NATIONAL CENTER FOR SCIENTIFIC RESEARCH
"DEMOKRITOS"

INSTITUTE OF BIOLOGY

2000

ANNUAL REPORT

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INTRODUCTION

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

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 interested 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 to the research and development activities that occur within the framework of the three Research Programmes, 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

These Service Units provide experimental animals for research activities carried out at the NCSR "Demokritos" and other research institutions of the country, and human tissue transplants to hospitals surgical units, respectively. Additional information about the activities of these Units are also presented in other pages of this Annual Report.

This year, 143 persons in total contributed to the R&D and service activities of the Institute. Amongst them are included 29 staff scientists, 27 postdoctoral fellows, research associates and other collaborators, 50 graduate students, 23 undergraduate students, 12 technicians and 2 administrative employees. The Institute's research activities have been supported by research funds provided by the Greek government, the European Union, non-profit organizations and industrial organisations. For this year, the total external (competitive) funding support amounted to 389,4 million GRD (approximately 2,7 million Euro).

Despite the fact that the year was marked by retirement-related reductions in our permanent staff (5 researchers and 3 technicians), the overall productivity of the Institute increased significantly relative to that of 1999. Amongst the productivity indices that showed significant increases are those for competitive funding (by 100%), undergraduate and graduate students (by 230%) and pre-degree students (by 77%). The most important contributing factor for the observed increase in the productivity indices was the success of the applications that the Institute's researchers submitted at the end of 1999 to the Greek General Secretariat of Research and Development (GSRT) in the context of relevant grant competition calls. We do hope that new competitions of the same type, in the context of the third 6-year Support Framework Programme (also termed National Competitiveness Programme), will be implemented without undue delays and will provide our researchers with additional opportunities to attract R&D funding in 2001.

This year was also marked by the completion of the installation of new major instrumentation that has increased significantly the capabilities and competitiveness of the Institute's researchers for leading edge research.. Prominent among the new instruments are the new confocal laser microscope and the new 500 MHz NMR unit. These instruments, together with the relatively new units of Protein Crystallography, Circular Dichroism Spectrometry, DNA Synthesis, DNA Sequencing and Fluorescence Activated Cell Sorting, provide the Institute researchers with increased capabilities for conducting high quality specialised research at a molecular, supramolecular and cellular level and, at the same time, allow our Institute to support relevant needs of research groups in other academic and

research institutions of the country.

One of the most significant events that took place during last year was the evaluation of the activities and performance of the Institute by an International Review Committee. The evaluation was part of the general evaluation of all Research Institutes in Greece organized by the GSRT. The evaluation process provided the opportunity for an in-depth appraisal of the achievements of the Institute during the five-year period 1996-2000, as well as for the submission of planning proposals for the next five-year period. The planning of the future activities of the Institute will continue to be discussed in depth, and it is predicted that it will result in the further improvement of the competitiveness of the Research Programs in areas that are determined by the rapid advances in the Biological Sciences and the social demands that are generated by them. The Director of the Institute expresses his gratitude to the staff of the Institute both for their contribution to the effort for a successful presentation of the activities to the Evaluation Committee and their continuing participation in discussions and reflections that are related to the future progress of the Institute.

The Third Annual Scientific Retreat of the Institute took place, in January 2001, at a hotel outside Athens, at the Mati region of Attika. During the retreat, all members of the Institute had the opportunity to come a bit closer to their colleagues, interact with one another, become updated on the activities and the achievements of the various research groups, discuss topics of common interest, and enjoy a few moments of peace in a hospitable environment away from the daily routine of the work place. It was felt that this year's retreat was one of the most successful ever. One of its highlights was the award of commemorative plaques to all its Scientists who retired during the period 1998-2000.

Finally, several colleagues, including Drs. I. Agyroudi-Akogiouvoglou, V. Vomvogianni, A. Kappas, E. Sideris and G. Papageorgiou and the members of the Technical Staff A. Alygizaki-Zorba, E. Kaparos and N. Terzakis, retired after a long and productive career at the Institute. To these colleagues, the Institute expresses gratitude for their long term contributions to the advancement of the Institute's activities and objectives. We wish them good health, success in their future endeavours and every personal happiness.

In closing this introduction, I wish to thank all the personnel of the Institute for their efforts toward the achievement of the goals of the Institute for 2000, and the presentation of the collective activities during the Review process. I am convinced that the year 2001 will be marked by additional achievements of our Scientists and by the development of new activities that will allow the Institute to fulfil more effectively its mission for creation of 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)

Akoyunoglou-Argyroudi Ioanna	Biochemist
Iatrou Kostas	Professor of Biochemistry and Molecular Biology
Kappas Andreas	Geneticist
Manoukas Athanassios	Nutritionist
Mazomenos Vassilios	Biologist
Papageorgiou George	Biochemist
Sekeri Kalliope	Biochemist
Sideris Eleftherios	Geneticist
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 Dimitrios	Biologist
Labropoulou Vassiliki	Biochemist
Pelecanou Maria	Pharmacist
Piperakis Stelios	Biologist
Prombona Anastassia	Biologist
Stamatakis Konstantinos	Biologist
Voutsinas Gerassimos	Biologist

Research Associates

Tzinia Athina	Biochemist

Senior Researchers

Vomvoyanni Vassiliki	Geneticist

Senior Research Specialists

Skarlou-Alexiou Vassiliki	Agronomist

Technical Specialists

Vavouraki Helen	Radiopharmacist

Research Specialists

Stefanou Dimitra	Agronomist

RESEARCH TECHNICIANS

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

POSTDOCTORAL FELLOWS

Fellow	Supervisor
Andreadaki Fotini	Iatrou K.
Arkas Michael	Akoyunoglou I.
Charitou Maria	Loukas S.
Dedos Skarlatos	Iatrou K.
Douris Vassilios	Iatrou K.
Gargalidis-Mountanos Harilaos	Prombona A.
Georgakilas Alexandros	Sideris E.
Georgakopoulos Ioannis	Akoyunoglou I.
Gazouli Maria	Georgoussi I.
Giannoulaki Eleni	Havredaki M.
Haveles Kostas	Sideris E.
Kitsiou Paraskevi	Tsilibary E.
Konstantopoulou Maria	Mazomenos V.
Kravariti Eleftheria	Iatrou K.
Krokos Fragoulis	Mazomenos V.
Lioupis Alexis	Iatrou K.
Petrakou Eftihia	Piperakis S., Voutsinas G.
Pratsinis Haris	Kletsas D.
Samiotaki Martina	Havredaki M.
Sourlingas Thomae	Sekeri K.
Stravopodis Dimitris	Iatrou K.
Swevers Luc	Iatrou K.
Visvardis Evaghelos-Efstathios	Sideris E.
Zografou Eleni	Mazomenos V.

GRADUATE STUDENTS

Student	Supervisor
Agheli Athanassia	Tsilibary E.
Argyrou Eleftheria	Sophianopoulou V.
Chryssou Aikaterini	Stassinopoulou Ch. - **PhD obtained in 2000**
Economou Kostas	Tsilibary E.

Erpapazoglou Zoi	Sophianopoulou V.
Giannouli Christina	Kletsas D.
Handris Panagiotis	Kletsas D.
Kaldis Athanassios-Dimitrios	Prombona A.
Karakatsanis Ioannis	Kletsas D.
Karamessinis Panayotis	Tsilibary E.
Koveou Ourania	Rotation
Ladas Nektarios	Papageorgiou G.
Lallas George	Havredaki M.
Massas Ioannis	Skarlou V.
Mazarakou Georgia	Georgoussi I.
Megaritis George	Georgoussi I.
Morou Evaghelia	Georgoussi I.
Nikolaou Christoforos	Rotation
Tavoularis Stefanos	Sophianopoulou V.
Thomadaki Ellinida	Havredaki M.
Kypreou Aikaterini	Sekeri K.
Lagoudakou Stavroula	Georgoussi I.
Sdralia Konstantia	Iatrou K.
Tartas Athanassios	Vlassi M.
Tsapali Dimitra	Sekeri K.
Visvardis Evaghelos-Efstathios	Sideris E. - **PhD obtained in 2000**
Zervolea Irini	Kletsas D. - **PhD obtained in 2000**
Zaharioudakis Stelios	Tsiropoulos G.

COLLABORATING RESEARCH SCIENTISTS

Collaborating Research Scientist	**Laboratory**
Ignatiadou Lydia (Dr. Hydrobiologist)	Iatrou K.
Leonidas Dimitrios (Dr. Chemist-Crystallographer)	Vlassi M.
Papageorgiou Spyros (Dr. Physicist)	Almirantis I.
Stathakos Dimitrios (Dr. Biochemist)	Kletsas D.

GRADUATE RESEARCH ASSOCIATES

Fellow	**Supervisor**
Douvara Despina	Mazomenos V.
Hatzis Michalis	Tsiropoulos G.
Kallionaki Argiro	Papageorgiou G.
Kefala Georgia	Vlassi M.
Kesanopoulos Kostas	Prombona A.
Konstantopoulou Maria	Papageorgiou G.
Koussidis Panagiotis	Prombona A.
Laskaris Evaghelos	Tsilibary E.
Maniou Vassiliki	Papageorgiou G.
Seferi Maria	Vlassi M.
Sofou Georgia	Akogiounoglou I.
Talamagkas Anargiros	Tsilibary E., Iatrou K.
Tsapardonis Christos/Kostas	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.
Koutalidi Sofia (Univ. of Athens)	Mazomenos V.
Metaxatou Aghelina (Univ. of Eagean)	Iatrou K. (Ignatiadou L.)
Michalopoulos N. (Univ. of Athens)	Voutsinas G.
Siskos Elias (Univ. of Cardiff)	Mazomenos V.

UNDERGRADUATE STUDENTS

Student (University)	Supervisor
Apostolaki Agheliki (Univ. of Paris)	Sideris E.
Charalambidou Maria (Univ. of Athens)	Vomvoyanni V.
Dimaras Ioannis (Univ. of Athens)	Piperakis S.
Dimitroglou Evanthia (Univ. of Athens)	Piperakis S.
Gali Eleana (Univ. of Athens)	Iatrou K.
Ghikopoulos Triantafillos (Univ. of Cardiff, Wales)	Prombona A.
Giannakopoulou Foteini (Agricultutal Univ. of Athens)	Skarlou V.
Gioti Anastasia (Univ. of Athens)	Sofianopoulou V.
Kalariti Niki (Univ. of Athens)	Mazomenos V.
Karatsa Maria (Univ. of Athens)	Vomvogianni V.
Maniati Maria (Univ. of London)	Mazomenos V.
Morout Paraskevi (Univ. of Athens)	Sideris E.
Papadopoulou Dimitra (Univ. of Athens)	Mazomenos V.
Papageorgopoulou Irini (Univ. of Athens)	Iatrou K.
Papassaikas P. (Univ. of Athens)	Voutsinas G.
Perpirakis Manolis (Agricultutal Univ. of Athens)	Skarlou V.
Psimadas Dimitrios (Univ. of Athens)	Piperakis S.
Tatsis Miltiadis (Agricultural Univ. Athens)	Skarlou V.
Tsilimigkaki Smaragdi (Univ. of Athens)	Piperakis S.
Tsoulou Aikaterini (Inst. of Nuclear Physics, NCSR “D”)	Sideris E.
Valogiannis Spyros (Agricultural Univ. of Athens)	Skarlou V.
Vatopoulou Th. (Univ. of Cardiff, Wales)	Tsilibary E.
Volakakis Nikolaos (Univ. of Thessalonika)	Iatrou K.

OTHER TECHNICAL STAFF

Staff	Supervisor
Aligizaki-Zorba Aikaterini	Papageorgiou G.
Avgeris Sokratis (Research Technician)	Kappas A.
Kriaras Alexandros	Tsilimbary E.

BIOCHEMISTRY, CELL AND MOLECULAR BIOLOGY

RESEARCH GROUP: Signal Transduction Mechanisms –Molecular Pharmacoloy

Research Staff

Iro Georgoussi, Associate Research Scientist
Maria Gazouli, Postdoctoral Fellow
Stavroula Lagoudakou, Graduate Student
Georgia Mazarakou, Graduate Student
George Megaritis, Graduate Student
Evaghelia Morou, Graduate Student
Socratis Avgeris, Technician

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.

2000 Findings

Identification of the structural and functional determinants of opioid receptors.

