker histone variant, H1o, at the protein synthesis and mRNA levels was studied in the *in vitro* artificial ageing cell system of human diploid fibroblasts, where the histone deacetylases inhibitors, sodium butyrate and trichostatin A, were used as inducers of artificial ageing and compared with fibroblast cell cultures aged physiologically. At the protein synthesis level, no differences were discerned amongst the two systems. Differences were found amongst the two systems with respect to H1o mRNA levels. These latter differences were further investigated in both systems in correlation with the number of cell population doublings that each of the two types of cultures had gone through till they reached the post-mitotic senescent state. Further investigation of the relationship amongst H1o mRNA levels in correlation with the cell population doubling number is under way.

The effect of the histone deacetylase inhibitors, sodium butyrate and trichostatin A, on H1o expression, histone H4 acetylation and apoptosis were studied in peripheral blood lymphocytes. Furthermore, a comparative study was accomplished on the effect of the inhibitor, trichostatin A, on H1o expression, histone H4 acetylation and inhibition of total histone synthesis in peripheral blood lymphocytes from donors of different ages. It was found that the effect of trichostatin A was dependent on the age of the donor. More specifically, the effect of the histone deacetylase inhibitor increased with increasing age of the donor. From these results it was concluded that histone deacetylases showed differential sensitivity to the inhibitor as a function of increasing age. These results may possibly be due to the appearance of different deacetylase molecules during senescence from those that are found in young cell types.

2001 Publications

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RESEARCH GROUP: Molecular Genetics and Biotechnology

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Konstantia Sdralia, Graduate Student
Aghelina Metaxatou, Collaborating Graduate Student
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Research Interests

Molecular analysis of the developmental program that directs follicular cell differentiation during oogenesis in silkmoths : use of *in vitro* culture of ovarioles to determine factors that stimulate follicle development, molecular analysis of the response to the steroid hormone 20-hydroxyecdysone by RNA interference technology.

Development of methods for control of insect pests: development of baculoviruses as new efficient transformation tools, improved pest control agents and gene therapy vectors, exploration of transposable elements for insect transformation.

Functional genomics : development of transformed lepidopteran tissue culture cell lines as screening systems for biologically active substances and producers of recombinant proteins of commercial and therapeutical value.

2001 Findings

Previous research on silkmoth oogenesis has led to the isolation of many regulatory factors (transcription factors, nuclear hormone receptors, vitelline membrane proteins, serine proteases, adaptor molecules in signal transduction) that are involved in the regulation of follicular cell differentiation during oogenesis. However, so far the assessment of the function of the regulatory factors during silkmoth oogenesis has been hampered *in vivo* by the lack of a robust silkmoth transformation system. Recently, the technique of double-stranded (ds) RNA-mediated RNA interference (RNAi) has emerged as a valuable tool to assess gene function in organisms that are not easily amenable to genetic analysis. To test the function of RNAi in the silkmoth, dsRNA was prepared from cloned sequences encoding nuclear receptors such as the ecdysone receptor (BmEcR) and its heterodimerization partner (BmCF1) and the orphan receptors BmHR3A and BmE75C. It was observed that introduction of dsRNA into Bm5 cells, a silkmoth-derived tissue culture cell line, results in strong, specific and dose-dependent inhibition of protein expression as judged by the activation of appropriate reporter (GFP or CAT) constructs and by Western blot analysis. Our results show that RNAi occurs in Lepidopteran cells and that it can be used as a valuable tool in clarifying signal transduction pathways such as the response to the steroid moulting hormone ecdysone. The potential of dsRNA to interfere with specific gene expression is currently being tested on growing silkmoth ovarioles in organ culture.

To obtain an improved biological insecticide, a new recombinant baculovirus (*Bombyx mori* nuclear polyhedrosis virus or BmNPV) expressing the AaIT insect specific neurotoxin from the scorpion *Androctonus australis* under the control of the silkmoth cytoplasmic actin promoter (A.AaIT) was constructed (BmNPV/A.AaIT). The insertion of the expression cassette occurred into a neutral position of the virus genome that is not essential for viral function and the purified recombinant baculoviruses that were obtained formed proteinaceous crystals (occlusion bodies) in the nuclei of infected cells, a phenotype that is essential for transmission of viral infection between different hosts. AaIT toxin expression was detected by Western blot analysis in the hemolymph of silkmoth larvae infected with recombinant BmNPV/A.AaIT. Preliminary experiments show that infection of silkworm larvae with BmNPV/A.AaIT leads to a dramatic reduction in feeding and time to death, in comparison to infection with control viruses.

Recombinant occlusion body-positive viruses generated by the insertion of the actin promoter-based expression cassette into a neutral position of the baculoviral genome are considered environmentally stable viruses that can spread efficiently among larvae by oral infection and therefore show great promise for their development as improved biological pesticides. The construction of similar viruses that express other harmful proteins such as juvenile hormone esterase (a hormonal disrupter) and collagenase (for destruction of the cellular basal membrane) is currently in progress.

The cloning of hormone receptors and the elucidation of their intracellular transduction pathways has opened new possibilities for the development of High Through-Put (HTP) screening systems for ligand analogues. These are based on cell lines that constutively express a ligand-activated receptor and are transformed with a reporter construct whose

activity is induced by the hormone-activated receptor. Using this approach, a HTP system was developed for the detection of ecdysone (moulting hormone) analogues in plant extracts. Bm5 cells, which express constitutively the two components of the ecdysone receptor heterodimer, BmEcR and BmCF1, were stably transformed with an ecdysone-responsive GFP reporter construct. Screenings (collaboration with the laboratory of Chemical Ecology and Natural Products, NCSR "Demokritos", and Vioryl Chemical Co) resulted in the identification of ecdysone mimetic compounds in extracts from spinach and *Chenopodium* of which one was characterized by mass spectrometry as 20-hydroxyecdysone. Antagonistic substances were shown to be present in extracts from several *Citrus* species and their purification by HPLC is in progress. A similar rationale is currently applied to develop cell-line based screening systems for juvenile hormone (JH)-like compounds and for serotonin mimetic substances.

2001 Publications

Eystathioy, T., Swevers, L. and Iatrou, K. (2001). The orphan nuclear receptor BmHR3A of *Bombyx mori*: hormonal control, ovarian expression and functional properties. Mech. Dev. **103**, 107-115.

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Iatrou, K. (2001). Methods of Expressing Proteins in Insect Cells and Methods of Killing Insects. USA Patent No. 6,221,632 (April 24, 2001).

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Swevers, L., Kravariti, L., Xenou-Kokoletsi, M., Sdralia, N., Mazomenos, V., Ragousis, N. and Iatrou, K. (2001). Development of new screening systems for hormonal compounds using transformed insect cell lines, 2001 Congress on In Vitro Biology, St. Louis, Missouri, USA (June 2001). In Vitro Cellular & Developmental Biology (Animal) 37, 6-A.

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RESEARCH GROUP: Theoretical and Developmental Biology

Research Staff

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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.

2001 Findings

During this year, the clustering and non-randomness in the nucleotide distribution of genomic sequences, is systematically studied by our group. For this purpose we use the "Modified Standard Deviation" (MSD), a quantity measuring the clustering of nucleotides, which is able to filter, during its computation, only the clustering in a specific length scale. In the middle scale of a few tenths to hundreds of nucleotides, non-coding sequences are found to be, in general, more non-random (clustered) than coding sequences. We apply this property in the assessment of the computer-derived annotation of the new sequences, massively produced by the ongoing genome projects. The MSD has been used recently in the formulation from our group of an evolutionary scenario for the genome evolution.

