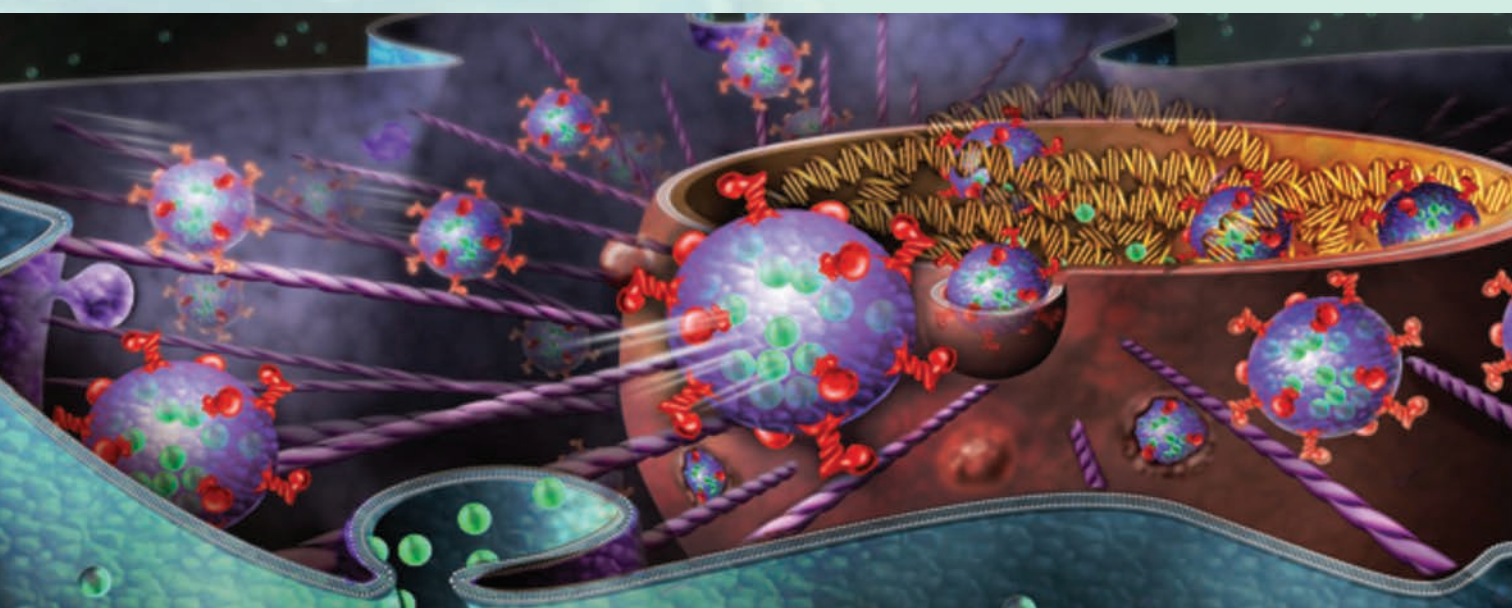


2012 ANNUAL REPORT

INSTITUTE OF BIOSCIENCES & APPLICATIONS



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***NATIONAL CENTRE FOR SCIENTIFIC RESEARCH
“DEMOKRITOS”***

***INSTITUTE OF BIOSCIENCES
& APPLICATIONS***

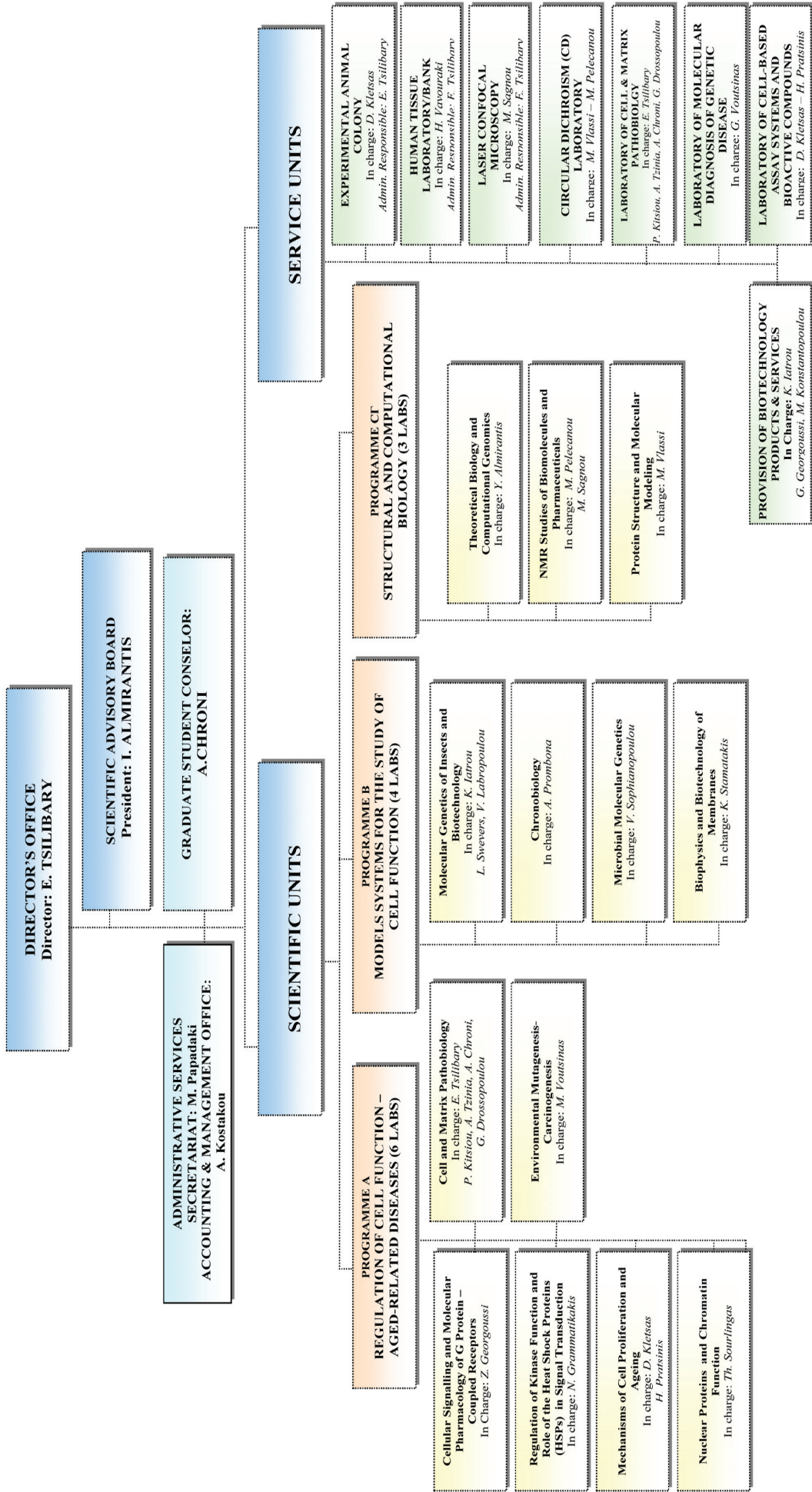
***2012
ANNUAL REPORT***

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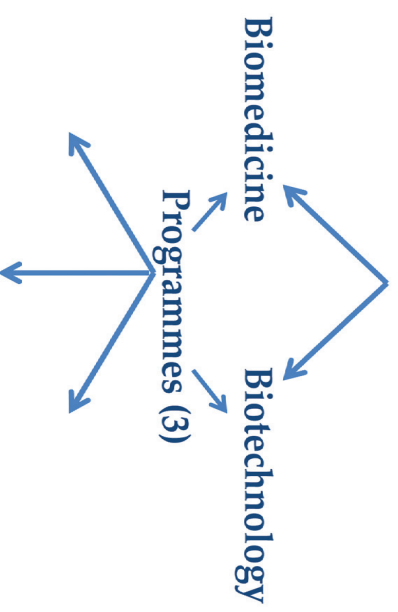
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ORGANISATION CHART



Institute of Biosciences & Applications

Research Directions (2)



Regulation of Cell Function/
Aged- related Diseases

Models Systems for
the Study of Cell Function

Structural and Computational
Biology

PERSONNEL

DIRECTOR

Tsilibary Effie

MD, Cell Biologist

ACTING DIRECTOR

Pelecanou Maria

Physicist-Chrystallographer

SCIENTIFIC STAFF

Research Directors

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Kletsas Dimitris

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Sophianopoulou Vassiliki

Swevers Luc

Tsilibary Effie

Vlassi Metaxia

Chemist

Biochemist

Professor of Biochemistry and Molecular Biology

Biologist

Pharmacist

Biologist

Biologist

MD, Cell Biologist

Physicist-Chrystallographer

Senior Researcher

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Kitsiou Paraskevi

Konstantopoulou Maria

Labropoulou Vassiliki

Prombona Anastasia

Sourlingas Thomae

Stamatakis Konstantinos

Tzinia Athina

Vavouraki Helen

Voutsinas Gerassimos

Biologist

Cell Biologist

Biologist

Biologist

Biochemist

Biologist

Biologist

Biologist

Biochemist

Radiopharmacist

Biologist

Researchers