To dissect the structural and functional features of opioid receptor-G protein-effector interactions and understand the activation process at the molecular level, we were able to constuct a minigene from the entire third intracellular loop (pDORi3) of the δ-opioid receptor. Cellular expression of pDORi3 minigene results in specific receptor antagonism by impairing protein interaction at the receptor-G-protein-effector interface. This form of receptor antagonism might provide a model for the development of a class of antagonists that specifically block signal transduction on homologous or heterologous G-protein coupled receptors. In parallel, site-specific point mutations, were used as tools to map the sites of interaction of these receptors with G proteins-effectors and other downstream component regulation following receptor activation. In this regard, mutation of the highly conserved asparatate D145 within the DRY motif, located at the second intracellular loop of the δ-opioid receptor, to alanine did not alter δ-opioid agonist binding, but looses its ability to inhibit adenylyl cyclase. Morever, mutations of the conserved asparagine N95 of the δ-opioid receptor and of the tyrosine Y339 at the C-terminal tail of the μ-receptor, did not impair ligand binding or signal transfer of these receptors.

Molecular mechanisms of addiction. Identification of the molecular signaling circuits through which drug abuse leads to alterations in mitogenic pathways and gene expression

Transcriptional regulation upon stimulation of the adenylyl cyclase signaling pathway has been shown to be mediated by a family of cAMP-responsive nuclear factors acting as activators or repressors. In this regard, we were able to observe that chronic morphine treatment of cells, transformed to express stably the μ-opioid receptor, results in CREB phosphorylation via a PKA mediated pathway. Acute morphine treatment alters the levels of the phosphorylated CREB in a similar manner. Moreover, the involvement of the βγ subunits in morphine-activated nuclear CREB phosphorylation was delineated using a βγ scavanger. The involvement of novel signaling pathways and the role of alternative transcriptional factor activation upon chronic and acute opioid treatment is further explored. These data will provide novel information on the mechanisms through which opiates alter gene expression in specific target neurons and thereby induce tolerance and dependence.

2000 Publications

Megaritis G., Merkouris M. and Z. Georgoussi (2000). Functional domains of δ- and μ-opioid receptors responsible for adenylyl cyclase inhibition, *Receptors and Channels*, 7:199-212

2000 Presentations at International Scientific Conferences

Georgoussi, Z. “New insights into the δ- and μ- opioid receptor signaling”, Neurochemistry Winter Conference, Solden, (Oetzal), March 2000.

Mazarakou G., Merkouris M., and Georgoussi G. "Induction of multiple effects on adenylyl cyclase and CREB phosphorylation upon exposure to morphine of cells transformed with the μ- opioid receptor", 31th International Narcotic Research Conference (INRC), Seattle, USA, July 2000.

Morou E., Prombona A. and Georgoussi Z. "Strategies for studying the roles of opioid receptor signaling", 31th International Narcotic Research Conference (INRC), Seattle, USA, July 2000.

Mazarakou G., Merkouris M., and Georgoussi G., "Induction of multiple effects on adenylyl cyclase and CREB phosphorylation upon exposure to morphine of cells transformed with the μ- opioid receptor", NATO / FEBS Advanced Study Institute "Protein Modules In Cellular Signalling" St-Martin-de-Londres, France, September 2000.

Morou E., Prombona A. and Georgoussi Z. "Expression of the third intacellular loop of the δ-opioid receptor alters G protein signaling", NATO/ FEBS Advanced Study Institute "Protein Modules In Cellular Signalling" St-Martin-de-Londres, France, September 2000.

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.

2000 Findings

Biological and biochemical studies of a new opioid peptide are completed. This peptide is derived from the second transmembrane loop of the opioid receptor with the sequence Tyr-Ile-Phe-Asn-Leu. This sequence is unique for the opioid receptor and conserved in all species and all types of the opioid receptor. Receptorphin is presenting kappa-receptor selectivity and inhibits cell proliferation of a number of human breast and prostate cancer lines at the picomolar and nanomolar range.

The study of low power laser effects at the single cell level is being continued. The aim of this study that is performed at the single cell level and at real time, is the understanding of the molecular mechanisms of action at the cellular level. Confocal microscopy is used for irradiation and observation of the same area of interest allowing the imaging of low power laser effects in subcellular components and functions, using specific fluorescent vital probes. So far the experiments have shown that biostimulation causes changes such as temporary increase of the mitochondrial membrane potential $\Delta\Psi_m$, temporary alkalization of the intracellular pH_i, and global calcium oscillations.

2000 Publications

Yova, D., V. Atlamazoglou, N. Kavantzas and S. Loukas (2000). Development of a fluorescence-based imaging system for colon cancer diagnosis using two novel rhodamine derivatives. Lasers Med. Sci. 15 (2): 140-147.

Haritou, M., D.Yova, S.Loukas (2000), Agents facilitating the electric field-induced fusion of intact rabbit erythrocytes Bioelectrochemistry, 52: 229-238.

Alexandratou, E., D.Yova, V.Atlamazoglou, P.Handris, D.Kletsas and S.Loukas (2000). Low Power Laser Effects at the Single Cell Level. A Confocal Microscopy Study. In Effects of Low-Power Light on Biological Systems V, Proceedings of the International Society for Optical Engineering (SPIE), 4159: 25-33.

Atlamazoglou, V., Yova, D., Kavantzas, N. and Loukas, S. Fluorescence labelling and microscopic imaging of colonic mucosal transformations. In: "Confocal Laser Scanning, Fluorescence and Image Contrast Microscopy", Proceedings of SPIE. In Press (2001).

Atlamazoglou, V., Yova, D., Kavantzas, N. and Loukas, S. (2000). 'Microscopical examination of the localization patterns of two novel rhodamine derivatives in normal and neoplastic colonic mucosa', Lasers Med. Sci. Accepted

Atlamazoglou, V., Yova, D., Kavantzas, N. and Loukas, S. (2000). "Texture analysis of fluorescence microscopic images of colonic tissue sections", Med. Biol. Eng. Comp. (Incorporating Cellular Engineering). Accepted

Kampa, M., S.Loukas, A.Hatzoglou, A.Tsapis, E. Castanas. (2000). «Receptorphin: A new opioid peptide with kappa receptor affinity and potent antiproliferative action, derived from the sequence of the opioid receptor». Biochem. J. Accepted

2000 Presentations at International Scientific Conferences

Atlamazoglou, V., Yova, D., Kavantzas, N. and Loukas, S.: 'Fluorescence labelling and microscopic imaging of colonic mucosal transformations'. In Confocal Laser Scanning, Fluorescence and Image Contrast Microscopy., European Symposium on BiOS Europe - EBiOs 2000, 4-8 July 2000, Amsterdam, The Netherlands.

Alexandratou, E., D.Yova, V.Atlamazoglou, P.Handris, D.Kletsas and S.Loukas. "Low Power Laser Effects at the Single Cell Level. A Confocal Microscopy Study", In Effects of Low-Power Light on Biological Systems V, European Symposium on BiOS Europe - EBiOs 2000, 4-8 July 2000, Amsterdam, The Netherlands.

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

Research Staff

Dimitrios Kletsas, Assistant Research Scientist
Dimitrios Stathakos, Collaborating Research Scientist
Haris Pratsinis, Postdoctoral Fellow
Irini Zervolea, Graduate Student
Panagiotis Handris, Graduate Student
Christina Giannouli, Graduate Student
Ioannis Karakatsanis, Graduate Student
Eleni Sevaslidou, Research Technician

Research Interests

The role of growth factors 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. Non-classical 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 an opposite to the cancerous cell – are also investigated, aiming at the elucidation of the mechanisms of ageing and longevity, as well as those of malignant transformation.

2000 Findings

During 2000 we have continued the study of the action of the multifunctional growth factor TGF-β on human fibroblasts. It has been found that TGF-β regulates in a differential manner the proliferation of human fibroblasts, depending on the developmental stage of the donor, as it stimulates the proliferation of adult cells and it inhibits embryonic fibroblasts. We have especially studied the involvement of the SMAD signaling pathway – the main pathway used by the members of the TGF-β superfamily. Our data indicate that the specific action of TGF-β is not due to a differential expression/activation of SMAD proteins, as the latter are equally up-regulated and translocated into the nucleus, after TGF-β treatment, in both embryonic and adult cells. Currently, we are studying the involvement of other signaling pathways – mainly the MAPK pathways – as well as their cross-talk with the SMAD pathway, in order to elucidate the this differential action of TGF-B.

Furthermore, we have investigated the interplay between autocrine growth factors and the extracellular matrix, and we have shown that human fibroblasts are able to regulate, locally and in an autonomous manner, normal tissue turnover and, consequently, tissue homeostasis, in general. Moreover, we have continued our studies on the response of cells to mechanical tensions and we have found that the latter induce the expression and activation of c-Fos and c-Jun proteins – members of the AP-1 transcription factor – through the MAPK and RhoKinase pathways. At the same time, we have investigated the anti-apoptotic action of growth factors and the regulation of their action by the transmembrane enzyme NEP (neutral endopeptidase-24.11).

Finally, we have continued the investigation of the structure and function of the senescent (non-proliferating) cell, in opposition to the cancer cell. We have developed a novel assay system of controlled cellular senescence/immortalisation (through the expression of the SV40 LT Ag) in which we have studied the expression of pivotal cell cycle regulators, such as the mdm2/p53/$p21^{WAF1}$ pathway, and the transcription factors of the AP-1 family, as well as autocrine growth factors, during the ageing process. Furthermore, the morphological and functional changes of the nucleus of the senescent cell have been investigated: 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.

2000 Publications

Hsieh, J.-K., Kletsas, D., Hughes, A., Schachter, M., Sever, P., Demoliou-Mason, C. Characterization of human vascular smooth muscle cells immortalized with a temperature-sensitive SV40 Large T antigen: an in vitro model for the study of cell-cycle control and apoptosis. Arterioscl. Thromb. Vascular Biol. 20 (2000) 973-981

Ottaviani, E., Barbieri, D., Franchini, A., Kletsas, D. PDGF and TGF-β partially prevent 2-deoxy-D-ribose-induced apoptosis in fat body cell line IPLB-LdFB from the insect *Lymantria dispar*. J. Insect. Physiol. 46 (2000) 81-87

Caselgrandi, E., Kletsas, D., Ottaviani, E. Endopeptidase-24.11 (NEP) de-activates PDGF- and TGF-β-induced cell shape changes in invertebrate immunocytes. Cell Biol. Int. 24 (2000) 85-90

Kletsas, D., Pratsinis, H., Zervolea, Ir., Chandris, P., Sevaslidou, E., Ottaviani. E., Stathakos, D. Fibroblast responses

to exogenous and autocrine growth factors relevant to tissue repair. Ann. N.Y. Acad. Sci. 908 (2000) 155-166
Pratsinis, H., Demoliou-Mason, C., Hughes, A., Kletsas, D. A novel in vitro model of conditionally immortalized human vascular smooth muscle cells: A tool for ageing studies. The effect of aging. Ann. N.Y. Acad. Sci. 908 (2000) 321-323
Zervolea, I., Kletsas, D., Stathakos, D. Autocrine regulation of proliferation and extracellular matrix homeostasis in human fibroblasts" Biochem. Biophys. Res. Commun. 276 (2000) 785-790
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. (2001) (in press)
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
Alexandratou, E., Yova, D., Atlamazoglou, V., Handris, P., Kletsas, D., Loukas, S.: "Low Power Laser Effects at the single Cell Level. A confocal Microscopy Study" In Effects of Low-Power Light on Biological Systems V, Proceedings of SPIE, vol. 4159: 25-33
Pratsinis, H., Konstantinidou, A., Liaouri, A., Kletsas, D., and Davaris, P. (2001). "*In vitro* studies of cell proliferation and cell death for the evaluation of natural products and synthetic compounds" Epitheor. Klin. Pharm. Pharmakokin.-Int. Ed. (in press).

2000 Presentations at International Scientific Conferences

H. Pratsinis, Ir. Zervolea, D. Kletsas "Differential response of fetal and adult human fibroblasts to TGF-β: Mechanistic aspects and interplay with the extracellular matrix" 10th Annual Meeting of the European Tissue Repair Society, May 24-27, 2000, Brussels, Belgium.

Ir. Zervolea, H. Pratsinis, D. Stathakos, D. Kletsas "Autocrine regulation of proliferation and extracellular matrix formation in human fibroblasts" XVIIth Meeting of the Federation of the European Connective Tissue Societies, July 1-5 , 2000, Patras.