2001 Publications

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RESEARCH GROUP: mRNA 3′- end Formation

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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.

2001 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.

2001 Publications

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RESEARCH GROUP: Environmental Mutagenesis-Carcinogenesis

Research Staff

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Sokratis Avgeris, Research Technician

Research Interests

Genetic and epigenetic alterations in genes involved in cellular metabolism, cell cycle, DNA repair and apoptosis
Involvement of apoptotic pathways in carcinogenesis and drug resistance
Protein-protein interactions as pharmaceutical targets

2001 Findings

Structural alterations and transcript variant analysis of Fas (APO-1/CD95) gene in breast cancer

In 43 breast cancer samples, we detected no structural changes in exons 9 and 6 of *Fas* gene, coding for the cytoplasmic death and the transmembrane domains. No difference in the ratio of transmembrane tmFas vs soluble sFas mRNA expression was detected between breast tumor and normal breast samples. Therefore, inhibition of the Fas pathway in breast cancer seems not be due to structural alterations in exons 9 and 6 of the gene, whereas the observed high levels of sCD95 in the serum of breast cancer patients are not produced by the cancer cells but they may rather be attributed to a systemic immune response against the tumor. The work was submitted for publication.

Microsatellite instability in Greek FAP patients

In 22 adenomas and 12 adenocarcinomas (34 samples) from 10 patients 10/22 and 6/12 mutations were found in *K-ras* codon 12, as well as 3/22 and 7/12 mutations in *p53* exons 5-8. The most important finding of the work is the detection of microsatellite instability in genetic loci BAT26, D5S346 and TβRII in two unrelated adenocarcinomas, which is a typical feature of the Hereditary Non-Polyposis Colorectal Cancer and not FAP. The work was submitted for publication.

p53 mutation detection and expression in non-melanoma skin cancer

In 35 samples, the majority was found to exhibit high levels of protein expression, while 6/24 putative mutations were detected. Three of the sequence alterations detected were verified and identified by direct sequencing.

Genetic polymorphisms in genes CYP17, COMT and ERα, involved in hormone metabolism, and breast cancer appearance

Collection of samples was completed and genotype analysis was carried out in 50 breast cancer patients. At present, genotype analysis in another 50 patients and 116 samples from normal women of the same age group.

Development of a new system for the study of protein-protein interactions based on Fluorescence Resonance Energy Transfer technology

After designing of the system, part of the plasmid constructs necessary for its validation and subsequent use were generated.

2001 Publications

Voutsinas G. (2001) Mutagenesis, apoptosis, basic relation to carcinogenic models. Folia Histochem. Cytobiol. 39, suppl. 2, 56-57.

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G. Voutsinas (2001). Mutagenesis, Apoptosis, Basic relation to carcinogenic models. NATO Advanced Research Workshop on Endocrine Disrupters and Carcinogenic Risk Assessment, 8-12 May 2001, Bialystok, Poland.

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RESEARCH GROUP: Microbial Molecular Genetics and Radiation Genetics

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Demitra Bouzarelou, Graduate Research Associate
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Research Interests

Study of the molecular mechanisms involved in the transport of nucleobases, ascorbate and amino acids across the plasma membrane. Structure-function analysis of specific transmembrane transporters that mediate such transport. Cloning and functional characterization of putative nucleobase/ascorbate transporters from parasitic protozoan, bacteria, plants and humans, using *Aspergillus nidulans* as a novel model system.

Study of induced, mainly from exposure to α- or γ- radiation, biophysical and biochemical lesions on the DNA of eukaryotic cells associated with the induction of cancer.

2001 Findings

Functional characterization of Leaf permease 1 (LPE1), a plant representative of the large and ubiquitous Nucleobase/Ascorbate Transporter family (NAT), by expression in *Aspergillus nidulans*. Our results define the biochemical function of LPE1. In addition, *A. nidulans* is introduced as a novel model system for the cloning and/or functional characterization of "foreign" transporter genes.

Functional expression and cellular localization of a GFP-tagged proline transporter (PrnB) in *A. nidulans.* This work shows that the Green-Fluorescent-Protein fusion technology is a unique tool to study the expression and cellular localization of low-abundance transmembrane transporters expressed from their native promoters. In addition our results demonstrate that the length of the amino acid linker, between the transporter protein and the GFP, is critical for the proper expression of the resulting chimeric protein molecules.

Mutational analysis of the major (PrnB) proline transporter of *A. nidulans*. This study shows that the distribution of critical for function amino acids in PrnB, is similar to that found in homologous Amino acid Polyamine Organocation (APC) transporters of bacteria, yeast and mammals.

The human Peripheral Blood Mononuclear Cells (PBMC) are the most commonly internationally used probe for PBMC monitoring and evaluating the radiation induced damage in humans. In our work with a significantly higher frequency radiation induced-programmed cell death (apoptosis) seems to be caused by the indirect effect of ionizing radiation than the direct effect on the cells DNA. The induction phase of apoptosis is a late phenomenon in human PBMC, evolving after DNA damage induction, while the execution phase of apoptosis is carried out significantly later than the DNA repair. The presence of differentiated PBMC sub populations during the induction and the execution phase was verified. A linear relationship between the percentage of cells carrying average and intense fragmentation after repair and the percentage of apoptotic cells during the post-exposure incubation was found for both indirect as well direct induced damage. In general the levels of initial DNA fragmentation, as well as the rejoining capacity do not directly relate to the levels of the late appearing apoptosis in the population of PBMC.

2001 Publications

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Qi Li, E. Argyrou, V. Sophianopoulou, G. Diallinas, N. Schultes (2001). Characterization of Plant Nucleobase-Ascorbate Transporters. Annual Meeting of the American Society of Plant Biologists (ASPB), 21-25 July, Providence, Rhode Island, USA.

E. Argyrou, G. Diallinas, N. Schultes, V. Sophianopoulou (2001). Functional characterization of a maize purine transporter by expression in *Aspergillus nidulans*. 2nd International Symposium on Nitrogen metabolism in Ascomycetes, March 21-25, 2001, San Jost Vistahermosa, Molero, Mexico.

S. N. Tavoularis, C. Scazzocchio, V. Sophianopoulou (2001). Functional Expression and Cellular Localization of a Green Fluorescent Protein-Tagged Proline transporter In *Aspergillus nidulans.* 19th International Meeting on Yeast Transport and Energetics (SMYTE), September 14-17, 2001, Chania, Greece. Page 51.

Visvardis, E. E., Haveles, K. S., Sideris, E. G., Sophianopoulou, V. (2001)**.** DNA damage, repair and induction of apoptosis in lymphocytes of patients with chronic lymphocytic leukemia. 48th Annual Meeting, Radiation Res. Soc., April 21-25, 2001, Puerto Rico, USA.

Haveles, K. S., Visvardis, E. E., Georgakilas, A. G., Sophianopoulou, V., Sideris, E. G. (2001). Distribution of DNA strand breaks in γ-irradiated human lymphocytes. 48th Annual Meeting, Radiation Res. Soc., April 21-25, 2001, Puerto Rico, USA .