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Pratsinis Haris

Sagnou Marina

Biologist

Chemist

Biologist/ Chemist

Technical Specialists

Kotsopoulou Eleni

Panagiotopoulou Angeliki

Stefanou Dimitra

Biologist

Biochemist

Agronomist

RESEARCH TECHNICIANS

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Avgeris Socratis
Giannakas Nikolaos
Doulgeridis George
Kakkos Stilianos
Kopanelis Dimitris (Retired)
Meristoudis Christos
Pantazi-Mazomenou Anastassia (Retired)
Zafiroopoulos Ioannis

ADMINISTRATIVE STAFF

Girlemis Dimitrios

Kostakou Athanassia
Papadaki Margarita
Pragastis Apostolos

Technician for electronic equipment &
maintenance of equipment

Accountant
Secretary
Administrator

EMERITUS & COLLABORATING SCIENTISTS

Emeritus Scientists

Ignatiadou Lydia (Dr. Hydrobiologist) - *Emeritus*
Papageorgiou George (Dr. Biophysicist) - *Emeritus*
Papageorgiou Spyros (Dr. Physicist) - *Emeritus*
Sekeris Kalliope (Dr. Biochemist) - *Emeritus*
Sideris Eleftherios (Dr. Geneticist) - *Emeritus*
Stathakos Dimitrios (Dr. Biochemist) - *Emeritus*
Tsimilli – Michael Meropi (Dr. Biologist) – *Collaborating
Scientist*

Laboratory

Iatrou K.
Stamatakis K.
Almirantis I.
Sourlingas Th.
Sophianopoulou V.
Kletsas D.

Stamatakis K.

POSTDOCTORAL FELLOWS

Fellow

Benaki Demetra (NCSR "Demokritos")
Dafnis Ioannis (Programme)
Gournas Christos (Programme)
Kythraioti Georgia (Programme)
Lambidonis Antonios (IKY)
Mavrogonatos Eleni (Programme)
Repouskou Anastasia (Programme)
Tsitoura Panagiota (Programme)
Vamvakas Sotirios - Spiridon (NCSR "Demokritos")

Supervisor

Pelekanou M.
Chroni A.
Sophianopoulou V.
Iatrou K.
Voutsinas G.
Kletsas D.
Prombona A.
Iatrou K.
Kletsas K.