D. Kletsas, H. Pratsinis, Ir. Zervolea, D. Stathakos "The effect of TGF-β on human fibroblasts depends on their developmental stage: The role of extracellular matrix" XVIIth Meeting of the Federation of the European Connective Tissue Societies, July 1-5, 2000, Patras.

H. Pratsinis, Ir. Zervolea, D. Stathakos, D. Kletsas "Fibroblast responses related to wound repair persist during ageing *in vivo*" XVIIth Meeting of the Federation of the European Connective Tissue Societies, July 1-5 , 2000, Patras.

Alexandratou, E., Yova, D., Atlamazoglou, V., Handris, P., Kletsas, D., Loukas, S.: "Low Power Laser Effects at the single Cell Level. A confocal Microscopy Study" In Effects of Low-Power Light on Biological Systems V, European Symposium on BiOS Europe – EbiOS 2000, 4-8 July 2000, Amsterdam, The Netherlands.

M.A. Konstantopoulou, F.D. Krokos, H. Pratsinis, D. Kletsas, B.E. Mazomenos "Pheromone binding protein of *Sesamia nonagrioides*" 13th International Symposium on Olfaction and Taste & 14th European Chemoreception Research Organisation Congress, July 20-24 , 2000, Brighton, UK.

RESEARCH GROUP: Pathobiology of the Exracellular Matrix

Research Staff

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Argiris Talamaghas, Graduate Research Associate
Th. Vatopoulou, Undergraduate Student

Research Interests

Functional properties of macromolecules of basement membranes, which are differentiated connective tissue structures, focusing on cell-matrix interactions.

Matrix receptor (integrin)-mediated cell-matrix interactions, matrix-mediated cell signaling and regulation of gene expression.

Functional properties of the specialized cell surface sialoprotein podocalyxin of renal glomerular epithelial cells, and regulation of podocalyxin expression by matrix components..

Modulation of cellular functions and matrix structural/functional properties in diseases involving the matrix, such as diabetes mellitus and Alzheimer's.

2000 Findings

Differential Integrin recognition by isoform collagen IV (basement membrane collagen) chains.

The α1, α2, α3, and β1 integrin subunits from different kinds of renal cells preferentially bound to the type of isoform chain, α1(IV), or α2(IV), which occurs in their native basement membranes *in situ*. In addition to the approach of affinity chromatography, preferential adhesion to isoform chains was also observed in solid phase-cell binding assays, corroborating the previous results. These data suggest an important functional property of isoform collagen IV chains, and help explain their presence in specialized basement membranes.

Regulation of the expression and functional properties of the specialized protein podocalyxin.

The expression of podocalyxin by renal glomerular epithelial cells was induced by intact, isolated basement membrane and the basement membrane glycoprotein, laminin, but not by type IV collagen, with western analysis. By immunofluorescence, podocalyxin was observed on the cell surface, only when the cells were grown on whole basement membrane or laminin substrates. By confocal microscopy, when the cells were grown on whole basement membrane or laminin, there was punctate, focal distribution of podocalyxin on the basal surface, indicating the possible formation of podocytic cell processes, which are observed *in situ*. Therefore basement membranes, and in particular the basement membrane component, laminin, are important for induction of the differentiated, specialized morphology of glomerular epithelial cells.

Modulation of integrin-mediated cell functions in the presence of increased glucose concentrations.

In the presence of high glucose concentrations which simulate diabetic conditions, cultured glomerular and proximal tubular epithelial cells had altered integrin expression, and altered expression of matrixins and their specific inhibitors, TIMPs. In addition, high glucose resulted in modulation of cell adhesion on collagen IV substrates, with was mediated by different integrins, compared to the control. Therefore, in diabetic conditions, there exist changes of the expression of connective tissue components, which are regulated by integrin receptors.

Modulation of integrins of monocytes in diabetic patients suffering from diabetic complications.

Monocytes from peripheral blood samples of diabetic human subjects suffering from vascular complications (nephropathy, retinopathy, atherosclerosis, hypertension, etc.) had significantly decreased expression of the β1 integrin subunit by western analysis. The possibility exists that this modulation of β1 may represent a familial/genetic trend, and could contribute to the early prediction of vascular diabetic complications.

2000 Publications

Krishnamurti, U., Zhou, B., Fan, W., Sasaki, S., Tsilibary, E.C., Rondeau, E., Sraer, J., Wayner, E., Kim, Y., Kashatn, C., Michael, A.F. Puromycin Aminonucleotide (PAN) suppresses integrin expression in cultured glomerular epithelial cells. J. Amer. Soc. Nephr. In Press

Kitsiou, P., Tzinia, A., Stettler-Stevenson, W., Michael, A.F., Fan, W., Zhou, B., Tsilibary, E. C. Glucose-induced phenotypic modulation of glomerular epithelial cells J. Biol. Chem. Accepted (Pending revision)

2000 Presentations at International Scientific Conferences

Aggeli, P. Kitsiou, B. Hudson, A. Boutaud, A.F. Michael, E.C. Tsilibary (2000): Differential Interactions of Integrins from Different Renal Cell Types with Isoform Collagen IV Chains. JASN, 11: 523A

Kitsiou P., P. Karamessinins, A.F.Michael, B. Zhou, W. Fan, M. Mauer, Y. Kim, E.C. Tsilibary (2000): Integrin Modulation in Renal Cells Cultured in High Glucose. JASN, 11: 645A

Kitsiou P., P. Karamessinins, A. Tzinia, M. Mauer, Y. Kim, A.F.Michael, W. Fan, B. Zhou, E.C. Tsilibary (2000): Altered Integrin Expression and Function in Diabetic Conditions: A phenotypic modulation shared by different renal cell types, which may be linked to Basement Membrane Thickening. 13th Meeting of the European Diabetic Nephropathy Study Group (EDNSG)

Tzinia A , A. Theodorou, V. Laskaris, S. Loukas, A. Michael, A. Charonis. Tutulointerstitial Nephritis Antigen (TIN-ag) exhibits enzymatic activity. 33rd Annual Meeting of American Society of Nephrology (October 2000-Toronto-Canada).

Karamessinis P.M, P. Kitsiou, A. Tzinia, K.G. Economou, A.F. Michael, W. Fan, B. Zhou, W. Stetler-Stevenson,

RESEARCH GROUP: Nuclear Proteins and Chromatin Function

Research Staff

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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.

2000 Findings

Using the in vitro model ageing human fibroblast cell system, we studied the mRNA levels of the histone variant, H1o, in mitotically active cell populations as a function of increasing age. Results showed that while in young and mid-aged cell populations (C.P.D.s 30 and 40, respectively) the H1o mRNA levels remained constantly high, in the more aged cell population of C.P.D. 50, where, based on the kinetics of DNA synthesis, the mitotic potential of this population begins to decrease, the H1o mRNA levels also decrease. These results show that the H1o mRNA levels follow the DNA synthesis levels. This is in contrast to the protein's synthesis levels which remain constant. This uncoupling of mRNA and protein levels indicates that H1o synthesis is regulated at the post-transcriptional level.

Sodium butyrate was used as a means to artificially age in vitro fibroblast cell populations so as to compare results from these post mitotic populations to those obtained from naturally aged fibroblast cell populations with respect to the histone H1o synthesis rate and the mRNA levels of this specific histone variant. From the results obtained, it would seem that these two types of in vitro ageing cell populations differ with respect to the mechanisms involved in each type of ageing process.

Within the framework of a research program involving the Psychiatric Clinic of the University of Athens, the Neurobiological Institute of the Th. Th. KOZZIKA Foundation and our laboratory we have continued our study of the nuclear proteins of peripheral blood lymphocytes of patients with bipolar disorder with the analysis of a much larger number of samples. Our previous results, referring to the use of the biosynthetic histone variant pattern for the characterization of the cell state of lymphocytes during the three phases of the illness and the association of histone H2B on the cytoplasmic membranes of patients revealing lymphocyte activation, were confirmed. Moreover, the same experiments were conducted on patients suffering from schizophrenia. The same results were obtained from this group of patients as those obtained from patients with bipolar disorder. These results are in agreement with those from other recent findings which indicate that both these psychosis have common elements.

2000 Publications

Zotos, T., Marinos, E., Sekeri-Pataryas, K.E. and T. G. Sourlingas. A morphological study of the effects of chlorambucil during the S and G2 phases of the cell cycle of synchronized HEp-2 cancer cell populations using computerized morphometry. *Micron*, 31: 741-754, 2000.

Tsapali, D.S., Sekeri-Pataryas, K.E. and T.G. Sourlingas. Study of the linker histone variant H1o during the in vitro ageing of human diploid fibroblasts. Ann. N.Y. Acad. Sci., 90B: 336-341, 2000.

Tsapali, D.S., Sekeri-Pataryas, K.E., Prombona, A. and T.G. Sourlingas. mRNA levels of the linker histone variant, H1o, in mitotically active human diploid fibroblasts as a functioin of the phases of the cell cycle and cumulatine population doublings. Mech. Ageing Dev. In press, (Vol. 121, 2000).

Varvarigou, A.D., Scopinaro, F., Leontiadis, L., Corrrleto, V., Schillaci, O., De Viricentis, G., Sourlingas, T.G., Sekeri-Pataryas, K.E., Evangelatos, G.P., Leonti, A., Datseris, J., Xanthopoulos, S., Delle Fave, G., and S.C. Archimandritis. Synthesis, chemical, radiochemical and radiobiological evaluation of a nerw ^{99m}Tc-labelled bombesin-like peptide. Cancer Biother. Radiopharm. Accepted

2000 Presentations at International Scientific Conferences

Sourlingas, T.G., Tsapali, D.S. and K.E. Sekeri-Pataryas, Immunology and Ageing in Europe, First Imagine Conference, Tuebingen, 2000.

RESEARCH GROUP: Molecular Genetics and Biotechnology

Research Staff

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Eleana Gali, Collaborating Graduate Student
Erini Papageorgopoulou, Collaborating Graduate Student
Nikos Volakakis, Undergraduate Student
Dimitrios Kopanelis, Research Technician

Research Interests

Developmental regulation of gene expression during insect oogenesis: hormonal control, transcription factors, evolutionary aspects of regulatory mechanisms controlling differential gene expression during oogenesis.

Molecular biology of nuclear polyhedrosis viruses: engineered viruses as biopesticides and expression vectors, regulation of host range specificity, development of transformation vectors.

Functional genomics: expression systems for production of therapeutic proteins and subunit vaccines for control of infectious diseases, drug discovery from plant extracts, identification of new pharmacological targets, DNA vaccination, gene therapy and cellular immunization.

2000 Findings

We have extended our studies on the functional characterization of the BmGATAβ transcription factor, a regulator of chorion (egg shell) gene transcription, whose synthesis and function in the follicular cells of the silkmoth follicle is regulated both transcriptionally and post-transcriptionally. Through the use of the yeast two-hybrid system, we have identified six proteins that interact with different domains of the BmGATAβ factor. Based on the sequence characteristics and tissue and temporal expression patterns of the corresponding genes, three of the cloned sequences were selected for further analysis. The first one encodes a putative Ser/Thr kinase that is expressed in follicular cells only during late vitellogenesis and early choriogenesis. The second one encodes a putative chaperone protein that is expressed during vitellogenesis but becomes down-regulated during late vitellogenesis. The third sequence encodes a novel protein with no close matches in the available databases, which is only expressed in choriogenic follicles. Our studies on these proteins are now extended through the determination of the complete sequences of the encoding cDNAs, the development and use of specific antibodies for the detection of specific protein complexes in the follicular epithelium and the determination of the function of the cloned factors by transcriptional assays.

We also continued the functional characterization of the orphan nuclear receptors BmHR3A, BmE75A, BmE75C and BmE75D, whose expression in follicular cells is induced following exposure to the steroid hormone 20-hydroxy-ecdysone (20E). Through the use of a functional assay we have established that BmHR3A is a constitutive activator of target genes containing appropriate response elements in their promoter regions. Using the same type of assay we have also established that the BmE75 receptors are powerful repressors of the activation function of BmHR3A. The functional analysis of deletion mutants and the mapping of the interaction domains of the two

classes of receptors allowed us to propose a model for the mechanism of inhibition of BmHR3A function by BmE75. The model predicts that the recruitment of transcriptional co-activators by the activation domain of BmHR3A (a prerequisite for transcriptional activation) is prevented by the direct binding of BmE75 (through its F-domain) to it. Further studies on the identification of the residues that are responsible for the functional properties of BmHR3A and BmE75 are in progress.