RESEARCH GROUP: Biogenesis and Function of the Photosynthetic Membrane

Research Staff

Anastassia Prombona, Assistant Research Scientist
Athanasios-Dimitrios Kaldis, Graduate Student
Kostas Kessanopoulos, Graduate Research Associate
Panagiotis Koussidis, Graduate Research Associate
Panagiotis Athanassopoulos, Undergraduate Student
Michalis Barkoulas, Undergraduate Student

Research Interests

Study of the biological clock function in plants. Molecular mechanisms of its synchronization/entrainment with the varying light-conditions of the environment. The role of PvLHY, a putative transcription factor and component of the central oscillator, in bean. Interaction of PvLHY with other transcription factors in order to elucidate its involvement in the induction and the repression of transcription.

2001 Findings

The performed experiments aimed to answer the question how the biological clock of plants is synchronized to varying light conditions. Etiolated bean seedlings were exposed to 2-min white-light pulses, applied at specific time points of the rhythm observed in *Lhcb* (light-harvesting complex of photosystem II) gene expression. Seedlings were also exposed to different photoperiods (day-night conditions). We studied the transcription pattern of PvLHY, a putative transcription factor and element of the central oscillator in bean, induced by the flashes and the day-night light conditions. Our results showed that 1. The rhythmic expression of *Lhcb* is due to the oscillations in the expression of *PvLHY*. 2. The differentiated response following the application of each new flash shows phase-dependence on the pre-existing rhythm. 3. These observed rhythms are always composed of two distinct rhythmic phenomena, the acute response cycle and the circadian cycle that are in strong antagonism. Thus, an enhancement in the amplitude and the period length of the acute response cycle correlates with a reduction in the amplitude of the circadian cycle and *vice-versa*. 4. The variability of the acute response cycle has as a consequence the advance or delay of the following circadian cycle. The total of these phenomena explains how the biological clock is synchronized with the light flashes. 5. *PvLHY* gene expression pattern at different photoperiods indicates for the first time that the rhythm under day/night conditions also consists of the two elements, the acute response, which takes place during the day, and the circadian response cycle, which occurs during the night. The circadian-phase at dawn of the new day determines the magnitude of the new acute response. Thus, the reciprocal control of the two cycles under the photoperiod as well, synchronizes the biological clock with the natural light conditions of the environment (work submitted for publication).

In the project aiming the identification of new clock elements in bean, we subtracted the two constructed cDNA libraries that correspond to the peak and the trough of the rhythm in the expression of *Lhcb*. The subtracted clone libraries were screened differentially and the obtained clones are under analysis.

2001 Publications

E. Morou, A. Prombona and Z. Georgoussi: *Expression of the third intracellular loop of the δ-opioid receptor alters G protein signaling*. In Protein Modules in Cellular Signaling, L. Heilmeyer and P. Friedrich (Eds.), IOS Press, Series A: Life Sciences, Vol. 318, p.114-122 (2001)

RESEARCH GROUP: Biophysics and Biotechnology of Membranes

Research Staff

Kostas Stamatakis, Assistant Research Scientist
George Papageorgiou, Collaborating Research Scientist
Maria Konstantopoulou, Graduate Research Associate
Vassiliki Maniou, Collaborating Graduate Student
Aikaterini Alygizaki-Zorba, Technician

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. Effects of allelochemicals on growth and photosynthesis of N_2-fixing cyanobacteria.

2001 Findings

In the research project of 2001 we examined the relation between Chl *a* fluorescence and osmotic volume changes of cyanobacterial cells. The excitation energy transfer from the phycobilisomes to the photosystems II and I depend on the osmotic conditions of the cytoplasm. At hyper-osmotic conditions more excitation energy transferred from phycobilisome to photosystem I. We demonstrated a quantitative relation between osmotically-induced cell volume changes (ΔV) and Chl *a* fluorescence changes (ΔF). During a salinity upshock episode cells of freshwater cyanobacterium *Synechococcus* undergo transient changes in the osmotic volume (ΔV_{OSM}), and the intensity of phycobilisome-sensitized chlorophyll *a* (Chla) fluorescence (ΔF_{OSM}), with $\Delta F_{OSM} \propto V_{OSM}$. We examined also the effect of epicuticular substanses from *Dittrichia viscosa* on the growth and photosynthesis of N_2-fixing cyanobacteria. The epicuticular substanses exerted no effect on the light-induced photosynthetic electron transport (from water to ferredoxin) but they prevented cell proliferation. This indicates inhibitory action on dark anabolic processes, such as CO_2 fixation.

2001 Publications

Stamatakis K., and Papageorgiou G. C. (2001). The osmolality of the cell suspension regulates phycobilisome-to-photosystem I transfers in cyanobacteria. Biochim. Biophys. Acta (Bioenergetics) 1506: 172-181

Papageorgiou GC (2001) Assessing cytoplasmic water deficit in cyanobacteria with chlorophyll fluorescence. In Modern Problems of Cellular and Molecular Biophysics (SN Ayrapetian & ACT North, eds), pp. 167- 176, UNESCO Biomedica.

2001 Presentations at International Scientific Conferences

S. Stavrianakou, E. V. Kapaxidi, P. Karageorgou, M. Konstantopoulou, E. Levizou, V. Liakoura, A. Markoglou, G. TH. Papadoulis, K. Stamatakis, G. A. Karabourniotis, Y. Manetas (2001). *Dittrichia viscosa*: A hostile neighbour? 1st International Congress in Allelopathy. Book of Abstracts 2001. Vigo, Spain.

K. Stamatakis and G. C. Papageorgiou (2001). Osmolality of cell suspension regulates the excitation transfer in cyanobacteria. Light Stress and Photosynthesis UVb and Visible Light Effects, 13-17 August 2001, Heron Island, Australia.

Stamatakis, K., and Konstantopoulou M. 2001 Allelopathy effects of aqueous rinses of *Dittrichia viscosa* (L.) on the photosynthesis and cell proliferation of N_2-fixing soil cyanobacteria. Proceedings of the 12th International Congress of Photosynthesis, Brisbane, Australia, 18-24 August 2001.

Papageorgiou, GC, Alygizaki-Zorba, A., Maniou, VG, Stamatakis, K. Na^+ and water fluxes across cell membranes of the freshwater cyanobacterium *Synechococcus* as reported by chlorophyll a fluorescence. Proceedings of the 12th International Congress of Photosynthesis, Brisbane, Australia, 18-24 August 2001.

ENVIRONMENTAL BIOLOGY

RESEARCH GROUP: DNA Repair Systems and Cancer

Research Staff

Stelios Piperakis, Assistant Research Scientist
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Evanthia Dimitroglou, UnderGraduate Student
Panagiotis Kanavetas, UnderGraduate Student
Niki Kandri, UnderGraduate Student
Kyriaki Maridaki, UnderGraduate Student
George Xristopoulos, UnderGraduate Student
Demetrios Psimadas, UnderGraduate Student
Nikolaos Psiouris, 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.

2001 Findings

We completed the experimental analyses of the results of the programme “pesticides effects on humans”. This programme which is been coordinated by me and in which participate with Greece, Spain Hungary and Poland is financed by E.U. We are now in the process of publishing these results.

We analyzed the results from the programme “Dietary habits: Programme of education in the Biology, Psychology of diet and misleading advertisements” which I coordinate with the participation of the University of Thessaly and the Education administration of the prefecture of Karditsa. We now prepare the results for publication.

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 accepted for publication.

The study "effects of high voltage pylons on DNA" is now completed. The results are under preparation for submission to publication.

In the study "DNA damage-repair and mental disorders" the samples have been collected from the Psychiatric Hospital and analyzed. The results are under preparation for submission to scientific journal.