GRADUATE STUDENTS

Student

Aliberti Sofia (NCSR "Demokritos")
 Apostolou – Karabelis Konstantinos (NCSR "Demokritos")
 Argyri Letta (Programme)
 Athanassopoulos Alexandros (NCSR "Demokritos")
 Daniil George (NCSR "Demokritos")
 Galeou Aggeliki (NCSR "Demokritos")
 Georganta Irene (Programme)
 Georgomanolis Theodore (NCSR "Demokritos")

Golfinopoulou Christina (Programme)
 Ioannidis Konstantinos (NCSR "Demokritos")
 Kaminari Archontia (Programme)
 Kapodistria Katerina (NCSR "Demokritos")
 Koliopoulou Anna (NCSR "Demokritos")
 Kostomiri Mirto (NCSR "Demokritos")
 Leontiadis Leonidas (NCSR "Demokritos")
 Papadopoulou Adamadia (Programme)
 Papakonstantinou Maria (NCSR "Demokritos")
 Vaggelatos Ioannis (NCSR "Demokritos")
 Xidous Marios (NCSR "Demokritos")

Supervisor

Grammatikakis N.
 Almirantis I.
 Chroni A.
 Sophianopoulou V.
 Chroni A.
 Prombona A.
 Georgoussi Z. –*PhD obtained*
 Iatrou K./ L. Swevers –*PhD obtained*
 Chroni A.
 Iatrou K.
 Tsilibary E.
 Kitsiou P.
 Iatrou K.
 Pelecanou M.
 Georgoussi Z. –*PhD obtained*
 Kletsas D.
 Georgoussi Z.
 Sophianopoulou V.
 Sourlingas Th. –*PhD obtained*

GRADUATE RESEARCH ASSOCIATES

Fellow

Anagnostakos Kostas (MD)
 Kyriazi Avgi (MD)
 Lefaki Maria (*MSc*)
 Raptopoulos Dimitris (Dr. Biologist)
 Sellis Diamadis (*MSc*)

Supervisor

Vavouraki H.
 Vavouraki H.
 Kletsas D.
 Iatrou K.
 Vlassi M.

COLLABORATING GRADUATE STUDENTS

Student (University)

Aggelopoulou Maria (Univ. of Athens, *MSc*)
 Delimitsou Aggeliki (Univ. of Athens, *MSc*)
 Geranios Pavlos (Univ. of Athens, *MSc*)
 Konstantatou Evmorphia (Univ. of Athens)
 Konstantonis Dimitris (Univ. of Athens)
 Kostopoulou Eleni (Univ. of Athens)
 Lagopati Nefeli (Athens Polytechnic School)
 Liakou Maria (Univ. of Athens, *MSc*)
 Polichronopoulos Dimitris (Univ. of Athens)
 Peristeri Eleftheria (Univ. of Athens, *MSc*)
 Vayenos Dimitris (Athens Polytechnic School, *MSc*)
 Verouti Sofia (Univ. of Athens)
 Sklirou Aimilia (Univ. of Athens, *MSc*)

Supervisor

Kletsas D.
 Voutsinas G.
 Sophianopoulou V.
 Voutsinas G.
 Kletsas D. – *MSc obtained*
 Sourlingas Th. – *MSc obtained*
 Tsilibary E./Tzinia A.
 Kletsas D. – *MSc obtained*
 Almirantis I.
 Voutsinas G. – *MSc obtained*
 Stamatakis K.
 Tsilibary E.
 Kletsas D. – *MSc obtained*

UNDERGRADUATE STUDENTS AND OTHER IN TRAINING

Student (University)

Androulakis Dimitris (Univ. of Central Greece)
Bassoyianni Angeliki – Stephania (Univ. of Athens)

Giannimaris Dionyssi (Univ. of Athens)
Diakatou Markella (Univ. of Athens)
Iglezos Dionysis (Univ. of central Greece)
Kapolou Konstantina (Univ. of Athens)
Kafkoutso Alexia (Univ. of Thessaly)
Karoussiotis Christos (Univ. of Alexandroupolis)
Karoumalis Anastasios (Univ. of Athens)
Kritharidou Anna (Univ. of Patras)
Kolovou Mariana (Univ. of Crete)
Liu Jisheng (Univ. of Gent, Belgium)
Nikolopoulou Chrissoula (Univ. of Athens)
Panteleri Rafaela (Univ. of Thessaloniki)
Tiniakou Ioanna (Univ. of Crete)
Tsoutsi Lamprini (Univ. of Athens)
Zoi Iliana (Univ. of Athens)
Zvintzou Evaggelia (Univ. of Patras)
Vassiliadou Mariliza (Univ. of Thessaly)
Verykiou Maria (Univ. of Athens)
Vrana Evi – Stavroula (Univ. of Athens)

Supervisor

Voutsinas G.
Voutsinas G. - *undergraduate
dissertation completed*
Chroni A.
Voutsinas G.
Voutsinas G.
Georgoussi Z.
Tsilibary E.
Georgoussi Z.
Kletsas D.
Georgoussi Z.

Swevers L.
Chroni A.
Konstantopoulou M.
Chroni A.
Georgoussi Z.
Sophianopoulou V.
Chroni A.
Pelecanou M.
Georgoussi Z.
Georgoussi Z.

INTRODUCTION

Following the merging of the previously existing eight institutes of the Center, the Institute of Biosciences & Applications (IB-A) is one of presently five institutes of NCSR "Demokritos". The Center, among the best for research in Europe and Greece is multidisciplinary and its earmark is the unique blending of different fields of sciences as well as numerous collaborations between scientists of different disciplines. The aim is an optimal advance of research, innovation and technology in the fields of research of scientists from the five different institutes.

The IB-A with 23 faculty members, managed to be maintained intact, as a separate institute following the merging process in March 2012, exactly due to its multidisciplinary nature and its outstanding scientific presence in the area of Life Sciences, as marked by the "RAND" report and the evaluation by the General Secretariat for Research & Technology (GSRT). It is the first time in the history of evaluations that the institute receives a positive evaluation outcome (as it should). This remarkable success is the result of the collective effort of IB-A's researchers whose continuous efforts have led to upgrading, despite the deep financial crisis and today's adverse conditions in general. The scientific personnel of IB-A deserves congratulations for its successful efforts.

A recent success was the approval for funding of the "KRIPIS" programme of the IB-A, on the amount of €1.039.000 from 2013 to 2015, which will further contribute to the upgrading of the institute by enabling the hiring of new scientific personnel, renewal of existing equipment, etc.