In parallel studies we have developed a cell-based high throughput (HTP) assay system allowing the fast detection of the presence of ligands capable of binding specifically to the 20E receptor (20E agonists and antagonists) in plant extracts. The system is based on the functionality of a reporter gene construct that directs expression of Green Fluorescent Protein (GFP) and consequent appearance of fluorescence in tissue culture cells exposed to 20E or 20E mimics. Silkmoth tissue culture cells containing the 20E receptor and stably transformed with the reporter construct showed the predicted appearance of fluorescence upon exposure to 20E and known 20E mimics. In collaboration with the Laboratory of Chemical Ecology, these cell lines were used for the detection and purification of substances with ecdysone agonist and antagonist activities from plant extracts. The presence of 20E in spinach extracts was established by mass spectroscopy, while ecdysone antagonists have been detected in extracts of various *Citrus* species and are currently purified by HPLC. The development of additional HTP systems allowing detection of mimetics for juvenile hormone (a regulator of insect development) and human serotonin (a hormone acting on the central nervous system) in plant extracts is in progress.

Our laboratory also continued its studies on the use of baculoviruses (or nuclear polyhedrosis viruses - NPVs), insect viruses of narrow host range, as means for environmentally safe control of lepidopteran insect pests and tools for genetic transformation of insect and non-insect cells. Using as our model BmNPV, the baculovirus that infects the domesticated silkworm *Bombyx mori*, we have generated recombinant viruses expressing three proteins, juvenile hormone esterase, scorpion toxin and matrix metalloproteinase, whose expression in the infected host is predicted to cause accelerated physiological incapacitation (cessation of feeding). The assessment of the functional properties of these viruses with regard to their effects on the infected insects and non-target animal species is in progress.

An additional objective, the generation of deficient baculoviruses capable of infecting and multiplying into their hosts but incapable of killing them, thus, serving as host transducing and transformation vectors, was also pursued. Our efforts concentrated on the generation of BmNPVs deficient for the function of the *lef-8* gene, which encodes a factor implicated in the regulation of post-replicative functions of the virus. To achieve production of disabled BmNPVs that are unable to infect productively their silkworm hosts, transformed insect cells expressing constitutively the *lef-8* gene that is missing from the mutant virus genome were generated. The virulence of the mutant viruses generated through the use of these cell lines is currently under evaluation.

2000 Publications

Keith, M.B.A., Farrell, P.J., Iatrou, K. and Behie, L.A. (2000). Use of flow cytometry to rapidly optimize the transfection of animal cells. Bio/Techniques. 28, 148-154.

Wang, W., Swevers L. and Iatrou, K. (2000). Mariner (Mos 1) transposase and genomic integration of foreign gene sequences in *Bombyx mori* cells. Insect Mol. Biol. 9, 145-155.

Prevost, J.M., Farrell, P.J., Iatrou, K. and Brown, C.B. (2000). Determinants of functional interaction between the soluble GM-CSF receptor and the GM-CSF receptor beta-subunit. Cytokine 12, 187-197.

Szerencsei, R.T., Tucker, J.E., Cooper, C.B., Winkfein, R.J., Farrell, P.E., Iatrou, K. and Schnetkampf, P.P.M. (2000).

Minimal domain requirement for cation transport by the potassium-dependent Na-Ca+K exchanger: comparison with a NCKX homologue from *Caenorhabditis elegans*. J. Biol. Chem. 275, 669-676.

Farrell, P.J., Behie, L. and Iatrou, K. (2000). Secretion of cytoplasmic and nuclear proteins from insect cells using a novel secretion module. Proteins: Struct. Function Genet. 41, 144-153.

Eystathioy, T., Swevers, L. and Iatrou, K. (2001). The orphan nuclear receptor BmHR3A of *Bombyx mori*: hormonal control, ovarian expression and functional analysis. Mech. Dev. In press.

Patents

Iatrou, K., P.J. Farrell, L. Behie (2000). Insect Sequences for Improving the Efficiency of Secretion of Non-Secreted Proteins in Eukaryotic Cells. USA Patent No. 6,037,150.

Iatrou, K., P.J. Farrell, L. Behie (2000). Insect Sequences Useful to Improve the Efficiency of Processing and Secretion of Non-Secreted Genes in Insect Systems. PCT Application PCT/CA98/00796.

Iatrou, K., P.J. Farrell, Y. Hashimoto (2000). Baculovirus Artificial Chromosomes and Methods of Use. USA Patent No. 6,090,584.

2000 Presentations at International Scientific Conferences

Swevers, L., K. Ito, T. Eystathioy, K. Iatrou. Functional interactions of the nuclear receptors BmHR3 and BmE75. XIV International Ecdysone Workshop, Rapperswil, Switzerland, July 24-27, 2000.

Ito, K., L. Swevers, K. Iatrou. BmSH3 is a multiple SH3-domain containing protein that interacts with the Proline-rich N-terminus of the nuclear receptor BmE75C. XIV International Ecdysone Workshop, Rapperswil, Switzerland, July 24-27, 2000.

Iatrou, K., Gene regulation during ovarian development. Symposium on “Molecular Aspects of Insect Reproduction”, XXI International Congress of Entomology, Iguassu Falls, Brazil, August 20-26, 2000.

Iatrou, K., Insect transgenesis: baculoviruses meet transposons for beneficial invasions. Symposium on “Non-drosophilid insect transgenesis: the state of the art and future possibilities”, XXI International Congress of Entomology, Iguassu Falls, Brazil, August 20-26, 2000.

Iatrou, K., Baculovirus artificial chromosomes: a new generation of baculovirus expression vectors. IV Annual Meeting on “Baculovirus & Insect Cell Culture”, The Williamsburg BioProcessing Foundation, San Antonio, Texas, February 26-March 1, 2001.

Farrell, P.J., L. Behie and K. Iatrou. Secretion of intracellular proteins from animal cells using fusion proteins. IV Annual Meeting on “Protein Expression”, Cambridge Healthtech Institute, Mclean, Virginia, April 2-4, 2001.

RESEARCH GROUP: Theoretical and Developmental Biology

Research Staff

Ioannis Almirantis, Associate Research Scientist
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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.

2000 Findings

An evolutionary scenario related to the genome structure is formulated. It consists of biologically plausible steps (events or sequences of events in evolutionary time) which account for several observed statistical features of genomic sequences. These features are: **(i)** the non-randomness as expressed at different length scales, **(ii)** the long-range order observed in the form of the size distributions which characterise different levels of genome organisation and **(iii)** the fractality and self-similarity in the alternation of coding and non-coding regions found in long genomic sequences of higher eukaryotes. Our model reproduces the observed statistical properties while remaining a minimal proposition, thus avoiding as far as possible *ad hoc* hypotheses for the remote evolutionary past.

2000 Publications

Almirantis Y. Pattern formation in a Turings' type model with minimal reactional complexity. *Computers and Chemistry (2000)* **24**, 159-170.

A.Provata & Y. Almirantis. Fractal Cantor patterns in the sequence structure of DNA. *Fractals (2000)* **8**, 15-27.

Vargesson N., Kostakopoulou K, Drossopoulou G, Papageorgiou S, Tickle C. Characterisation of hoxa gene expression in the chick limb bud in response to FGF. *Dev. Dyn.* (2001) **220**, 87-90.

Papageorgiou S. A physical force may expose Hox genes to express in a morphogenetic density gradient. *Bull Math Biol* (2001) **63**, 185-200I.

2000 Presentations at International Scientific Conferences

A.Provata & Y.Almirantis. International Conference on Non-linear Science: Dynamics and Stochasticity, Statistical dynamics of clustering in the genome structure, Brussels, Belgium, June 1999. J.Stat.Phys. (2000) 101, 709.

RESEARCH GROUP: mRNA 3′- end Formation

Research Staff

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Ellinida Thomadaki, Graduate Student
George Lallas, Graduate Student

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.

2000 Findings

PAP phosphorylation-dephosphorylation precedes and may control PAP proteolysis during cell apoptosis. On the basis of this finding we extended our interest to investigate further how external cellular stimuli (chemotherapeutic agents, biological response modifiers, hormones, or their combinations) could influence gene expression via mRNA polyadenylation-deadenylation reactions. Chemotherapeutic agents (rIFNα, 5FU, Tamoxifen, 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]. These findings yield information on a possible use of PAP as a tumor marker involved in cell commitment and/or execution to apoptosis and may assist to improve our understanding of the PAP role during cell cycle arrest and/or apoptosis induced by a variety of chemical agents. Furthermore, attempts to correlate PAP modulations in structure and/or function upon the action of anticancer drugs with the ability of cancer cells to acquire drug resistance, is based on the recent hypothesis that 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.

2000 Publications

Atabassides, H., Tsiapalis, C.M. and Havredaki, M. (2000) Poly(A) polymerase specifically implicated in the mechanism of chemotherapeutic drug action during cell apoptosis. Int. J. Biol. Markers 15(1), 10-14.

Balatsos, N.A.A., Havredaki, M., Tsiapalis, C.M. (2000) Anticancer drug action on poly(A) polymerase activity and isoforms during HeLa and WISH cell apoptosis. Int. J. Biol. Markers 15(2), 171-178.

Balatsos, N.A.A. Havredaki, M., Tsiapalis, C.M. (2000) Early 5-fluorouracyl-induced changes of poly(A) polymerase in HeLa and WISH cells. Int. J. Biol. Markers 15(4), 294-299.

Samiotaki, M., Balatsos, N.A.A., Courtis, N. and Tsiapalis, C.M. (2000) Assignment of the 160-kDa subunit of cleavage and polyadenylation specificity factor (CPSF1) to human chromosome 8q24.23 by radiation hybrid mapping. Cytogenet. Cell Genet 90, 234-235.

Samiotaki, M., Balatsos, N.A.A., Courtis, N. and Tsiapalis, C.M. (2000) Assignment of the 100-kDa subunit of cleavage and polyadenylation specificity factor (CPSF2) to human chromosome 14q31.3 by radiation hybrid mapping. Cytogenet. Cell Genet, 90, 328-329.

2000 Presentations at International Scientific Conferences

Thomadaki H., Atabasides H., Tsiapalis C.M. and Havredaki M. (2000) Mammalian poly(A) polymerase regulation during U937 and HeLa cell apoptosis. Proceedings of the 3rd Symposium of the International Cell Death Society "Mechanisms of Cell Death 2000", May 6-10, 2000. El Escorial Monastery, Spain.

Lallas G., Balatsos N., Tsiapalis C.M. and Havredaki M. (2000) Chemotherapeutic drug action on poly(A) polymerase activity and isoforms. Proceedings of the 3rd Symposium of the International Cell Death Society "Mechanisms of Cell Death 2000", May 6-10, 2000. El Escorial Monastery, Spain

RESEARCH GROUP: Environmental Mutagenesis-Carcinogenesis

Research Staff

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Research Interests

Genetic and epigenetic alterations in cellular metabolism, cell cycle and apoptosis
Involvement of apoptotic pathways in carcinogenesis and drug resistance
Gene expression in cancer
Mutation spectra of environmental agents

2000 Findings

Structural alterations or changes in the expression pattern of the CD95 gene that lead to loss of the CD95 apoptotic function may favour clonal selection of highly malignant cells, resulting in resistance to chemotherapy and poor prognosis. In our work, we focused on two mechanisms of resistance against CD95-mediated apoptosis in breast cancer. We dealt with the identification of putative CD95 gene mutations leading to loss of CD95 function, as well as the analysis of the transcription pattern of transmembrane (tm) versus soluble (s) CD95 mRNA species. In 73 breast cancer samples, we detected no structural changes in exons 9 and 6 of CD95, coding for the cytoplasmic death and the transmembrane domains. No difference in the ratio of tm vs sCD95 mRNA expression was detected between breast tumor and normal breast samples. Therefore, inhibition of the CD95 pathway in breast cancer seems not be due to structural alterations in exons 9 and 6 of the CD95 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 an immune response against the tumor.

2000 Presentations at International Scientific Conferences

Voutsinas G. "Concurrent exposures to genotoxic agents: molecular targets and their relations in carcinogenesis", CCMS/NATO Pilot Study Meeting on Advanced Cancer Risk Assessment Methods, Santorini, Greece, 17-21 November 2000.