The collection of the samples for the study "stress and DNA damage-repair" has been completed and analyzed. The results have been submitted for publication.

The study "DNA damage-repair and effects of solar seasonal variations" has finished. The results have been submitted for publication.

2001 Publications

S. Pastor, S. Gutierrez, A. Creus, N. Xamena, S.M. Piperakis and R. Marcos. (2001). Cytogenetic analysis of Greek farmers using micronucleus assay in peripheral lymphocytes and bucal cells. Mutagenesis, 16, 539-545

2001 Presentations at International Scientific Conferences

E. Dimitroglou, M. Zafiropoulou, N. Messini-Nikolaki, S. Doudounakis, S. Tsilimigaki and S.M. Piperakis. "DNA damage-repair in a population with chronic psychogenic stress". 31st European Environmental Mutagen Society meeting, Ghent, Belgium, September 2001.

D. Psimadas, N. Messini-Nikolaki, A. Fortos, S. Tsilimigaki and S.M. Piperakis. "DNA damage and repair efficiency in schizophrenic patients". 31st European Environmental Mutagen Society meeting, Ghent, Belgium, September 2001

S. Tsilimigaki, N. Messini-Nikolaki, M. Kanariou and S.M. Piperakis "A study on the effects of seasonal solar radiation on exposed populations". 31st European Environmental Mutagen Society meeting, Ghent, Belgium, September 2001

J. Delimaris, S. Tsilimigaki, N. Messini-Nikolaki, G. Ziros and S.M. Piperakis "Effects of low-frequency electromagnetic fields in human lymphocytes". 31st European Environmental Mutagen Society meeting, Ghent, Belgium, September 2001.

RESEARCH GROUP: Chemical Ecology and Natural Products

Research Staff

Vassilios Mazomenos, Research Scientist
Vassiliki Labropoulou, Assistant Research Scientist
Maria Konstantopoulou, Research Specialist
Dimitra Stefanou, Technical Specialist
Mary Fletser, Collaborating Research Scientist
Ioannis Georgakopoulos, Postdoctoral Fellow
Despina Douvara, Graduate Research Associate
Magda Xenou-Kokoletsi, Graduate Research Associate
Morteza Movahedy Feizal, Collaborating Graduate Student
Aurelien Tellier, Collaborating Graduate Student
Elias Siskos, Collaborating Graduate Student
Niki Kalariti, Undergraduate Student
Flora Lepturgidou, Undergraduate Student
Maria Maniati, Undergraduate Student
Demetra Papadopoulou, 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).

The study of molecular mechanisms involved in insect chemical communication and generally in olfaction of *Sesamia nonagrioides*. We are currently studying the expression of pheromone binding proteins PBPs and general odorant binding proteins GOBPs in an attempt to identify and clone new genes for this protein family.

Another aspect of our research is the isolation and typing of entomopathogenic microorgasnisms. The identification of bacteria and fungi isolates is carried out mainly by sequence comparison of the rRNA gene. Genetic fingerprints and genetic variation of the fungi isolates are analyzed using the RAPD PCR and RFLP methodologies.

2001 Findings

The isolation and identification of the toxic (to insects) metabolites produced by the fungi species *Mucur 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 Mucor *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*.

Two bioactive substances that act as antagonist to the insect hormone ecdysone were isolated from the peels of the beet orange *Citrus aurantium* fruits. Studies are under way for the collection of sufficient quantities in order to proceed with the identification of these bioactive chemicals.

Biosynthesis of 1,7-dioxaspiro (5,5) undecane major pheromone of the olive fruit fly. The objective of this study was to investigate the origin of spiroacetal oxygen's using fruit flies exposed to an atmosphere enriched in [^{18}O]-oxygen. ^{18}O- Oxygen incorporation into the spiroacetal was monitored by GC-MS analysis of headspace volatiles collected with a SPME fibre (carboxen/PDMS). MS spectra indicated that ^{18}O was incorporated into the spiroacetal molecule. We are now directing attention to the origin of 9-keto-alcohol the likely precursor of the spiroacetal and to establishing the generality of the biosynthetic relationship between keto-alcohols and the corresponding spiroacetals.

The study of molecular mechanisms involved in insect chemical communication and generally in olfaction of *Sesamia nonagrioides*. We are currently studying the expression of pheromone binding proteins PBPs and general odorant binding proteins GOBPs in an attempt to identify and clone new genes for this protein family.

Another aspect of our research is the isolation and typing of entomopathogenic microorgasnisms. The identification of bacteria and fungi isolates is carried out mainly by sequence comparison of the rRNA gene.

Genetic fingerprints and genetic variation of the fungi isolates are analyzed using the RAPD PCR and RFLP methodologies.

2001 Publications

F.D. Krokos , M.A. Konstantopoulou and B.E. Mazomenos 2001 Alkadienes and Alkenes, sex pheromone components of the almond seed wasp *Eurytoma amygdali* (Hymenoptera:Eurytomidae). J. Chem Ecol, 27: 2169-2181.

R. Albajes, M. Konstantopoulou, O. Etchepare, M. Eizaguirre, B. Frérot, A. Sans, F. Krokos, A. Améline, B. Mazomenos. 2001. Mating disruption of the corn *borer Sesamia nonagrioides* (Lepidoptera: Noctuidae) using sprayable formulations of pheromone. Crop Protection (in press).

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. (in press*)*.

2001 Presentations at International Scientific Conferences

M.A Konstantopoulou, E.N. Zografou, and B.E Mazomenos,. Susceptibility of the olive fruit fly *Bactrocera oleae* and the Mediterranean fruit fly *Ceratitis capitata* to fungi species isolated from infected insects. *8th European Meeting IOBC/WPRS "Insect Pathogens and Insect parasitic Nematodes"*, Athens Greece 29 May- 2 June 2001.

V. Labropoulou, I. Douvara, M. Konstantopoulou, B. Mazomenos. Zygomycete Mucor species isolated from field collected insect larvae: genetic analysis of five isolates by RAPD-PCR and partial analysis of SSU rDNA. *8th European Meeting IOBC/WPRS "Insect Pathogens and Insect parasitic Nematodes"*, Athens Greece 29 May- 2 June 2001.

M. Guillon, R. Albajes, B. Mazomenos, B. Frerot, and O. Etcheparre. Mating disruption of Mediterranean corn borer *Sesamia nonagrioides* (lepidoptera: Noctuidae) using sprayable formulations of pheromone in France. *18th Ann. meeting Intern. Soc. of Chemical Ecology*. Granlibakken, Resort, Lake Tahoe CA July 7-12, 2001.

RESEARCH GROUP: Biology, Ecology and Behaviour of Insects - Trapping Systems for Control of Insects Pests.

Research Staff

George Zervas, Associate Research Scientist

Research Interests

The aims of the program are to develop, to improve and to compare traps or trapping systems for insect mass trapping or monitoring. The aim of that is to minimize or to stop sprays with insecticide mostly against Olive and Medflies. In particular, the research concerns in the traps of the Vial-sac type traps. Moreover, concerns in the Trimedlure-Dry traps of reverse cup-type. The study concern the:

A. The behavior of materials which the traps are made to the solar and UV light and other environmental conditions, in order to increase the life span of the traps.
B. The replacement of the same material (Metallic or plastic) with other ones which are environmentally safe.
C. Improvement and development of new attractants which increase the attribution of the traps.

2001 Findings

For the second year the Ministry of Agriculture have tested in the field the Vial-type trap baited with the attractant Z1. (The Vial-type trap and the attractant Z1 have been developed at the N.R.C. “Democritos”). Those methods had been used against the Olive fly with very satisfactory results.