The two major service laboratories, the Experimental Animal Colony and the Laboratory for Human Tissue grafts, both certified by ISO 9001/2008 have recorded a significant number of sales. Most importantly, continuously increasing sales of bone fragments for use in the field of Dentistry-Implantology since the beginning of 2012, represent a recent, extremely positive development of the "Human Tissue Laboratory" of the IB-A. The newly established extended market has already secured sales to two different companies as well as many practicing dentists/implantologists. The latter is the outcome of the initiative and un-relented effort of Dr. Sagnou, who volunteered for this activity, with the help of Mrs. M. Papadaki, the secretary of the institute, and the signed underneath. The remarkable effort of Dr. Vavouraki, the scientist in charge of the production, to positively respond to the increasing demands of the market for sales, should not go unnoticed. Income from sales of the two service laboratories continually contributes to the upgrade of their space (new office, new room for animal maintenance, new space for animal experimentation, etc.).

In addition to services, the IB-A holds significant other independent and collaborative efforts aiming at internationally competitive research and further development of excellence. A major feature of the IB-A, albeit not the only one, is multidisciplinary biomedical and biotechnological research in the areas of Life Sciences and the Environment. This is a significant advantage/specialization ("earmark") for the Institute and the Center as well, focused on the environment, the development of new molecules and biomolecules for diagnostic, medical and imaging use, as well as for targeted drug delivery, among other. The above-mentioned research activities include intense and dynamic efforts of a large part of the scientific personnel. Several independent activities of the Institute focus on biomedical research with the use of biochemical, cell biological, molecular, pharmacological, proteomic, and other approaches, while other activities include biotechnological and environmental research, making use of similar approaches. Finally, structural, computational and molecular modeling approaches are one more research direction of the IB-A.

Retired, emeritus researchers proved to be very active once again contributing publications, seminars, participation to IB research projects etc, thus contributing significantly to the productivity of the IB-A.

In 2012, the PhD candidate awardee of the "Akogionoglou Award" (in honor of the internationally recognized scientist who was a researcher and Head of the IB-A [(1969-1975, 1979-1982), was: E.M. Georganta/ It should be noted that all submitted applications were extremely competitive, an indication of research of outstanding quality which is performed at the IB-A.

Apart for the hopeful news for nascent researchers-scientists, the IB was faced with the extremely saddening event, to bid farewell to two young PhD candidates, Anastasia Dimozi and Andreas Armatas, who passed away due to an accident on 15/1/2012. To honor and memory, the Center inaugurated in 2012 awards and two studentships, on in each of their names. It is hoped that these awards and studentships will reflect the promise of quality research which was demonstrated by the two unjustly lost young people.

I wish to thank the members of the scientific advisory committee of the IB-A (Dr. I. Almirantis, Dr. A. Chroni, Dr. M. Pelecanou, Dr. C. Stamatakis, and Dr. M. Vlassi) and the Educational Committee (Dr. A. Chroni, Dr. M. Sagnou) who supported the task of upgrading the IB. I am also thankful to all researchers who participated in various committees, as well as the Vice-Director of the IB, Dr. Pelecanou, who substantially contributed to a smooth operation of the IB-A and helped with my numerous administrative tasks, especially under extremely stressful conditions.

Despite the various obstacles and difficulties, the support and confidence constantly provided by the majority of researchers is a main source of optimism and confidence in successfully achieving the aim of upgrading the IB-A, towards excellence. It is my belief that based on objective indexes such as: funding, number of publications, citations, etc., the IB-A becomes continually upgraded, despite the adverse conditions imposed by the financial crisis, resulting in significantly reduced budget allocated by the government. However, continuous efforts by the institute's researchers attracted substantial funding from competitive programs from the GSRT, EU as well as the Industry. Today the IB-A is already recognized as an internationally competitive institute. With positive attitude, persistence to the aim of excellence and un-relented efforts, the unprecedented financial crisis which we experience during the last four years, can be reverted to a series of opportunities for all; more so for young scientists who represent our main hope for an immediately better future and promising perspectives.

Finally, I wish to heartfully thank Ms. Athanasia Kostakou, the IB-A accountant, and Ms. Margarita Papadaki, the IB-A secretary.

Effie C. Tsilibary, MD, PhD

Director of the IB-A

November 2013



PROGRAMME A:
Regulation Of Cell Function
Aged-Related Diseases

Research Group: **Cellular Signalling and Molecular Pharmacology of G Protein-Coupled Receptors**

Research Staff

Iro Georgoussi, Research Director

Leonidas Leontiadis, Graduate Student - *PhD obtained in 2012*

Eirini- Maria Georganta, Graduate Student - *PhD obtained in 2012*

Maria- Pagona Papakonstantinou, Graduate Student

Evi-Stavroula Vrana, Undergraduate Student

Konstantina Kapolou, Undergraduate Student

Christos Karoussiotis, Undergraduate Student

Lamprini Tsoutsi, Undergraduate Student

Anna Kritharidou, Undergraduate Student

Maria Verykiou, Undergraduate Student

Research Interests

The laboratory research is focused on the elucidation of the molecular mechanisms governing G protein-coupled receptor (GPCR) signaling using as model the opioid receptors because of their involvement in pain perception and in the addictive processes upon prolonged drug administration.

More specifically we aim in the:

- Identification of novel opioid-interactive proteins in an attempt to a) elucidate new signaling pathways mediated upon activation of these receptors and b) define new pharmacological targets.
- Identification of new transcription factors and genes which action is altered upon opioid receptor activation with selective opioid agonists
- Characterization of new bioactive molecules which bind to opioids or other GPCRs on cell based assays in an attempt to define "smart drugs" to alleviate pain or other diseases of the central nervous system.