RESEARCH GROUP: Radiation and Molecular Genetics

Research Staff

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Alexandros Georgakilas, Postdoctoral Fellow
Kostas Haveles, Postdoctoral Fellow
Eleftheria Argyrou, Graduate Student
Zoi Erpapazoglou, Graduate Student
Stefanos Tavoularis, Graduate Student
Anastassia Gioti, Undergraduate Student
Paraskevi Morout, Undergraduate Student
Aikaterini Tsoulou, Collaborating Graduate Student (Graduate Student of Institute of Nuclear Physics)
Agheliki Apostolaki, Collaborating Graduate Student
Areti Tsolomiti-Gourgou, Research Technician

Research Interests

The research interests of the Laboratory are focused on the 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 as well as the study of the molecular mechanisms involved in the transport of nucleobases, ascorbate and amino acids across the plasma membrane, mediated by specific transmembrane transporters and s;hructure-function analysis of such transporters.

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

2000 Findings

The resolution of the Multiple Microgel Comet Assay, developed by this Laboratory, and the nature of the of the classical comet assay were elucidated. The corresponding ratio of the DNA double strand breaks over the single strand breaks was related to the effective dose of ionizing radiation and to the heterogeneity of the breakage distribution which is probably associated to the high order chromatin structures found in the human lymphocytes used in this work or the antioxidant capacity of each particular cell. It was established that the induction of apoptosis, which follows the DNA breakage, in these cells as well their malignant counterparts, the human chronic lymphocytic leukemia cells, is not determined only by the DNA repair capacity and the extend of the remaining DNA damage but also by the redox status of the cells. The redox potential of these human cells can render some of the cells tolerant or delay their commitment to apoptosis even though they carry increased amount of non-repaired DNA damage.

Functional characterization of Leaf permease 1 (LPE1), the first isolated plant representative of a large and ubiquitous transporter family, a protein that is necessary for proper chloroplast development in maize, by functional expression in the model system *Aspergillus nidulans*. Our results demonstrate that LPE1 is a highly specific and efficient uric acid-xanthine transporter that can also bind, but is unable to transport, ascorbate at high concentrations.

Functional expression and cellular localization of a GFP-tagged proline transporter in *A. nidulans.* Our study shows that the **G**reen**F**luorescent**P**rotein fusion technology is a unique tool to study the expression and cellular localization of low-abundance transmembrane transporters expressed from their native promoters and in addition demonstrates that the length of the amino acid linker between the transporter protein and the GFP is critical for proper expression of the resulting chimeric protein molecules.

2000 Publications

Haveles K. S., A. G. Georgakilas, E. G. Sideris and V. Sophianopoulou. (2000) Effects Of Radical Scavengers On Radiation Induced Double Strand Breaks. *Int. J. Radiat. Biol.* 76:51-59

Georgakilas A. G., K. S. Haveles, V. Sophianopoulou, l. Sakelliou, G. Zarris and E. G. Sideris. (2000) Alpha – Particle – Induced Changes In The Stability And The Size Of The DNA. *Rad . Res.* 153:258-262

Visvardis E.-E., K. S. Haveles, T.A. Pataryas, l. C. Margaritis, V. Sophianopoulou and E. G. Sideris. (2000) Peripheral

Blood Mononuclear Cells, Pbmc, Diversity As Revealed By A Novel Multiple Microgel "Comet" Assay, Mmca. *Env. Mol. Mutag.* 36:32-39

Georgakilas A.G., A. A. Konsta, l. Sakelliou and E.G. Sideris.. (2000) Dielectric And Uv Spectrophotometric Study Of Physicochemical Effects Of Ionizing Radiations On Mammalian Macromolecular DNA. Trans. Dielectr. Electr. Insul. (in press)

Argyrou E., V. Sophianopoulou, N. Schultes and G. Diallinas (2001). Functional characterization of a maize purine transporter by expression in *Aspergillus nidulans*. *Plant Cell (accepted for publication).*

2000 Presentations at International Scientific Conferences

Sophianopoulou V., E. Argyrou, N. Schultes and G. Diallinas (2000). Functional characterization of a maize purine transporter by expression in *Aspergillus nidulans*. Proceedings of the 18th International Meeting on Yeast Transport and Energetics (SMYTE), Ouro Preto, Brazil, page 35.

Argyrou E., G. Diallinas, Qi Li, N. Schultes and V. Sophianopoulou (2000). Functional characterization of Plant Nucleobase-Ascorbate Transporters. Abstract of the International Congress of Plant Molecular Biology, Quebec, Canada (in press)

Haveles K.S., E.E. Visvardis, V. Sophianopoulou and E.G. Sideris (2000). DSB's/SSB's ratio as a function of the antioxidant capacity of human peripheral blood mononuclear cells exposed to γ-rays. Abstract of the 47th Annual Meeting of the Radiation Research Society, Albuquerque, New Mexico, USA, P WP 022.

Visvardis E.E., K.S. Haveles, V. Sophianopoulou and E.G. Sideris (2000). Differentiation of p53 accumulation and apoptosis in human peripheral blood mononuclear cells submitted to direct and indirect DNA breakage. Abstract of the 47th Annual Meeting of the Radiation Research Society, Albuquerque, New Mexico, USA, P WP 021.

RESEARCH GROUP: Biogenesis and Function of the Photosynthetic Membrane

Research Staff

Ioanna Akoyunoglou-Argyroudi, Research Scientist
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Michalis Arkas, Postdoctoral Fellow
Ioannis Georgakopoulos, Postdoctoral Fellow
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Athanasios-Dimitrios Kaldis, Graduate Student
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Kostas Kessanopoulos, Graduate Research Associate
Panagiotis Koussidis, Graduate Research Associate
Triantafillos Ghikopoulos, Undergraduate Student
Eleni Sevaslidou, Research Technician

Research Interests

Our research interests aim the understanding of the regulatory mechanisms underlying the biogenesis / differentiation of the chloroplast. Especially, we try to elucidate the function of the biological clock in plants by studying its interaction with external signals, the role of the photoreceptors in resetting and the molecular mechanisms controlling rhythmic gene expression.

2000 Findings

We have tried to isolate and further characterize the thylakoidal protease implicated in LHCII stabilization during chloroplast biogenesis. We found that all LHCII preparations, prepared from thylakoids of broken or intact bean chloroplasts, show "autolysis" to a variable extent, but the LHCII trimer, separated by ultracentrifugation on SDS-sucrose density gradients displays the highest, much higher than its monomeric form separated on the same gradient. On such gradients, the protease as detected on gelatin-containing activity gels, comigrates with the LHCII trimer. In thylakoids isolated during greening of etiolated bean leaves, deficient in LHCII, the activity peaks in similar fractions. The activity increases in thylakoids during chloroplast development, it is inhibited by Cd or Zn, and enhanced in the presence of Mg. Addition of the isolated LHCII trimer to a PSII core protein preparation results in degradation of the D1 protein as well. When plants are transferred to high light the activity in the LHCII trimer is drastically increased; the opposite is found in plants transferred to low light. Similarly, the LHCII trimer obtained from thylakoids of *Scenedesmus obliquus* displays high proteolytic activity against LHCII. Addition of the polyamine putrescine or the thylakoid lipids MGDG and DGDG (at low concentrations) to the LHCII trimer results in the inhibition of the proteolytic activity.

We have separated the protease activity from the LHCII trimer by PAGE or by ultracentrifugation on sucrose density gradients containing 0.1 M NaCl. The activity zone, released from the trimer by PAGE, is located just above the LHCII trimer; extraction into 1% SDS and further analysis on SDS gels (7% or 12-17% gradient gel) shows the presence of a protein at about 180 kDa, and a series of proteins at 68, 24, 23 and 21 kDa. Since the latter proteins are components of CPIa – the Photosystem I complex -, which we found to be proteolytically inactive, we suppose that the 180 kDa protein is the protease. The close association of the protease with the LHCII complex suggests that this protease may be a component of the PSII super-complex, playing a major role in its biogenesis and adaptation.

Experiments aiming the study of synchronization/resetting of the biological clock in young etiolated bean seedlings, using as external signal 2min light pulses, showed that 1. The newly induced rhythmic transcription of the *Lhcb* genes (detected as steady-state levels of mRNA) differs during the initial acute response, depending on the phase of the preexisting rhythm 2. When the new light signal is applied at preexisting troughs, there is a phase shift of about 6 hours. The duration of the acute response is adjusted in favor of the phase correction. Our data show that the differentiated acute response is part of the mechanism responsible for the entrainment of the endogenous oscillator to the changing environmental light conditions. 2. Additional experiments indicate the involvement of the photoreceptor phytochrome and of specific transcription factors in the regulation of this mechanism. Application of RT-PCR, using primers designed from heterologous sequences available from other plants, we were able to clone bean *phyB* (phytochrome B) and *lhy* (transcription factor of *Lhcb*/clock element) cDNAs as a first step to investigate their roles in the synchronization of the biological clock of plants. 3. We constructed two cDNA libraries of bean leaf mRNA corresponding to the peak and the trough of *Lhcb* expression. By subtractive hybridization we intend to identify new plant clock elements.

2000 Publications

Hoober, K. and Argyroudi-Akoyunoglou, JH (2000) Assembly of Light-Harvesting Complexes with specific emphasis on the role of chlorophyll b. In: Chlorophyll Fluorescence: The signature of green plant Photosynthesis. (Papageorgiou, G. and Govindjee, eds). Kluwer Academic Publishers (in press)

Tsapali, D.S., Sekeri-Pataryas, K.E., Prombona A. and T.G. Sourlingas (2000) mRNA levels of the linker Histone variant, H1o, in mitotically active human diploid fibroplasts as a function of the phases of the cell cycle and cumulative population doublings. Mech. Ageing Dev. 121, 101-112

2000 Presentations at International Scientific Conferences

Argyroudi-Akoyunoglou, JH, Sevaslidou, E., and Prombona A. (2000) The biological clock in *Phaseolus vulgaris*. 12th FESPP Congress, August 22-27, Budapest Hungary, Plant Physiol. Biochem. Vol 38-Supplement. p 569

Georgakopoulos, J., Sokolenko, A., Arkas, M., Sofou, G., Herrmann, RG and Argyroudi-Akoyunoglou, JH (2000) Protease activity associated to LHCII. 12th FESPP Congress, August 22-27, Budapest-Hungary. Plant Physiol. Biochem. Vol 38-Supplement p 5103

Argyroudi-Akoyunoglou, JH, Sevaslidou, E. and Prombona, A. (2000) The biological clock in *Phaseolus vulgaris*, as reported by the capacity of the etiolated leaf to accumulate chlorophyll in the light. Workshop of the European Society for Chronobiology. Sept 15-17, Markgrafenheide, Germany

Kaldis, A.D., and A. Prombona (2000) Circadian control by light in the expression of *Lhcb* in *Phaseolus vulgaris seedlings*, 2nd Workshop of the Europ. Society for Chronobiology, News from the Plant Chronobiology Research, Markgrafenheide, Germany 15-17 September, Abstract book in press

Morou, E., A. Prombona and Z. Georgoussi (2000) *Strategies for studying the roles of opioid receptor signaling*, 31st Int. Narcotic Research Conference (INRC), July 15-20, Seattle/USA

Morou E., Prombona A., and Georgoussi Z (2000) *Expression of the third intracellular loop of the δ-opioid receptor alters G protein signaling*, NATO/FEBS Advanced Study Inst. "Protein Modules In Cellular Signaling", St-Martin-de-Londres, France, September

RESEARCH GROUP: Biophysics and Biotechnology of Membranes

Research Staff

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Nektarios Ladas, Graduate Student
Argiro Kallionaki, Graduate Research Associate
Vassiliki Maniou, Graduate Research Associate
Aikaterini Alygizaki-Zorba, Research 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. Construction of an optical osmo-biosensor based on gel-immobilized cyanobacteria.

2000 Findings

In the research project of 2000 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. In collaboration with National Institute for Basic Biology (Prof. N. Murata), we examined the changes in the thermotropic behavior of cyanobacterial cells in the presence of osmolyte glycine betaine. We demonstrated a quantitative relation between osmotically-induced cell volume changes (ΔV) and Chl *a* fluorescence changes (ΔF). We examined also the effect of allelochemicals from *Dittrichia viscosa* on the growth and photosynthesis of N_2-fixing cyanobacteria

2000 Publications

Allakhverdieva Y, Ferimazova N, Mamedov MD, Papageorgiou GC (2000). The effect of glycine betaine on heat stability of photosystem I and photosystem II electron transport in spinach thylakoid membranes. Photosynthetica 37: 423-432.