RESEARCH GROUP: Insect Ecophysiology

Research Staff

George Tsiropoulos, Research Scientist
Mihalis Hatzis, Graduate Research Associate
Vassilios Papadopoulos, Research Technician

Research Interests

Development of plant growth and protection systems using trapping systems containing food and sex attractants, antimetabolites and photosensitization substances, as well as, the use of agrochemicals acceptable for biological cultures.

2001 Findings

During 2.001 the experimental work related to *Dacus* and Cerattitis attraction to new attractants as well as to various combinations of them, was concluded.

At the same time, the work related to the study of the chemoreceptory organs of *Dacus*, as well as the study of their function and response to various chemicals, using electrophysiological methods, was continued.

Also, during 2001, the tobacco growing and protection system was further developed with the introduction of new agrochemicals acceptable for biological cultures.

2001 Publications

E.N. Zografou, G.J. Tsiropoulos and L.H. Margaritis (2001). Effect of Phenylalanine and Tyrosine analogues on Bactrocra oleae Gmelin (Dipt. Tephrititae) reproduction J. Appl. Ent. 125, 365-369.

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.

2001 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.

2001 Presentations at International Scientific Conferences

A.G. Manoukas and M. N. Hassapidou.(2001). Vitamin E: a-, b- and gamma tocopherol content of Greek table olive fruits. 17th International Congress of Nutrition (ICN). Modern Aspects of Nutrition: Present Knowledge and Future Perspectives. August 27-31, 2001. Vienna, Austria. Annals of Nutrition and Metabolism 45:595.

A.G. Manoukas and J. Massas. (2001). Dietary efficiency nad mineral utilization of the Mediterranean fruit fly (*Ceratitis capitata).* XXXI Annual Meeting of European Society for New Methods in Agriculture (ESNA). 8-12 September 2001. Chania, Crete Greece. Proceedings: in Press.

RESEARCH GROUP: Radionuclide Transfer in the Soil-Plant System

Research Staff

Vassiliki Skarlou, Senior Research Specialist
Ioannis Massas, Graduate Student
Fotini Giannakopoulou, Undergraduate Student
Spiros Valogiannis, Undergraduate Student
Miltiadis Tatsis, Undergraduate 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.

2001 Findings

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

1. If ^{134}Cs uptake by one plant grown on a soil type was high, it was also high for all other crops grown on the same soil type and the opposite.
2. ^{134}Cs transfer factors for all the studied crops were higher in the volcanic – marginal soils than in the representative agricultural soils of the country.
3. In the framework of an IAEA Coordinated Research Program a first attempt was made to classify the soil types according to ^{134}Cs uptake by reference plants.
4. In all studied soil systems it seems that there is a constant ratio between ^{134}Cs transfer factor for corn and the leafy crops.

In collaboration with the Institute of Nuclear Technology and Radioprotection a Data Base of radionuclides for Mediterranean cultivation is created.

2001 Publications

Massas I., V. Skarlou and C. Haidouti. 2001. ^{134}Cs uptake in relation to soil properties and time. J. Env. Radioact. (in press).

Haidouti C., Th. Karyotis, I. Massas and Ath. Charoulis. 2001. The red soils of Thrace (Greece): Properties, development, and productivity. Comm. Soil Sc. Plant Anal. 32, 617-632.

Arapis, G., Massas, I. and Skarlou, V. 2001. Limitations and perspectives of radioecological assessment for soil/ plant systems in Greece. NATO Science Series. Series 2: Environmental Security. Assessment and Management of Environmental Risks: Cost-efficient methods and applications. Editor I. Linkov (in press).

2001 Presentations at International Scientific Conferences

Massas I., V. Skarlou, C. Haidouti and G. Arapis. 2001. ^{134}Cs transfer factors for Greek soil/plant systems; the sunflower case. Proceedings of the First European Bioremediation Conference, Chania, Crete. July 2-5, 2001, p. 519-522.

Skarlou, V., I. Massas, I. Anoussis, C. Haidouti and G. Arapis. 2001. ^{134}Cs uptake for crops grown on representative and volcanic Greek soils. European Society for new Methods in Agricultural Research. 8-12 September, MAICH, Chania, Crete, Greece. Proceedings of the XXXI Annual Meeting. (in press).

Skarlou, V. and M.J. Frissel. 2001. Generic TF-Values for Cs and Sr. European Society for new Methods in Agricultural Research. 8-12 September, MAICH, Chania, Crete, Greece. Proceedings of the XXXI Annual Meeting. (in press).

Manoukas, A. and I. Massas. 2001. Dietary efficiency and mineral utilization of the Mediterranean fruit fly (*Ceratitis capitata*). European Society for new Methods in Agricultural Research. 8-12 September, MAICH, Chania, Crete, Greece. Proceedings of the XXXI Annual Meeting. (in press).

Gasparatos, D., D. Myloni, C. Haidouti and I. Massas. 2001. Heavy metals distribution in soils from Eleonas area, Athens, Greece in relation to land use. European Society for new Methods in Agricultural Research. 8-12 September, MAICH, Chania, Crete, Greece. Proceedings of the XXXI Annual Meeting. (in press).

Gasparatos, D., A. Papafilippaki, C. Haidouti and I. Massas. 2001. Evaluation of Pb, Cu and Zn bioavaliability in contaminated from an urban – industrial area in Greece. European Society for new Methods in Agricultural

Research. 8-12 September, MAICH, Chania, Crete, Greece. Proceedings of the XXXI Annual Meeting. (in press).

STRUCTURAL BIOLOGY

RESEARCH GROUP: Protein Crystallography

Research Staff

Metaxia Vlassi, Associate Research Scientist
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Athanassios Tartas, Graduate Student
George Nikolopoulos, Collaborating Graduate Student
Georgia Kefala, Graduate Research Associate
Maria Seferi, Graduate Research Associate

Research Interests

Our research activities are focused on structural studies of proteins based on x-ray diffraction experiments and Biocomputing (3D Modelling) with the aim of: 1) elucidating the TPR mediated protein-protein interaction mechanism, using the Ssn6/Tup1 protein complex as model-system, 2) rational design of inhibitors of the enzyme GAPN identified in pathogenic bacteria but not in humans and 3) elucidation of the role of calcium ions in stabilizing TIN antigen in the extracellular matrix.

2001 Findings

In a previous work and in order to study the TPR mediated protein-protein interaction, we have expressed and purified deletion mutants of Ssn6 (ScB=aa:1-175) and Tup1 (TN72=aa:1-72 and TCA=aa:1-250) containing their interaction domains with the aim to study their structure crystallographically.

In order to overcome proteolysis problems we have now expressed ScB, TN72 as well as a new clone of Tup1 (TN108=aa:1-108) as GST-fused proteins using a protease-deficient *E.coli* strain (BL21). Subsequently we purified GST-ScB, GS-TN72 and GST-TN108 using affinity chromatography followed by cleavage ςιτη thrombin. The purified ScB, Tn72 and TN108 were then used in circular dichroism experiments in order to analyze their secondary structure and thermal stability. A JASCO-J715 spectrophotometer was used for this purpose. Analysis of the CD spectra using the CDNN program showed that: a) TN72 and TN108 have a high α-helical content that does not change upon addition of TFE, even at concentrations up to 50%. TFE has been shown to induce α-helical structure to peptides and proteins with propensity for α-helix formation. This finding in conjunction with secondary structure predictions suggests both TN72 and TN108 be completely folded. Analysis of thermal unfolding CD spectra gave for both proteins a Tm=36ºC that makes them thermally unstable. b) On the other hand ScB has a lower α-helical content than expected from secondary and tertiary structure predictions. Addition of TFE at concentrations up to 50% raised the α-helical content of the protein up to the expected value. The conformational changes seen by the CD experiments with TFE demonstrate a significant potential for acquisition of α-helicity in ScB, suggesting that this fragment is only partially folded in solution. This was also shown in thermal unfolding studies where raising of temperature from 10 to 90 ºC was not followed by significant change of the CD spectrum. Crystallization of ScB is thus predicted impossible.