2012 Findings

Novel opioid receptor interactive proteins implicated in signalling and internalization

In an attempt to identify new interacting proteins involved in opioid receptor function we investigated the role of **spinophilin** in the δ - and μ -opioid receptor signaling. Spinophilin, a neuronal scaffolding protein, is found in dendritic spines and acts as an organizer for the actin-based cytoskeleton regulating spine morphology, synaptic plasticity and neuronal migration, which stabilizes GPCRs to the cell membrane among others. Our results demonstrate that spinophilin associates directly with δ - and μ - opioid receptors and its expression alters the levels of the intracellular cAMP accumulation and phosphorylation of the ERK1,2 kinases upon activation of these receptors in HEK293 and neuronal cells. Spinophilin participates also in a multimeric signaling complex composed of selective members of $G\alpha$ subunits, $G\beta\gamma$ and RGS4 using as platform the carboxyl- termini of the δ - and μ - opioid receptors. These results suggest that spinophilin may be considered as a novel pharmacological target to optimize the action of opioid drugs devoted of the adverse effects leading to tolerance and dependence.

Additionally, we also investigated the mode of action of **Regulators of G protein Signaling (RGS proteins)** such as RGS2 and RGS4 in opioid signalling and defined whether coupling selectivity between the μ -OR, δ -OR and κ -OR with members of RGS and G proteins exists. Our findings indicated that RGS2 and RGS4 proteins interact with the κ -opioid receptor to differentially modify receptor signalling. Our results confirm for the first time the role of RGS proteins as new pharmacological targets in opioid receptor signaling.

Activation of STAT5B transcription factor by δ -opioid receptor agonists leads in neurite outgrowth and differentiation of neuronal cells

In an attempt to elucidate the molecular mechanisms involved in the phosphorylation of transcription factors upon opioid administration in neuronal cells, we demonstrated that the δ -opioid receptor (δ -OR) serves as a platform for the formation of a multi-component signalling complex, "signalosome" consisting of p-STAT5B- c-Src kinase and specific G α and G $\beta\gamma$ subunits of proteins. This "signalosome" is implicated in cell survival and plays a critical role in neurite length, outgrowth and differentiation suggesting that δ -OR activation leads to a number of neurotropic events via a Gai/o-linked and STAT5B dependent manner.

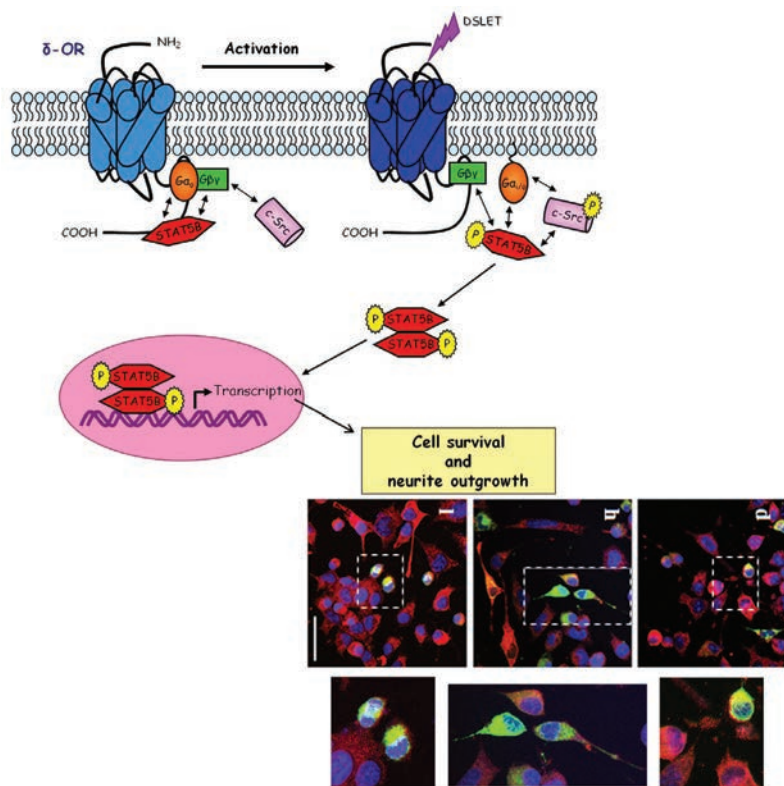


Figure 1: Upper panel: Proposed putative signaling pathway of the δ -opioid receptor-STAT5B-G protein complex, implicated in STAT5B phosphorylation leading to cell survival and neurite outgrowth. Lower pane: Confocal image indicating that activation of δ -OR leads to increased neuronal cell adhesion molecule NCAM (green) in Neuro-2A cells which is abolished upon expression of a dominant negative mutant of STAT5B, (red) represents nuclei stained with TO-PRO (Georganta et al. 2010, & Georganta et al., 2013: doi: 10.1111/jnc.12386).

Pharmacological characterization of new selective compounds/development of high throughput screening systems for G protein coupled receptors

By participating in the in the EU consortium "NORMOLIFE" (LSHC-CT2006-037733) and the Normolife -Network, our group characterized pharmacologically new opioid ligands (synthesized by other members of the consortium), that bind to the μ -, δ - or κ -opioid receptors, expressing mixed agonistic and antagonistic properties on cell based assays. These analogs display potent in vivo analgesic effects as assessed using brain-region specific primary network co-cultures and microelectrode neurochip technology prior to animal studies.

In collaboration with the Laboratory of Molecular Genetics & Biotechnology of the Institute of Biosciences

and Applications (Prof. K. Iatrou) and under the 7th EU Consortium «ENAROMaTIC», we study the signalling mechanisms of the mosquito *Anopheles gambiae* odorant receptors, and develop novel cell based screening platforms expressing selective serotonin receptor subtypes.

Publications

Fourla D.D., Papakonstantinou M.P., Vrana S.M., Georgoussi Z. (2012) Selective interactions of spinophilin with the C-terminal domains of the δ - and μ -opioid receptors and G proteins differentially modulate opioid receptor signaling. *Cell Signal.* Dec;24(12):2315-2328.

Georgoussi, Z., Georganta, E-M., Milligan, G. (2012). The other side of opioid receptor signalling: Regulation by protein-protein interaction. *Curr. Drug Targets*, Vol. 13, 80-102.