Ladas NP, Papageorgiou GC (2000). Cell turgor: A critical factor for the proliferation of cyanobacteria. Photosynth. Res. (in press 2001).

Ladas NP, Papageorgiou GC (2000). The salinity tolerance of freshwater cyanobacterium Synechococcus sp. PCC 7942 is determined by its ability for osmotic adjustment. Photosynthetica (in press 2001).

Papageorgiou G.C. (2001) Fluorescence of Photosynthetic Pigments in Vitro and in Vivo. In: Advances in Photosynthesis and Respiration Series, Chlorophyll fluorescence: A Signature of Photosynthesis (Ed. George C. Papageorgiou and Govindjee), Kluwer Academic Publishers, Dordrecht, NL (In press)

Papageorgiou G. C. and Stamatakis K. (2001). Water and Solute Transport in Cyanobacteria as Probed by Chlorophyll fluorescence. In: Advances in Photosynthesis and Respiration Series, Chlorophyll fluorescence: A Signature of Photosynthesis (Ed. George C. Papageorgiou and Govindjee), Kluwer Academic Publishers, Dordrecht, NL (In press)

2000 Presentations at International Scientific Conferences

Papageorgiou, G. C. (Invited lecturer). Water and solute transport in cyanobacteria as probed by chlorophyll *a* fluorescence. *International Conference on Photobiophysics in Technlogy and Medicine*, Institute of Physics, Poznan Technical University, Poland, 26-30 June 2000.

ENVIRONMENTAL BIOLOGY

RESEARCH GROUP: Cytotoxic Drug Sensitivity in *Neurospora crassa*

Research Staff

Vassiliki Vomvoyanni, Senior Researcher
Maria Karatsa, Undergraduate Student
Maria Charalambidou, Undergraduate Student

Research Interests

Global cell response to enviromental stress. Genome organization-differential gene expression and stress. Multiple drug resistant phenotypes(MDR/PDR)-coordination of cell metabolism.

2000 Findings

Fungi may generally share some specific developmental regulators in different developmental pathways, which are called upon at different times in different tissues to coordinate events in development. Out of schedule release of repression and aberrant transcription of the genes for these regulators may block the normal progression of developmental events required for completion of fungal morphogenesis. Data obtained suggests that the aconidial phenotype of the strain ch97 in Neurospora crassa, controlled by a mutation in the cyhH gene (increased sensitivity to cyclohexmide), can be attributed to inappropriately turning on some genes concerned with conidiation, that rendered this pathway defective. The key role of the cyhH gene in the control of differential gene expression in the fungus life cycle, is probably extended to the altered regulation of global response to a variety of stresses in the pleiotropic phenotype of the ch97 mutant strain. In all organisms this global response depends upon cell's ability to sense and signal different stress conditions and finally alter growth and developmental programmes to counteract the effects of the stress and to ensure the survival of the cell or the organism. There is evidence that the ch97 mutant is unable to strictly regulate these programmes.

RESEARCH GROUP: DNA Repair Systems and Cancer

Research Staff

Stelios Piperakis, Assistant Research Scientist
Eftihia Petrakou, Postdoctoral Fellow
Ioannis Dimaras, UnderGraduate Student
Evanthia Dimitroglou, UnderGraduate Student
Smaragdi Tsilimighaki, UnderGraduate Student
Dimitrios Psimadas, 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 ageing as well.

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

2000 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 statistical analyzing these results.
We have already published the results from the project "the effects of air pollution to human health".

We are now analyzing 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.

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 sent for publication.

The study "effects of high voltage pylons on DNA" is now in progress. Samples have been collected and are been analyzed.

In the study "DNA damage-repair and mental disorders" most of the samples have been collected from the Psychiatric Hospital and analyzed.

The collection of the samples for the study "stress and DNA damage-repair" has almost been completed and we are now about to start analyzing them.

The study "DNA damage-repair and effects of solar seasonal variations" is in the final stages.

2000 Publications

Piperakis, S.M., E. Petrakou, S. Tsilimigaki. Effects of air pollution and smoking on DNA damage of human lymphocytes. Environm. Mol. Mutagenesis, 36, 243-249, 2000.

2000 Presentations at International Scientific Conferences

Petrakou E., Tronov V.A., Konstantinou E.M., Piperakis S.M. European Association for Cancer Research, XVI-2000, International Conference, Chalkidiki, May, 2000. "Lymphocytes exposed to hydrogen peroxide are protected from necrosis but not from apoptosis, by nicotinamide ".

Piperakis S.M., Petrakou E., Monogindis E., Haniotakis G., Karkaseli M., Sarikaki E. Swansea 2000-United Kinghton Environmental Mutagen Society, Swansea, Britain, June 2000. " Pesticides effects on humans: a molecular epidemiological study".

Piperakis S.M., Petrakou E. European Environmental Mutagen Society, Congress 2000, Budapest, Hungary , August 2000." Effects of air pollution and smoking on DNA damage of human lymphocytes of exposed populations".

RESEARCH GROUP: Chemical Ecology and Natural Products

Research Staff

Vassilios Mazomenos, Research Scientist
Vassiliki Labropoulou, Assistant Research Scientist
Maria Konstantopoulou, Postdoctoral Fellow
Fragoulis Krokos, Postdoctoral Fellow
Eleni Zografou, Postdoctoral Fellow
Dimitra Stefanou, Research Specialist
Despina Douvara, Graduate Research Associate
Magda Xenou-Kokoletsi, Graduate Research Associate
Sofia Koutalidi, Collaborating Graduate Student
Elias Siskos, Collaborating Graduate Student
Dimitra Papadopoulou, UnderGraduate Student
Niki Kalariti, UnderGraduate Student
Maria Maniati, 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 pheromone binding proteins PBPs and general odorant binding proteins GOBPs. Another aspect is the identification of microorganisms that infect insects using molecular methods (PCR and DNA sequencing).

2000 Findings

The virulence of a range of fungi isolated from infected larvae collected from the field was determined against the olive fruit fly Bactrocera oleae and the Med-fly Ceratitis capitata. Some of the isolated fungi were found to be efficient in killing adult flies and are promising to be used as control agents. Pathogenicity derives mainly from toxins produced by the fungi. The isolation and identification of the toxins produced is under investigation.

A study to screen plants of the Greek flora for the occurrence of chemicals that act as agonists or antagonists to the insect molting hormones (juvenile and ecdysone) was initiated this year. Results so far showed that natural plants of the Chenopodiacae family synthesize chemicals that mimic ecdysone activity. The structure of the active substance was determined by chromatographic and spectrometric methods and was found to be identical with that of synthetic 20-hydroxy-ecdysone. In addition plant species of Rutaceae synthesize chemicals that act as antagonist to ecdysone. The chemical structure of these active substances is studied.

The presence of 4 different bacteria was confirmed in infected insects isolated from the environment, using PCR analysis and subsequently DNA sequencing of the 16S RNA bacterial gene.

The study of the pheromone binding proteins (PBP) of Sesamia nonagrioides was continued. The molecular weight of the protein was determined using the antibodies PBP1 and PBP2 of Lymantria dispar and western analysis. Furthermore the molecular weight of a general odorant binding protein (GOBP) was determined using the GOBP2 of Lymantria. Immuno-detection experiments using antennal sections showed that these proteins were found to be localized in the antenna of Sesamia nonagrioides. Southern and Northern analysis were conducted to identify the PBP and GOBP nucleic acids of S. nonagrioides which in turn will be used for screening antennal cDNA libraries.

The study of PBP and GOBP expression was continued this year. Immunodetection experiments using Lymantria dispar antisera against PBP1 and PBP2 as well as GOBP2 in antennal sections, showed that these proteins were found to be localized in the antenna of Sesamia nonagrioides. Southern and Northern analysis were

conducted to identify the PBP and GOBP nucleic acid sequences of Sesamia nonagrioides which in turn will be used for screening antennal cDNA libraries.

The presence of 4 different bacteria was confirmed in infected insects isolated from the environment, using PCR analysis and subsequently DNA sequencing of the 16S rRNA bacterial gene.

2000 Publications

Krokos F., M. Konstantopoulou, and B.E. Mazomenos. (2000) Alkadienes and alkenes sex pheromone components of the almond seed wasp (*Eurytoma amygdali*) [Hymenoptera :Eurytomidae]. J. Chem. Ecol. (in press)

Krokos F., M. Konstantopoulou, and B.E. Mazomenos. (2000) Chemical characterisation of corn plant volatiles by different extraction techniques and the role of potent chemicals in the reproductive behaviour of the corn stalk borer Sesamia nonagrioides (lef.). *Use of Pheromones and other Semiochemicals in Intergrated Control*. IOBC wprs Bulletin. Witzgall P, Mazomenos B and Konstantopoulou M (eds) Vol. 24, (11) (in press).

Mazomenos B.E., A, Pantazi-Mazomenou, D. Stefanou. (2000) Attract and Kill of the Olive Fruit Fly Bactrocera oleae (Gmel.) in Greece as a Part of an Integrated Control System**.** *Use of Pheromones and other Semiochemicals in Intergrated Control*. IOBC wprs Bulletin. Witzgall P, Mazomenos B, and Konstantopoulou M. (eds) Vol. 24, (11) (in press)

Mazomenos B.E., M. Konstantopoulou, D. Stefanou and S. Skareas. (2000) Female calling behaviour and male response to the synthetic sex pheromone components of Palpita unionalis [Lepidoptera: Pyralidae]. *Use of Pheromones and other Semiochemicals in Intergrated Control*. Internat. Organisation of Biological Control wprs Bulletin. Witzgall P, Mazomenos B, and Konstantopoulou M. (eds) Vol. 24, (11) (in press).

2000 Presentations at International Scientific Conferences

Labropoulou V. and B. Mazomenos. Pheromone and odorant binding protein of Sesamia nonagrioides.) Joint International Meeting of ECRO, ISOT & ISOEN 2000 20-24, July Brighton U.K. 2000.

Konstantopoulou M.A. F.D. Krokos, H. Pratsinis, D. Kletsas and B.E. Mazomenos. Pheromone binding protein of Sesamia nonagrioides. Joint International Meeting of ECRO, ISOT & ISOEN 2000 20-24, July , Brighton U.K. 2000.

Ortiz A., M. Nogueras, A. Sánchez and B. E. Mazomenos. Development of a pheromone trap for monitoring of the olive tree branches borer *Euzophera pinguis* Haw. (Lepidoptera: Pyralidae). *25th Anniversary Jubilee Reunion Use of pheromones and other semiochemicals for pest control* Samos, Greece, September 25-29, 2000.

Hiskia A, M, Economou, N. Moratis, F. Krokos, E. Papakonstantinou, and B. Mazomenos. Determination of VOC's using Solid-Phase Micro extraction and Gas Chromatography in aqueous TiO_2 suspensions. *1st European Conference on Pesticides and Relative Organic Micropolutants in the Environment* Ioannina Greece 5-8 October, 2000.

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 research aim is the development, the improvement and the evaluation of attractants and traps in order to be used in mass trapping or monitoring systems of natural populations of harmful insects (ie olive- Mediterranean fruit flies) in order to reduce or abolish insecticide sprays. In particular, the research aims in the improvement of the designed and tested traps (bottle, bag and reverse cup type traps), the improvement concern:

The research of the resistance of the construction material (Plastic and Aluminum), under field conditions as the U.V. light and the rain in order to increase the life span of the traps.

The replacement of the insecticides which are used in the killing factors of the same traps(ie reverse cup and the bottle type traps), by Protoactive Dyes or Bioelectrostatic powers which have been found to be environmentally friendly.

The development and the improvement of different attractant in order to increase the efficiency of the traps.

2000 Findings

For a sixth year the application of the new developed mass trapping method, for control the Mediterranean fruit fly in an apricot and a Orange Orcharch was successful, although the natural population was extremely high. Some of the evaluated new attractants used in traps of the bottle or bag type sowed great results concerning their attractivity to the flies (Olive-Medfly) also concerning their duration of attractivity (over 6 months duration). Long life traps participating in mass trapping systems reduces applications costs. In the three years experiments in which different traps as the ECOTRAP® of Vioryl, the Valoplastiki® of Stavrakis, the ANEL® trap, the McPhail trap of Ganadakis® and the Demokritos traps of the Bottle type, baited with Z1® and the Dry trap baited with Trimedlure were compared. The two Demokritos traps showed the best efficiency in catching Mediterranean flies.