Therefore we are planning to design new deletion mutants of both Ssn6 and Tup1 and study them with CD with the aim to find those protein fragments that are folded, stable and thus able to crystallize.

The non-phosphorylating Glyceraldehyde–3-phosphate dehydrogenase (GAPN) involved in production of NADPH for biosynthesis has been recently also identified in some pathogenic bacteria (such as *Salmonella, Pneumonia, Clostridium*) but not in humans. This finding makes GAPN a very good target for drug design. For this purpose we have modeled the 3D structure of two GAPNs from pathogenic bacteria identified by prof. Soukri's group (Hassan II University, Morocco). The 3D models are going to be used in a structure-based rational design of potent inhibitors of this enzyme. In collaboration with prof. Soukri we have applied for a NATO grant (Cooperative Science & Technology program) in order to complete this work.

We have modeled the 3D structure of a region of TIN antigen (TubuloInterstitial Nephritis antigen) that, in a previous study, we have predicted to be responsible for calcium binding. The aim of this work is to elucidate the role of calcium ions in stabilizing TIN in the extracellular matrix. Experimental evidence that calcium ions may be important for stabilizing TIN in extracellular matrix was first given by our collaborator prof. Charonis (Univ. of Patras) and co-workers. Based on the 3D model and in order to verify our prediction we have proposed mutation of this region of TIN. Mutated peptides are going to be synthesized and their ability to bind calcium ions is going to be checked experimentally by prof. Charonis's group.

2001 Publications

Z. Sayers, I. Sagi, M. Vlassi (2001) Sesame workshop/school on bioinformatics and structural modeling. Synchrotron Rad. News 14: 24-25

RESEARCH GROUP: NMR Studies of Biomolecules and Parmaceuticals

Research Staff

Chariklia Ioannidou Stassinopoulou, Research Scientist
Maria Pelekanou, Assistant Research Scientist
Stamatia Tzanopoulou, Collaborating Graduate Student
Kalliope Kalokiri-Stilianidi, Research Technician

Research Interests

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

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

2001 Findings

Excellent quality high resolution ^{1}H NMR spectra of the hydrated Alzheimer's β-amyloid(1-28) fibril were obtained using the technique of high resolution magic angle spinning (HR-MAS). The spectral resolution achieved allowed the application of 2D NMR techniques providing evidence for the precise arrangement of the individual β-amyloid peptides in the fibril, an issue that was until recently elusive namely whether the arrangement of the β-sheet structure is parallel or antiparallel. Our data are in agreement with the parallel arrangement. In addition, in 2001 the NMR and CD conformational study of C-terminal 16-peptides from bacterial cytochromes was published.

In the field of oxorhenium and oxotechnetium complexes, the [SN][S][S] ligand system that gives stable, neutral complexes was further explored. In these studies, a novel complex of the ReO_2[SN][P] type was isolated in which a second oxygen is attached to the oxorhenium core. The phosphorous present in this type of complex comes from the precursor molecule used in the synthetic pathway. Furthermore, in the process of developing complexes targeting specific receptor sites, new complexes of the ReO[SNS][S] type for imaging of serotonin and dopamine were synthesized, characterized and biologically evaluated.

The detailed study of the chemistry of the widely used diaminedithiol (DADT) ligand system and the isolation of novel complexes of this ligand with the oxorhenium core was completed and is submitted for publication.

In the process of writing are the manuscripts describing our initial results on the synthesis of a radiodiagnostic for Alzheimer's disease, based on the structure of dyes that display selectivity for the amyloid plaque.

2001 Publications

Bouchayer, E., Stassinopoulou, C.I., Tzougraki, Ch., Marion, D., Gans, P. (2001). NMR and CD conformational studies of the C-terminal 16-peptides of *P. aeruginosa* c_{551} and *H. thermophilus* c_{552} cytochromes. J. Peptide Res. 57, 39-47.

Mikros, E., Benaki, D., Humpfer, E., Spraul, M., Loukas, S., Stassinopoulou, C.I., Pelecanou, M. (2001). High-Resolution NMR Spectroscopy of the β-Amyloid(1-28) Fibril Typical for Alzheimer's Disease. Angew. Chem. Int. Ed. 40, 3603-3605.

Bouziotis, P., Papagiannopoulou, D., Pirmettis, I., Pelecanou, M., Raptopoulou, C. P., Stassinopoulou, C. I., Terzis, A., Friebe, M., Spies, H., Papadopoulos, M., Chiotellis, E. (2001). Synthesis and structural characterization of two *cis*-dioxorhenium(V) ReO_2[SN][P] mixed-ligand complexes. Inorg. Chim. Acta 320, 174-177

Papagiannopoulou, D., Pirmettis, I., Maina, T., Pelecanou, M. Nikolopoulou, A., Chiotellis, E., Raptopoulou, C. P., Vlahos, A. T., Terzis, A., Papadopoulos, M., Chiotellis, E. (2001). "Development of novel mixed-ligand oxotechnetium [SNS/S] complexes as potential 5-HT_{1A} receptor imaging agents" J. Biol. Inorg. Chem. 6, 256-265

Bouziotis, P., Pirmettis, I., Pelecanou, M., Raptopoulou, C. P., Terzis, A., Papadopoulos, M., Chiotellis, E. (2001). "Novel oxorhenium and oxotechnetium complexes from an aminothiol[NS]/thiol[S] mixed ligand system" Chem. Eur. J. 7, 3671-3680

Pirmettis, I., Patsis, G., Pelecanou, M., Tsoukalas, C., Papadopoulos, A., Raptopoulou, C. P., Terzis, A., Papadopoulos, M., Chiotellis, E. (2001). "Synthesis of oxorhenium(V) and oxotechnetium(V) [SN(R)S/S] mixed ligand complexes containing a phenothiazine moiety on the tridentate SN(R)S ligand" Biorg. Med. Chem. Lett. 11, 1859-1862

2001 Presentations at International Scientific Conferences

I. C. Pirmettis, D. Papagiannopoulou, M. Pelecanou, M. Papachristou, C. Tsoukalas, C. P. Raptopoulou, A. Terzis, M. Papadopoulos, E. Chiotellis (2001). Oxotechnetium and oxorhenium complexes from a novel (NN)(SNO) mixed ligand system. Advanced Medicinal Chemistry Symposium, May 2001, Salonica, Greece

M. Papachristou, I. Pirmettis, D. Papagiannopoulou, M. Pelecanou, C. Raptopoulou, A. Terzis, T. Papastaikoudi, M. Papadopoulos, E. Chiotellis (2001). Synthesis and characterization of novel mixed ligand oxotechnetium and oxorhenium complexes $MO(NN)S_3$ and MO(NN)(SO)(S). 13^{th} Radiopharmaceutical Chemistry Symposium, June 2001, Interlaken, Switzerland. Extended abstract published in J. Labelled Cpd Radiopharm. *44* Suppl. 1 (2001) S515-S517