Pasquinucci L., Parenti C., Turnaturi R., Aric G., Marrazzo A., Prezzavento O., Ronsisvalle G., Georgoussi Z., Fourla D-D., Scoto G-M., and Ronsisvalle G.M (2012) The benzomorphan-based LP1 ligand is a suitable MOR/DOR agonist for chronic pain treatment *Life Sciences*, 90, 66-70.

Articles in Press

E-M. Georganta, L. Tsoutsi, M. Gaitanou and Z. Georgoussi (2013) δ -opioid receptor activation leads to neurite outgrowth and neuronal differentiation via a STAT5B-Gai/o pathway. *J. Neurochem.* 2013 Jul 31. doi: 10.1111/jnc.12386. [Epub ahead of print]

A. Kritharidou, Z. Georgoussi, C.Tsamis, and E. Makarona (2013) Zinc oxide nanostructures as low-cost templates for neuronal circuit. *Proc. SPIE 8765, Bio-MEMS and Medical Micro devices*, 87650G doi:10.1117/12.2017620

Presentations at Scientific Conferences

E.-M. Georganta, L. Tsoutsi and Z. Georgoussi Activation of the δ -opioid receptor leads to differentiation and neurite outgrowth via a STAT5B-Gai/o signaling pathway. *Pharmacology Symposium*, 12th April 2012, Thessaloniki, Greece

M.-P. Papakonstantinou, L. J. Leontiadis, F. Nikolos and Z. Georgoussi. Interactions of two members of the B/R4 subfamily of the Regulators of G protein Signaling with kappa and delta opioid receptors differentially modulate their signaling. *Pharmacology Symposium*, 12th April 2012, Thessaloniki, Greece

Z. Georgoussi, M.-P. Papakostandinou, D.-D. Fourla, S.-M. Vrana, A. Agalou Spinophilin a new interacting partner of δ - and μ -opioid receptors, 8th FENS Forum of Neuroscience, 14-18 July 2012, Barcelona, Spain

M.P. Papakonstantinou, D.D. Fourla, S.M. Vrana & Z. Georgoussi. Chimeric peptides corresponding to intracellular regions of opioid receptors as "baits" for screening novel receptor-interacting partners, 32nd European Peptide Symposium, 2nd to 7th September 2012, Megaron Concert Hall, Athens, Greece

Papakonstantinou M., Leontiadis L., Vrana, S.M., Tsoutsi L. and Georgoussi Z. Regulator of G protein signaling (RGS) proteins-opioid receptor interactions: drug targets modulating opioid receptor signaling. *International Conference on Chemistry for Health*, 9 – 14 September, 2012, National Hellenic Research Foundation, Athens, Greece (Oral Presentation)

Georgoussi Z., Papakonstantinou M.-P., Vrana S.-M., Tsoutsi L., Drug addiction and pain sedation: Two different sides of the same currency, *Researcher's Night*, 26 September 2012, National Hellenic Research Foundation, Athens, Greece

E.-M. Georganta, L. Tsoutsis, M. Gaitanou and Z. Georgoussi, Activation of the δ -opioid receptor leads to differentiation and neurite outgrowth via a STAT5B-Gai/o signalling pathway, Neurosciences Days 2-3 November 2012, Biomedical Research Foundation of the Academy of Athens.

Papakostantinou M.P., Fourla D.D., Vrana S.M., Agalou A., Georgoussi Z., Association of spinophilin with the δ - and μ -opioid receptors differentially modifies their signalling, Neurosciences Days, 2-3 November 2012, Biomedical Research Foundation of the Academy of Athens, Greece

Papakonstandinou M.P., Fourla D.D., Vrana, S.M. and Georgoussi Z. Spinophilin: a novel modulator of the δ - and μ -opioid receptor signaling 63rd Conference of the Hellenic Society of Biochemistry and Molecular Biology, 9-11 November 2012, Heraklion, Crete, Greece

E.-M. Georganta, L. Tsoutsis, and Z. Georgoussi Opioid receptor induced neurite outgrowth is mediated by STAT5B activation through a Gai/o signalling pathway, 63rd Conference of the Hellenic Society of Biochemistry and Molecular Biology, November 9 -11, 2012, Heraklion, Greece.

E. Makarona, A. Kritharidou, C. Tsamis and Z. Georgoussi "Zinc Oxide Nanostructured Substrates as Alternative Low-cost Templates for the Development of Cell-based Circuits" Euroensors XXVI, Krakow, Poland (Oral Presentation)

Educational Activities

PhD Theses

L. Leondiadis completed his PhD Thesis entitled "Opioid receptor signaling beyond the G protein paradigm", Department of Biology, Kapodistrian University of Athens – Defended 13 January 2012 with grade "Excellent"

I. Georganda completed her PhD Thesis entitled "Novel Signalling pathways leading to alteration in gene expression and synaptosomal plasticity", Department of Biology, Kapodistrian University of Athens – Defended 28th May 2012 with grade "Excellent"

M. Papakonstantinou: "RGS proteins novel players in opioid receptor signalling", Department of Pharmacy, University of Patras, in progress

Diploma Theses

E. Vrana, "*Spinophilin a new interactive partner of opioid receptors*", Department of Biology, Kapodistrian University of Athens, in progress

K. Kapolou, "*Novel interaction of opioid receptors with RGS proteins*", Department of Biology, Kapodistrian University of Athens, in progress

C. Karoussiotis, "*Pharmacological characterization of new opioid peptides*", Department of Molecular Biology, Demokrition University, Thraki, in progress

A. Kritharidou, "*Zinc oxide nanostructures as a platform for neuronal circuits*", Department of Physics, University of Patras, Patra, in progress

Z. Georgoussi:

Post-graduate Master's Program in Biochemistry of the University of Athens Department of Biochemistry and Molecular Biology, University of Athens, with title: *G protein-coupled receptors in health and disease*", 3 hours 15 students

Post-graduate Master's Program, «Molecular basis of Human Disease» of the University of Athens Department of Biochemistry and Molecular Biology, University of Athens, with title: *G proteins in health and disease*", 4 hours 15 students

Participation in the Postgraduate Course of the Polish Academy of Sciences, Warsaw, Poland

Participation in educational programme of School of Pharmacy, University of Catania, Catania, Sicily, Italia

Member of the Recruitment Committee for postdoctoral fellowships in I.B.A.