RESEARCH GROUP: Insect Ecophysiology

Research Staff

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

Research Interests

Research on the ecophysiology of economically important insects, targeting the development of integrated population control methods, with main points, the environmental and consumer safety.

2000 Findings

With the economic support of the cigarette industry SEKAP S.A., a pilot program for the production of 'Organic Tobacco' was started, including the varieties Virginia and Basmas. All tobacco protection chemicals, including fertilizers, were substituded by biorganic ones. The first year was successful and certified by the aythorized organization ΔΗΩ.

2000 Presentations at International Scientific Conferences

Zacharioudakis S.S., Tsiropoulos G.J. and Margaritis L.H. Morphological characteristics of communication organs of adult Bactrocera (Dacus) oleae (Diptera: Tephritidae) XXI International Congress of Entomology, Iguassu, Brazil, Aug. 20-26, 2000, Abstracts, book II, p. 807.

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.

2000 Findings

The composition and efficiency of the improved practical and low cost diet for the Mediterranian Fruit Fly reported in 1998, has been determined. 2.Allelochemicals are compounds which may be beneficial(kairomones) or detrimental(allomones) to behavior, metabolism and development of the receiving organism. The effect of certain allelochemics, such as C6-C10 fatty acids and amino acid analogs upon larval growth and survival of the olive fruit fly larvae has been investigated. It was found that larval survival was the most reliable criterion for determining the inhibition level of the chemicals tested. Further work, especially with amino acid analogs may lead to the development of environmentally friendly methods for the management of the olive fruit fly, because of the exclusive and unique relationship of this insect to olive fruit, free amino acids and bacteria, documented by studies in our Laboratory and elsewhere.

2000 Publications

Manoukas, A. G. 2000. The effect of C6 to C10 fatty acids on larval growth and survival of the olive fruit fly *Bactrocera oleae*.(Diptera: Tephritidae) Entomologia Hellenica. 13:17-22.

Manoukas, A. G. 2000. The effect of amino acid analogues on larval growth and survival of the olive fruit fly *Bactrocera oleae*.(Diptera: Tephritidae). Entomologia Hellenica. 13:23-30.

Manoukas, A. G. 2000. A practical, Efficient and Low Cost Diet for Rearing the Mediterranean Fruit Fly Larvae. (p.588-594). In: Area-Wide Control of Fruit Flies and Other Insect Pests. Edited by Keng-Hong Tan. CABI Publishing, CAB International. ISBN 983-861-195-6, 780 pages.

2000 Presentations at International Scientific Conferences

Manoukas, A. G. and E. N. Zografou.2000. Composition and Efficiency of a Yeast-Free Larval Diet for the Mediterranean Fruit Fly. XXI Iternational Congres of Entomology. Foz do Iguassu, Brazil 20-26 August. Absract Proc.Book 1: 398

RESEARCH GROUP: Radionuclide Transfer in the Soil-Plant System

Research Staff

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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.

2000 Findings

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

1. The different soils studied had a similar effect on ^{134}Cs uptake by sunflower and soybean plants.
2. ^{134}Cs transfer factors and daily ^{134}Cs plant uptake (fluxes) showed similar trends, but the use of fluxes provided less variable results and stronger function relationships.

A strong negative power relationship between exchangeable $K+NH_4$ expressed as a percentage of CEC and ^{134}Cs plant uptake was observed.

No consistent relationships with either ^{134}Cs TFs or fluxes and the other studied soil properties were found.

Soil and plant characteristics may affect ^{134}Cs plant uptake more than that of potassium.

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

2000 Publications

Massas I, Skarlou V and Haidouti C. "Time dependent ^{134}Cs uptake by sunflower and soya plants grown on different soils". Proceedings of XXXth Annual Meeting of European Society of New Methods in Agriculture [ESNA] and International Union of Radioecology [IUR], Kesztlely, Hungary, 26-30 August 2000, pp. 27-33.

Frissel N., D. Deb, M. Fathory, Y. Lin, A. Mollah, N. Ngo, I. Othman, W. Robison, V. Skarlou, S. Toycuoglu, J. Twuning, S. Ushida and W. Wasserman. "Generic values for Soil to Plant Transfer Factors of radiocesium". Journal of Environmental Radioactivity. Accepted.

Arapis G., I. Massas and V. Skarlou, 2000. "Limitations and perspectives of radioecological assessment for soil-plant systems in Greece. NATO Science Series. Serie 2: Environmental Security. Editor I. Linkov. Accepted.

STRUCTURAL BIOLOGY

RESEARCH GROUP: Protein Crystallography

Research Staff

Metaxia Vlassi, Associate Research Scientist
Dimitrios Leonidas, Collaborating Research Scientist
Athanassios Tartas, Graduate Student
Georgia Kefala, Graduate Research Associate
Maria Seferi, Graduate Research Associate
Christos-Kostas Tsapardonis, 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 and 2) identifying the role of specific mutations of the tumor-suppressor gene BRCA1 in breast/ovarian cancer.

2000 Findings

In order to determine the conformation of the interaction domains of Ssn6 and Tup1 proteins we have performed circular dichroism (CD) experiments on purified deletion mutants of both proteins corresponding to their interaction domain. The CD experiments, in conjuction with secondary structure predictions, based on primary sequence, showed that the interaction domain of both proteins is fully folded before the formation of the Ssn6/Tup1 complex. The CD experiments were performed using the CD spectrophotometer of IB and in collaboration with Dr. M. Pelecanou. In parallel crystallization experiments of the above deletion mutants were carried out. Certain conditions led to the formation of microcrystals in both cases which however did not grow any further due to proteolysis. Therefore we redesigned expression experiments using a different Ecoli strain. These experiments are in progress.

We have produced a 3D model of the BRCT domain of the tumor-supressor protein BRCT1 of humans. The model was further used in order to elucidate the role of specific muatations of the BRCA1 gene, identified by Dr. Yannoukakos's group (IRRP), in patients with breast/ovarian familly history in Greece.

In the framework of a general program of rational design of ribonuclease A(RNase) inhibitors, we have cocrystallized RNase with 3 inhibitors. We then collected x-ray diffraction data using our local x-ray system as well as synchrotron radiation (Erretra, Trieste).

2000 Publications

Zhou, B., Nelson, T.R., Kashtan, C., Gleason, B., Michael, A.F., Vlassi, M. and Charonis, A.S (2000). Identification of two alternatively spliced forms of Human Tubulointestitial Nephritis Antigen (TIN-ag). J. Am Soc Nephrol 11(4):658-68

Gounalaki N, Tzamarias D, Vlassi M.(2000). Identification of residues in the TPR domain of Ssn6 responsible for interaction with the Tup1 protein. FEBS Lett. 473(1):37-41

Tsolas, O., Papamarcaki, T., Christoforidis, S. and Vlassi, M. (2000). Molecular effects of ATP diphosphohydrolase on blood fluidity and blood clotting. In "Ecto-ATPases and Related Ectonucleotidases" pp 175-183. (Edited by L. Vanduffel & R. Lemmens) Shaker Publishing B.V. Maastricht, The Netherlands.

RESEARCH GROUP: NMR Studies of Biomolecules and Parmaceuticals

Research Staff

Chariklia Ioannidou Stassinopoulou, Research Scientist
Maria Pelekanou, Assistant Research Scientist
Aikaterini Chryssou, Graduate Student
Kalliope Kalokiri-Stilianidi, Research Technician

Research Interests

Molecular structure and dynamics in solution using NMR and other spectroscopic methods such as CD , ESR and FT-IR. Two types of molecules are considered:

I . Peptides and proteins

II. Complexes of transition state elements designed as potential pharmaceuticals

NMR studies in solution of the structural, conformational and dynamic properties of compounds with pharmacological and biological interest as well as of their interaction with biological substrates. Current activity: 1. Design, synthesis, and structural study of technetium and rhenium complexes for selective targeting in radiodiagnosis and radiotherapy. 2. NMR study of the fibrillar structure of the β-amyloid peptide

2000 Findings

Our detailed structural studies on technetium and rhenium complexes have allowed the recognition of trends in the NMR parameters which now can be used to obtain the structure in solution whenever the complexes cannot be crystallized.

We have proposed a mechanism for the retention of SNS/S mixed ligand TcO(V) and ReO(V) complexes in the brain involving the substitution of the monodentate S ligand by glutathione. The products of this reaction were characterized and certain kinetics aspects were studied.

During 2000 the work in the area of radiodiagnostic and radiopharmaceutic complexes of technetium and rhenium continued with emphasis on the design and study of complexes for specific target tissues. For example, a series of [SNS][S] complexes of technetium were designed for serotonergic receptor imaging (Publication #4). These complexes have as side chain the active part of the WAY 100635 molecule that is a competitor of serotonin. In addition, sufficient progress was made on the development of a technetium-99m radiodiagnostic agent for Alzheimer's disease based on the Congo red dye. Specifically, the investigation of the coordination sphere of technetium with prototype molecules that serve as models for the dye has been completed (Publication in preparation).

Finally, in 2000 research on a new subject was initiated. This is the study of the structure of the Alzheimer's β-amyloid fibril using HR-MAS NMR. Experimental data has been collected and their interpretation is currently being carried out.

2000 Publications

Pelecanou M., K.Chryssou and C.I.Stassinopoulou. (2000) "Trends in NMR chemical shifts and ligand mobility of TcO(V) and ReO(V) complexes with aminothiols" J. Inorg. Biochem. 79, 347-351

Nock B., T. Maina, A. Tsortos, M. Pelecanou, C.P. Raptopoulou, M. Papadopoulos, H. J. Pietzsch, C. I. Stassinopoulou, A. Terzis, H. Spies, G. Nounesis, E. Chiotellis. (2000) "Glutathione interaction with SNS/S mixed ligand complexes of oxorhenium(V): Kinetic aspects and characterization of the products" Inorg. Chem. 39,4433-4441

Rey A., I. Pirmettis, M. Pelecanou, M. Papadopoulos, C.P. Raptopoulou, L. Mallo, C.I. Stassinopoulou, A.Terzis, E. Chiotellis, A. Leon. (2000) "Synthesis and characterization of mixed ligand oxorhenium complexes with the SNN type of ligand. Isolation of a novel ReO[SN][S][S] complex" Inorg. Chem. 39, 4211-4218

Bouchayer E., C.I.Stassinopoulou, Ch. Tzougraki, D. Marion and P. Gans. (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

Papagianopoulou D., I. Pirmettis, T. Maina, M. Pelecanou, A. Nikolopoulou, E. Chiotellis, C.P. Raptopoulou, A.T. Vlahos, A. Terzis, M. Papadopoulos, E. Chiotellis. (2000) "Development of novel mixed-ligand oxotechnetium [SNS/S] complexes as potential 5-HT_{1A} receptor imaging agents" *J. Biol. Inorg. Chem.* In

press

2000 Presentations at International Scientific Conferences

Costopoulos B., A.D Varvarigou, M. Pelecanou, O. Scillachi, J. Datseris, S. C. Archimandritis "Study of a ^{188}Re decapeptide as potential therapeutic radiopharmaceutical" 5th Congress of the Italian Association of Nuclear Medicine Pesaro, Italy, June 2000

Kostopoulos B., M. Pelecanou, E. Mikros, C.I. Stassinopoulou, A.D. Varvarigou, S.C. Archimandritis "An oxorhenium-RGD derivative complex for radiopharmaceutical application" XVI International Symposium om Medicinal Chemistry Bologna, September 18-22, 2000

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 produced for the first time in our Bank, 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. Moreover we have tested the possibility of establishing a Cord Blood Bank with the aim of collecting, processing and storing unrelated stem cells to be used as an alternative to bone marrow for transplantation.

Service Unit Activities during 2000

The numbers of the various types of grafts which were produced and delivered to Hospitals, during 2000, are listed in the following table.

GRAFTS	DELIVERY
Cancellous Bone	680
Cortical bone	25
Mixed bone	9
Dura mater	85
Cartilage	3
Cranium bone	3
Collaborations	9

EXPERIMENTAL ANIMAL COLONY

Research Staff

Effie-Fotini Tsilibary, Research Scientist
Ioannis Zafiropoulos, Research Technician
Alexandros Kriaras, 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 2000 the colony provided the following numbers of experimental animals:

Users	Rats	Mice	Rabbits
Institute of Biology	11	80	6
Institute of Radioisotopes & Radiodiagnostics	326	605	8
Office of the Director of "D"	14	-	-
University of Athens	212	40	-
"ELPEN" Pharmaceuticals	329	-	-
Academy of Athens	7	-	-
Total of animals provided	**899**	**725**	**14**

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 Institutions.