D. V. Papagiannopoulou, I. Pirmettis, M. Pelecanou, C. Raptopoulou, A. Terzis, M. Papadopoulos, E. Chiotellis (2001). Synthesis and characterization of novel "3+2" mixed ligand oxorhenium complexes, ReO[NN]{S(Me)No]: *Syn* and *anti* isomerism. American Chemical Society National Meeting, August 2001, Chicago, USA

E. Mikros, D. Benaki, E. Humpfer, M. Spraul, S. Loukas, C.I. Stassinopoulou and M. Pelecanou (2001). High-Resolution 1H Magic Angle Spinning NMR of Alzheimer's β-Amyloid(1-28) Fibril. 14^{th} Conference of the International Society of Magnetic Resonance (ISMAR), August 20-24, 2001, Rhodes, Greece

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 continued to produce demineralised freeze-dried cancellous bone in order to be studied in vitro for its osteogenesis , osteoinduction and osteoconduction capacities and further to be used in dental surgery.

In addition we continue the development and evaluation. of bovine bone graft according to our established production method,

Service Unit Activities during 2001

The numbers of the various types of grafts which were produced and delivered to Hospitals, during 2001, are listed in the following table.

GRAFTS	DELIVERY
Cancellous Bone	720
Cortical bone	6
Mixed bone	2
Dura mater	86
Cartilage	3
Cranium bone	3

EXPERIMENTAL ANIMAL COLONY

Research Staff

Effie-Fotini Tsilibary, Research Scientist
Ioannis Zafiropoulos, Research Technician
Margarita Anagnostopoulou, Technician

Description

The colony provides inbred strains of experimental animals, carefully bred and checked free of disease. The following species are currently available:

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

The number and species available vary, depending upon the needs of research programs of NCSR "D", in particular the Institutes of Biology and Radio-Isotopes-Radiodiagnostics. When surplus is available, the animals are provided to other research laboratories, pharmaceutical companies, etc.

During 2001 the colony provided the following numbers of experimental animals:

Users	Rats	Mice	Rabbits
Institute of Biology	5	7	
Institute of Radioisotopes & Radiodiagnostics	209	297	
University of Athens	303	177	30
"ELPEN" Pharmaceuticals	266		
National Foundation of Researches	32		
University of Ioannina	24		
Total of animals provided	**630**	**481**	**30**

In addition, animals were propagated in appropriate weights and ages, depending on demand, and are in stock for any immediate needs by users, for reproduction, rejuvenation and programming of the colonies.

The staff prepared antibodies and helped with all aspects of needs of experimental animals. The also collaborated with other Institutions and gave information on animal maintenance. In addition, it provided nude mice to researchers of "D" from other Institutes.

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 2001 sixteen scientists were trained at the postdoctoral level at our Institute. Furthermore, 24 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 2001, one of our graduate students finished their thesis work and became PhDs.

Moreover, 23 students from the University are carrying out their pre-graduate project thesis work at the Institute. Additionally, 2 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 2001 the Biology Institute organized four new 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. Blasi, M. Pelecanou and H. Stassinopoulou (coordinator)].
- **Gene Structure and Expression** [course lecturers: B Lambropoulou, A. Prombona, K.E. Sekeri, V. Sophianopoulou and M. Havredaki (coordinator)].
- **Enviromental Biology** [course lecturers: B. Bombogianni (coordinator), M. Boutsinas and K. Stamatakis].

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:

- *Cell cultures – Tissue cultures* (**Dr. D. Kletsas**, Department of Biology, University of Athens).
- *Cell Cycle: Checkpoints and consequences for physiological cell function* (**Dr. T. Sourlingas,** Department of Biology, University of Athens).
- *Production of mutations: cellular and environmental factors, mutation detection techniques. Cancer genetics.* (**Dr. G.Voutsinas,** Specialization course, in the Graduate Course Programme in molecular Genetics – Cytogenetics, School of Medicine, University of Patras.
- *Radiobiology* (**Dr. E. Sideris,** European Course on Biomedical Engineering and Medical Physics of the EEC Programme, ERASMUS, University of Patras).
- *Methods of Studying of DNA* (**Dr. E. Sideris**, Interdepartmental Graduate Programme on Food Technology, Agricultural University of Athens).
- *General Biology* (**Dr. S.Piperakis**, School of Humanities, University of Thessalia)

Within the framework of the Graduate School Programme, are also organized, on a regular basis, bibliographical seminars and seminars presenting progress in current research work. These seminars are presented by all the graduate students of the Institute and supplemented by scientific seminars presented by other researchers of the Institute as well as invited guest speakers from other Greek or foreign Educational and/or Scientific Research Institutes. The seminars accomplished the past year (2001) are presented analytically in the following pages.

During July 2001, the first Biology Summer School of our Institute (Biology Days 2001) was held. This came to continue the long tradition of Summer Schools of the NCSR "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.
Of the eighty students who enrolled in the Summer School, approximately fifty 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.

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 2000

GRADUATE STUDENT	TITLE OF DOCTORAL THESIS	ADVISOR (in Institute of Biology)	UNIVERSITY
Dimitra Tsapali	«Study of histone H_{1o} during *in vitro* ageing»	Kalliope Sekeri	Department of Biology, University of Athens

LECTURE CONTRIBUTIONS TO THE 2001 SUMMER SCHOLL OF THE NCSR "DEMOKRITOS"

(July 2001)

SPEAKER	TITLE
I. GEORGOUSSI Institute of Biology, NCSR "Demokritos"	G proteins in health and disease
G. Milligan Univ. of Glasgow, UK	- G protein coupled receptors (GPCRs):Diversity function and regulation - GPCRs in health and disease. - Novel fluorescence technologies and their application to drug discovery
A. PAPAVASILEIOU Univ. of Patras	Cell Cycle regulation
G. VOUTSINAS Institute of Biology, NCSR "Demokritos"	Cancer Genetics
D. KLETSAS Institute of Biology, NCSR "Demokritos"	Cell Cycle regulation – Cellular senescence and carcinogenesis
G. PANAGIOTOU NCBR «A. Flemigk»	Use of proteomics in the study of cellular ageing and carcinogenesis
E. TSILIBARY Institute of Biology, NCSR "Demokritos"	The cell and its environement: The role of connective tissue in the control of gene expression
J. SAUS Foundation Valenciana de Investigaciones Biomedicas	Mechanisms of regulation of gene expression: the cell and its environment
M. VLASSI Institute of Biology, NCSR "Demokritos"	Introduction to Protein Structure -Structure determination by X-Ray Crystallography
M. KOKKINIDIS Univ. of Crete, IMBB	Applications of Crystallography to Biotechnology
E. ELIOPOULOS Agricultural Univ. of Athens	Prediction and model creation of the three-dimensional structure of proteins.
P. BENOS Washington Univ.	DNA and proteins: recognition codes
M. PELEKANOU Institute of Biology, NCSR "Demokritos"	Molecular structure studies by NMR
C.I. STASSINOPOULOU Institute of Biology, NCSR "Demokritos"	Protein structure determination by NMR
I. GEROTHANASSIS Univ. of Ioannina	Multinuclear NMR studies of interaction of biological macromolecules with small ligands: structural and thermodynamical approaches
A. POLITOU Univ. of Ioannina	Folding and thermodynamical stability of proteins
ST. ROBBINS Univ. of Calgary, Canada	- The genome projects and integrated genome maps. - Mutations and expression profiling in disease. - Assessing of biological function. - Gene and gene product interactions - Bionformatics
S. LEES MILLER Univ. of Calgary, Canada	- Proteomics- Introduction to proteomics, 2-D gels - Proteomics- Introduction to maldi-tof mass spectrometry, preparation of samples for maldi, database searching using maldi results, examples from current literature
K. IATROU Institute of Biology, NCSR "Demokritos"	Recombinant protein over-expression using novel eukaryotic systems
V. SOPHIANOPOULOU Institute of Biology, NCSR "Demokritos"	Use of model eukaryotic systems for the functional characterization of "foreign" transmembrane transporters