Research Seminars held by I. Georganta and M. Papakonstantinou under the postgraduate program of the IBA

Supervision of the Ph.D theses of the graduate students:

E. Georganta & L. Leontiadis (University of Athens) and M. Papakonstantinou (University of Patras)

Member of the advisory committee in the Department of Biology of the University of Athens for the Ph.D candidates E. Georganta and L. Leontiadis

Member of the advisory committee in the Department of Pharmacy of the University of Patras for the Ph.D candidate M. Papakonstantinou.

Other Scientific Activities

Z. Georgoussi:

National Representative of COST action-CM1207: "GLISTEN": GPCR-Ligand Interactions, Structures and Transmembrane Signalling: a European Research Network

Member of the International Research Network for palliative care "*Normolife-Network*", for the design, development and assessment of new analgesic compounds

Reviewer of manuscripts submitted to scientific Journals: Molecular Pharmacology, Journal of Neurochemistry, Journal of Biological Chemistry, Journal of Pharmacology and Experimental Therapeutics, Cellular Signaling, Neuropharmacology, Neuropharmacology Journal of Biotechnology, Journal of Neuroscience, BioMed Cell Biology

Reviewer of research grant proposals of the General Secretary for Research of Cyprus (RPF), Cyprus, General Secretary of Research and Technology, National Scholarship Foundation (IKY)

Member of "Periktioni" Network representing the N.C.S.R. "Demokritos" for the Greek Women Scientists

Co-founder together with Prof. K. Iatrou and Dr L. Swevers of the spin-off company GENEXPA

Other Distinctions and Awards

Akogiounoglou Award of the Institute of Biosciences and Applications, NCSR "Demokritos"

Irene Georganta has been awarded with the Akogionoglou Award of the Institute of Biosciences and Applications (IBA) for her excellent performance and significant scientific contribution on «*Novel signal transduction pathways leading to alterations of transcription factors implicated in synaptosomal plasticity*» (26/4/2012) .

Other Activities for the Institute of Biosciences & Applications

Z. Georgoussi:

Member of the evaluation committee for postdoctoral researchers

Supervisor for IBA infra structure for the ultracentrifuges Beckman L8 and Optima max, Speedvac concentrator and spectrophotometer Perkin Elmer

Impact factor (for 3 publications): 11,6

Citations for 2012 (without self citations): 37

Citations for 2008-2012 (without self citations): 135

h-factor: 15

Laboratory Equipment and Common Usage Equipment (IB-A)

- Tissue culture room: laminar air flow chamber, incubators, inverted microscope, microcentrifuge.
- Moleocular Pharmacology: BRANDEL apparatus for multiple experiments of receptor binding assays, chromatography columns for Adenyl-cyclase assays, DNA, RNA and protein Gel electrophoresis equipments, microcentrifuge, β -counter.
- Biochemistry and Molecular Biology: GST, MBP, 6xHis fusion recombinant protein purification, Affinity chromatography columns
- Cellular Biology: Flow cytometry (FACs), immunofluorescence microscope, confocal microscopy detection of bioactive factors and interactions of protein molecules, microtiter plate reader for fluorescent and chemiluminescence (ex. GFP, luciferase, β galactocidase)

Current external funding

Research Program entitled *ENAROMaTIC- European Network for Advanced Research on Olfaction for Malaria Transmitting Insect Control*, funded by the European Union as responsible Scientist and program Coordinator: Prof. K. Iatrou

Duration: 12/2008-12/2012

Total Funding of the program (consortium): 2.500.000 €

Total funding (lab): 50.000€

Total funding (lab) for 2011: 10.000 €

Greek-Hungarian bilateral entitled "SELF-ASSEMBLED ZnO NANOSTRUCTURES FOR ENGINEERED NEURONAL NETWORKS", funded by the General Secretary of Research and Technology, Greek Ministry of Education coordinated by Dr. E. Makorona (Institute for Advanced Materials, Physicochemical Processes, Nanotechnology & Microsystems)

Duration: 12/2011-12/2013

Total funding of the program: 15.000€

Total funding (lab) for 2012: 0 €

Note:

Pending proposals:

NO-ALGOS: "*Neuroprotective Opioid activity by Alternative G protein-coupled Opioid receptor Signaling leading to Neurite Outgrowth*", submitted June 2012, Minisrty of Education, Lifelong Learning and Religious Affairs, under the National frame of the action «EXCELLENCE II».

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

Research Staff

Nikos Grammatikakis, Senior Researcher

Sofia Aliberti, Graduate Student

Research Interests

A) Cell Signaling

Mechanisms of mammalian kinase regulation during normal differentiation and disease

Chemotherapeutical inhibition of oncogenic kinase activity

B) Cellular Responses to Stress and Nutrition

Regulation of Chaperone Protein Activity

Identification of Signaling Mediators (including kinases and transcriptional factors) which are modulated by the Chaperone Machinery in response to Stress and Dietary Factors

C) Cell Cycle Regulation

The Chaperone Machinery as an effector of cellular Stress in cell cycle progression

D) Novel Molecular Chaperones

Characterization and study of a group of novel Molecular Chaperones identified in our lab and their potential role as mediators of the assembly and activity of ErbB2, Raf, Akt, Cdk4 and I-kappaB kinases (IKK) in cell proliferation and cell cycle progression. Our study extends to learning how the activity of these novel signal modulators is regulated by Growth conditions and Stress (Radiation and chemotherapeutic drugs).