EDUCATIONAL ACTIVITIES

EDUCATION

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

Training of young scientists at the postdoctoral level
Pre-graduate and graduate thesis work
Courses at the graduate level
Summer School courses

During the year 2000 twenty- three scientists were trained at the postdoctoral level at our Institute. Furthermore, 28 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 2000, 3 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 three students from Greek Universities participated.

In the framework of Graduate Programme, during the year 2000 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: I. Almirantis, M. Blasi, M. Pelecanou and H. Stassinopoulou (coordinator)].
Gene Structure and Expression [course lecturers: B Lambropoulou, A. Prombona, K.E. Sekeri, B. 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:

Current Topics in Up-to-date Research "G proteins: structure and function. (**Dr. H. Georgoussi**, Department of Biochemistry-Molecular Biology of the University of Athens)
Mechanisms of cellular proliferation and differentiation (**Dr. D. Kletsas**, Univ. of Modena and Reggio Emilia, Italy).
Cell cultures – Tissue cultures (**Dr. D. Kletsas**, Department of Biology, University of Athens).
Cell ageing and carcinogenesis (**Dr. D. Kletsas**, Department of Biology, University of Athens).
Induction of messages by the extracellular substances: Role of growth factors, proteoglycans and integrins. (**Dr. E. Tsilibary**, Department of Biology, University of Athens).Seminar topic, *Expression and Role of Integrins during development* (**Dr. A. Tzinia,** Post Graduate course "Developmental Biology" Department of Biology, University of Patras).
Cell Cycle: Checkpoints and consequences for physiological cell function (**Dr. T. Sourlingas,** Department of Biology, University of Athens).
Radiobiology (**Dr. E. Sideris,** European Course on Biomedical Engineering and Medical Physics of the EEC Programme, ERASMUS, University of Patras).
Radiobiology (**Dr. E. Sideris**, Interdepartmental Graduate Programme on Medical Physics, University of Athens)
Methods of Studying of DNA (**Dr. E. Sideris**, Interdepartmental Graduate Programme on Food Technology, Agricultural University of Athens).
Methods of Molecular Genetic Studies of DNA (**Dr. E. Sideris**, Interdepartmental Graduate Programme on Bioanorganic Chemistry, University of Giannena).
Plant Biotechnology and Applications (**Dr. M. Blasi**, Graduate Programme of the Department of Mediterranean Agronomic, Institute of Hania).

Diagnostic Approach with Molecular Biology Techniques (Dr. V. Lambropoulou, Graduate Seminars of Medical Biochemistry, Department of Microbiology, University of Athens)

Graduate Course in Oceanography (Dr. L. Ignatiadou, Department of Biology, University of Athens)

Design, Synthesis and Determination of the Structure of Technetium and Rhenium Radiopharmaceuticals (Dr. C.I. Stassinopoulou, Graduate Programme, Department of Chemistry, University of Patras)

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 (2000) are presented analytically in the following two pages.

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

COMPLETION/AWARD OF DOCTORAL THESES IN 2000

GRADUATE STUDENT	TITLE OF DOCTORAL THESIS	ADVISOR (in Institute of Biology)	UNIVERSITY
Aikaterini Chryssou	«Synthesis and study of the structure and properties of oxorhenium (V) complexes with diaminothiols»	Chariklia Stassinopoulou	Department of Chemistry, University of Athens
Evaghelos Visvardis	«Study of normal and tumor lymphocytes response to oxidative DNA damage»	Eleftherios Sideris	Department of Biology, University of Athens
Irini Zervolea	«The interplay of growth factors and the extracellular matrix in the proliferation of normal mammalian cells»	Dimitris Kletsas	Department of Biology, University of Athens

SEMINAR PROGRAMME

DATE	SPEAKER	TITLE
3/1/00	**Dr. I. STROUBOULIS** *Department of Cell Biology,* *Faculty of Medecine,* *Erasmus Univ.,* *Rotterdam, The Nederlands*	«Regulation of the human beta-globin locus in transgenic mice»
4/1/00	**Dr. E. ROGAKOU** *National Institute of Health Bethesda, MD, USA*	«Megabase-long Chromatin domains are involved in DNA Double Strand Breaks»
19/1/00	**A. KALDIS** Institute of Biology, NCSR "Demokritos"	«Rhythmic expression of *lhcb*, as a reporter of the biological clock function in *Phaseolus vulgaris* seedlings»
19/1/00	**E. THOMADAKI** Institute of Biology, NCSR "Demokritos"	«Apoptosis regulation during mRNA polyadenylation»
21/1/00	**Dr. I. DRAGATSIS** *Columbia University,* *New York, USA*	«The gene for Huntington disease»
28/1/00	**S. TAVOULARIS** Institute of Biology, NCSR "Demokritos"	«Structure-function analysis of the main proline transporter (PrnB) of the filamentous heugus Aspergillus nidulans»
2/2/00	**E. ARGIROU** Institute of Biology, NCSR "Demokritos"	«Use of microbial model organisms (Apergillus nidulans, Escherichia coli) for functional characterization and structure-function analysis of maize and human nucleobase/ ascorbate transporters»
2/2/00	**P. HANDRIS** Institute of Biology, NCSR "Demokritos"	«Structural and biochmemical changes of cell nucleus during cellular ageing in vitro»
9/2/00	**E. MOROU** Institute of Biology, NCSR "Demokritos"	«Mapping the sites of opioid receptor-G protein and effector coupling»
16/2/00	**S. ZAHARIOUDAKIS** Institute of Biology, NCSR "Demokritos"	«Morphological and functional analysis of chemical communication organs on the insect Bactrocera (Dacus) oleae»
23/2/00	**K. OIKONOMOU** Institute of Biology, NCSR "Demokritos"	«Podocalyxin of glomerular epithelial cells: induction by basement membrane proteins and role in functional properties of renal glomerular epithelial cells»
1/3/00	**E. ZERVOLEA** Institute of Biology, NCSR "Demokritos"	«The interplay of growth factors and the exracellular matrix in the proliferation of normal mammalian cells»
1/3/00	**Α. ΧΡΥΣΟΥ** Institute of Biology, NCSR "Demokritos"	«Synthesis and structural studies of oxorhenium (V) complexes with diaminethiol ligands»
8/3/00	**A. AGHELI** Institute of Biology, NCSR "Demokritos"	«Interactions of isoform collagen IV chains with integrins from different renal cell types»
8/3/00	**G. LALLAS** Institute of Biology, NCSR "Demokritos"	«Chematherapeutic agents induced apoptosis and the development of cell-drug resistance:mRNA polyadenylation modulations»
15/3/00	**P. KARAMESSINIS** Institute of Biology, NCSR "Demokritos"	«High glucose-induced modulation of integrin and matrix components in immortalized renal proximal tubular epithelial cells»
22/3/00	**I. KARAKATSANIS** Institute of Biology, NCSR "Demokritos"	«Regulation of TGF-β action at different developmental stages»
22/3/00	**A. TARTAS** Institute of Biology, NCSR "Demokritos"	«Isolation of domains of the proteins Ssn6 and Tup 1 in order to determine their structure with X-ray crystallography»
5/4/00	**G. MAZARAKOU** Institute of Biology, NCSR "Demokritos"	«Molecular and structural determinants of the opioid receptor signaling»
5/4/00 12/4/00	**K. SDRALIA** Institute of Biology, NCSR "Demokritos" **S. LAGOUDAKOU** Institute of Biology, NCSR "Demokritos"	«Induction of GADD45 and INK/SAPK-Dependent apoptosis following inducible expression of BRCA1» «Targeting the receptor-Gq interface to inhibit in Vivo pressure overload myocardial hypertrophy»
12/4/00	**A. KIPREOU** Institute of Biology, NCSR "Demokritos"	«Activation of the apoptotic endonuclease DFF40 (Caspase-activated DNase or Nuclease»

19/4/00	**CH. GIANNOULI** Institute of Biology, NCSR "Demokritos"	«Mechanism of TGF-β action on fetal and adult human fibroblasts»
11/5/00	**Prof. N. ROBAKIS** *Director of Fishberg Research Center for Neurobiology* *Mount Sinai School of Medecine, N.Y.*	«Cellular and molecular biology of presenilin-1:Implications for Alzheimer disease»
13/6/00	**Dr. A. POLITOU** *Research Associate* *MRC Mill Hill,* *National Institute for Medical Research, London*	«Mosaic proteins: from structure to function»
14/7/00	**Prof. A. TSIFTSOGLOU** *Laboratory of Pharmacology* *Department of Pharmacy* *Univ. of Thessalonica*	«Regulatory control of differentiation and apoptosis in neoplastic cells»
27/9/00	**P. HANDRIS** Institute of Biology, NCSR "Demokritos"	«Centrosome-independent mitotic spindle formation in vertebrates. A combination of GFP-tagged proteins with laser microsurgery technology to unveil biological functions»
4/10/00	**A. TARTAS** Institute of Biology, NCSR "Demokritos"	«Quantitation and presentation of the domain motion and the confrormational changes of the proteins»
4/10/00	**A. KIPREOY** Institute of Biology, NCSR "Demokritos"	«Retinoblastoma protein represses transcription by recruiting a histone deacetylase»
11/10/00	**A. KALDIS** Institute of Biology, NCSR "Demokritos"	«Direct targeting of light signals to a promote element-bound transcription factor PIF3»
18/10/00	**CH. GIANNOULI** Institute of Biology, NCSR "Demokritos"	«The MEK pathway is required for the activation of p21WAF1/CIP1 by TGF-β»
18/10/00	**G. MAZARAKOU** Institute of Biology, NCSR "Demokritos"	«Src tyrosine kinase is a novel direct effector of G proteins»
25/10/00	**P. KARAMESSINIS** Institute of Biology, NCSR "Demokritos"	«Distinct roles of the adaptor protein Shc and focal adhesion kinase in integrin signaling to ERK»
25/10/00	**S. ZAHARIOUDAKIS** Institute of Biology, NCSR "Demokritos"	«Chemosensory proteins of Locusta migratoria»
1/11/00	**E. MOROU** Institute of Biology, NCSR "Demokritos"	«Apoptotic signaling through the β-adrenergic receptor: a new Gs effector pathway»
1/11/00	**K. SDRALIA** Institute of Biology, NCSR "Demokritos"	«A functionally conserved N-terminal domain of the friend of GATA-2 (FOG-2) protein represses GATA4-dependent transcription»
8/11/00	**G. LALLAS** Institute of Biology, NCSR "Demokritos"	«Poly(A) polymerase phosphorylation is dependent on novel interactions with cyclins»
8/11/00	**E. ARGIROU** Institute of Biology, NCSR "Demokritos"	«A new family of high-affinity transporters for adenine, cytosine and purine derivatives in arabidopsis gillissen »
15/11/00	**K. OIKONOMOU** Institute of Biology, NCSR "Demokritos"	«Pyk2 and FAK differentially regulate progression of the cell cycle»
15/11/00	**E. THOMADAKI** Institute of Biology, NCSR "Demokritos"	«Distinct caspase cascades are initiated in receptor-mediated and chemical-induced apoptosis»
29/11/00	**O. KOVEOU** Institute of Biology, NCSR "Demokritos"	«Human and mouse Fas (APO-1/CD95) Death receptor genes each contain a p53-responsive element that is activated by p53 mutants unable to induce apoptosis»
29/11/00	**CH. NIKOLAOU** Institute of Biology, NCSR "Demokritos"	«Underlying order in protein sequence organization. Periodic recurrence of methionines:Fossil of gene fusion?»
8/12/00	**Prof. C. TICKLE** *Univesity of Dundee, UK*	«Limb development as a universal model for vertebrate pattern formation»
13/12/00	**Z. ERPAPAZOGLOU** Institute of Biology, NCSR "Demokritos"	«Chromodomains are protein-RNA interaction modules»
18/12/00	**Prof. CH. ZERVAS** *Wellcome/CRC Institute of Cancer & Developmental Biology* *Univ. of Cambridge*	"The role of Integrin Linked Kinase-ILK in cell adhesion during the development of Drosophila melanogaster»