SEMINAR PROGRAMME

DATE	SPEAKER	TITLE
8/1/01	**Dr. E. ROGAKOU** Erasmus University Rotterdam	Histone γH2AX is induced in cellular functions mediated by double –strand DNA breaks
24/1/01	**P. KARAMESSINIS** Institute of Biology, NCSR "Demokritos"	Proximal tubular epithelial cell-matrix interactions in the presence of increased glucose concentrations
31/1/01	**S. ZAHARIOUDAKIS** Institute of Biology, NCSR "Demokritos"	Sensory apparatus, structural and functional analysis of chemical communication organs of *Bactrocera (Dacus) oleae*
7/2/01	**Α. ΤΑΡΤΑΣ** Institute of Biology, NCSR "Demokritos"	Overexpression and purification of the N' terminus of the protein Ssn6 of S. cerevisiae. Biochemical characterization and crystallization experiments
14/2/01	**K. OIKONOMOY** Institute of Biology, NCSR "Demokritos"	Functional properties of podocalyxin (PCLP) under physiological and pathological conditions. Regulation of its expression by basement membrane proteins.
19/2/01	**Dr. D. KOULOUGLIOTIS** Yale University	Protein structure-biological function correlation in solution: The cytochrom b5 case
21/2/01	**P. HANDRIS** Institute of Biology, NCSR "Demokritos"	Structural and biochemical changes in the nucleus of senescent cell
28/2/01	**E. ARGYROU** Institute of Biology, NCSR "Demokritos"	Functional characterization and structure-function analysis of nucleobase/ascorbate transporters in microbial model systems (*Aspergillus nidulans*, *Escherichia coli*)
7/3/01	**Cr. NIKOLAOU** Institute of Biology, NCSR "Demokritos"	Study and Quantification of Non-Randomness. Correlation of non-randomness and functionality of genomic sequences
14/3/01	**G. LALLAS** Institute of Biology, NCSR "Demokritos"	Anticancer drugs strategies and cell resistance: post-transcriptional modifications and apoptosis
21/3/01	**E. THOMADAKI** Institute of Biology, NCSR "Demokritos"	Apoptosis regulation through mRNA polyadenylation
4/4/01	**O. KOVAIOU** Institute of Biology, NCSR "Demokritos"	Retinoblastoma protein recruits histone deacetylase to repress transcription
18/4/01	**Cr. GIANNOULI** Institute of Biology, NCSR "Demokritos"	Differential effect of tgf-β on the proliferation of human fibroblasts
25/4/01	**E. MOROU** Institute of Biology, NCSR "Demokritos"	Mapping the sites of opioid receptor- G protein interface and their effectors
2/5/01	**Z. ERPAPAZOGLOU** Institute of Biology, NCSR "Demokritos"	Control of oligoclonal antipeptide antibodies against the major proline transporter (PrnB) of the filamentous fungus *Aspergillus nidulans*.
7/5/01	**Dr. G. THIRAIOS** IMBB, Univ. of Crete	Mechanisms of chromatin remodelling
9/5/01	**A. KALDIS** Institute of Biology, NCSR "Demokritos"	Study of the circadian clock in Phaseolus vulgaris
16/5/01	**A. KYPREOU** Institute of Biology, NCSR "Demokritos"	The role of histones in *in vitro* senescence and apoptosis of T lymphocytes
18/5/01	**Dr. L. GEDAMU** Univ. of Calgary, Biological Sciences	Survinal and pathogenesis of Leishmania parasites
23/5/01	**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*

6/6/01	**G. MAZARAKOU** Institute of Biology, NCSR "Demokritos"	G-Protein Coupled Receptor (GPCR): Activation of transcriptional factors
27/6/01	**S. TAVOULARIS** Institute of Biology, NCSR "Demokritos"	Structural and functional analysis of the major transporter of proline (PmB) in *Aspergillus nidulans*
10/10/01	**Cr. GIANNOULI** Institute of Biology, NCSR "Demokritos"	The activity of guanine exchange factor NET1 is essential for transforming growth factor-beta-mediated stress fiber formation
17/10/01	**Z. ERPAPAZOGLOU** Institute of Biology, NCSR "Demokritos"	Ammonia mediates communication between yeast colonies. Yeast colonies synchronise their growth and development.
24/10/01	**Ch. NIKOLAOU** Institute of Biology, NCSR "Demokritos"	Intra- and inter-specific nucleotide constitutional differences among eukaryotic genomes
24/10/01	**A. KALDIS** Institute of Biology, NCSR "Demokritos"	Positional cloning and functional characterisation of the mammalian circadian mutation *tau*
31/10/01	**E. MOROU** Institute of Biology, NCSR "Demokritos"	Oligomerization of opioid receptors with β2-adrenergic receptors: A role in trafficking and mitogen-activated protein kinase activation
31/10/01	**M. SIDERIDOU** Institute of Biology, NCSR "Demokritos"	Genomewide studies of histone deacetylase Function in yeast
7/11/01	**K. SDRALIA** Institute of Biology, NCSR "Demokritos"	RNAi mechanism: Role for a bidentate ribonuclease in the initiation step of RNA interference
7/11/01	**TH. GEORGOMANOLIS** Institute of Biology, NCSR "Demokritos"	RNAi in mammalian cells: Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells
14/11/01	**G. MAZARAKOU** Institute of Biology, NCSR "Demokritos"	Regulation of membrane targeting of the G Protein-coupled Receptor Kinase 2 by Protein Kinase A and its anchoring protein AKAP 79
21/11/01	**K. OIKONOMOU** Institute of Biology, NCSR "Demokritos"	Protein Vav2 is essential for cell spreading
26/11/01	**Dr. G. PANAGIOTOU** NCBR "A. Flemigk"	Analysis of DNA repair enzymes with Surface Plasmon Resonance biosensors
28/11/01	**A. APOSTOLIDOU** Institute of Biology, NCSR "Demokritos"	Spatio-Temporal images of growth factor induced activation of Ras and Rap1
5/12/01	**P. HANDRIS** Institute of Biology, NCSR "Demokritos"	The centrosome as a regulator of cell cycle progression
12/12/01	**L. LEONTIADIS** Institute of Biology, NCSR "Demokritos"	Integrins regulate the linkage between upstream and downstream events in G protein-coupled receptor signaling to mitogen-activated protein kinase
17/12/01	**Dr. D. THANOS** NCBR "A. Flemigk"	Mechanisms of transcriptional control in higher eukaryotes
18/12/01	**Dr. H. GIZELI** Univ. of Cambridge, Institute of Biotechnology	Study of membrane proteins with biosensors
19/12/01	**D. TSAPALI** Institute of Biology, NCSR "Demokritos"	Study of histone H_{1o} during *in vitro* ageing