2012 Findings

The first part of our work in 2004 was carried in our lab in the US (Harvard University/ BIDMC). Upon transferring to the Institute of Biology at the NCSR Demokritos we encountered a major hardship and delay in establishing a working environment, especially in regards to finding a proper laboratory space. Nevertheless, we have been able to get around this problem and establishing new collaborations with investigators from the Signaling field continue our experimental work in other labs in the area. The results of the work we have carried out both in the US and in Greece during the past year can be summarized as follows:

In general lines we have continued testing experimentally the model we had proposed 9 years ago, namely that "the activity of a select group of kinases might -in addition to the classic mechanisms- be possibly conformationally modulated by Cdc37 and the chaperone machinery". To this end, our recent data indicate that p50Cdc37 is the same molecule suspected since the 1980s to, together with Hsp90, bind to and possibly regulate Src (reviewed in: Brugge J., 1986: Interaction of the Rous sarcoma virus protein pp60src with the cellular proteins pp50 and pp90. *Curr Top Microbiol Immunol* 123:1-22). As our current data also show, p50Cdc37 is a crucial regulatory factor for a group of G1-specific kinases which is functionally dependent on interactions with the Chaperone Machinery (HSPs). This group of kinases includes Raf and Src, ErbB2, Akt, the I-kappaB kinases (IKKs), MLK3, the heme-regulated eIF-2alpha kinase (HRI) and, among the cyclin-dependent-kinases, Cdk4, Cdk6 and Cdk9. Further, as our overall data show, the entire assembly and coordinate regulation of at least three crucial signaling kinase modules (Raf>Mek>Erk, IKK>IkappaB>NFkappaB and Cdk4>Cdk4/CyclinD>pRb) might be mediated by the chaperone machinery under physiological conditions. In the above process, the ATPase/folding activity of the participating Hsp90 and Hsp70 plays a central role. Finally, we have reported a novel

tripartite connection, that of the MAPK kinase module and of the 14-3-3 family of adaptors with the Heat Shock Factor-1 (HSF-1) and formulated a hypothesis of how the nucleocytoplasmic localization, and as result the transcriptional activity of the later might be affected by upstream signaling.

Publications

El Hamidieh A, Grammatikakis N. and Patsavoudi E. Cell surface Cdc37 participates in extracellular Hsp90 mediated cancer cell invasion. PLoS One. 2012; 7(8):e42722.

Impact Factor (for 1 publication): 4,05

Citations 2012 (without self- citations): 56

Total Citations 2008-2012 (without self- citations): 382

h-factor: 18

Research Group: **Mechanisms of Cell Proliferation and Ageing**

Research Staff

Dimitris Kletsas, Research Director

Harris Pratsinis, Researcher

Eleni Mavrogonatou, Postdoctoral Fellow

Sotirios-Spyridon Vamvakas, Postdoctoral Fellow

Adamantia Papadopoulou, Graduate Student

Dimitris Konstantonis, Collaborating Graduate Student (*MSc*) - *MSc obtained in 2012*

Eleni Liakou, Collaborating Graduate Student (*MSc*) - *MSc obtained in 2012*

Maria Angelopoulou, Collaborating Graduate Student (*MSc*)

Aimilia Sklirou, Collaborating Graduate Student (*MSc*)

Maria Lefaki, Graduate Research Associate (*MSc*)

Anastasios Kouroumalis, Training Student

Research Interests

The Laboratory is focusing on tissue repair during development and ageing with an emphasis on the role of growth factors, and especially that of TGF- β . The action of growth factors on cell proliferation and extracellular matrix production, as well as the responsible signaling pathways are investigated. Alternative mechanisms of cell proliferation and differentiation, such as autocrine regulation, cell-matrix interactions, exogenous stresses and the effect of mechanical forces are also studied.

Main goal of the Laboratory is the investigation of the mechanisms of ageing and longevity. The structural and functional characteristics of the senescent cell - as a result of successive duplications or of exogenous stresses - in comparison to that of the young or the cancer cell are investigated. Especially, we are interested in the role of the senescent - somatic and stem - cell in the process of ageing and the development of age-related diseases, including cancer. In this direction, we study the interaction between senescent stromal fibroblasts and adjacent cancer cells. Emphasis is given on tissues, such as the intervertebral disc, the degeneration of which provokes severe dysfunctions during ageing.

Aim of our studies is the elucidation of the mechanisms underlying the regulation of tissue homeostasis, especially during ageing, and furthermore the contribution, through research networks, in the development of cell replacement therapies. Finally, we study natural products and new synthetic compounds with putative anti-cancer, anti-ageing/anti-oxidant and wound healing action, as well as their mode of action.

2012 Findings

In our laboratory the role of growth factors in tissue repair is being studied with a special emphasis on the action of TGF- β . We showed that TGF- β has differential effects in human fibroblasts regarding their proliferative potential, depending on the tissue, the developmental stage and the presence of extracellular matrix components, such as collagen and hyaluronic acid.

Main goal of the laboratory is the study of the functional features of senescent cells, as well as their role in the development of age-related diseases including cancer. We have observed that various known anticancer drugs induce premature senescence of the stromal cells, which in turn favours the growth of cancer cells. Furthermore, a new marker for the identification of senescent cells *in vitro* and *in vivo* was developed in collaboration with other research groups.

In addition, we continued our research on the cellular physiology of intervertebral disc degeneration, and especially the cellular responses to various stresses, such as hyperosmolality, oxidative stress, and cyclic mechanical stretching. It was observed that these stresses stimulate intracellular signalling pathways leading to the regulation of cell proliferation and to premature senescence. In parallel, bioinformatics' analysis of cDNA microarray experiments in intervertebral disc cells treated with high osmolality revealed the differential expression of numerous genes, the role of which is currently under investigation.

A further goal of our work is the use of cell therapies in age-related degenerative disorders, by employing either autologous somatic cells or mesenchymal stem cells (MSCs). We have shown that MSCs that have reached replicative senescence or senescence induced by the use of anticancer treatments possess a diminished differentiation capacity. This finding was also observed in fibroblasts of the periodontal ligament, which lose their differentiation capacity towards osteoblasts during senescence due to down-regulation of the relative transcription factors.

Finally, we identified a significant number of plant extracts and of their fractions with antioxidant activity and a capacity to protect human skin fibroblasts from the cytotoxic effects of UVB-radiation.

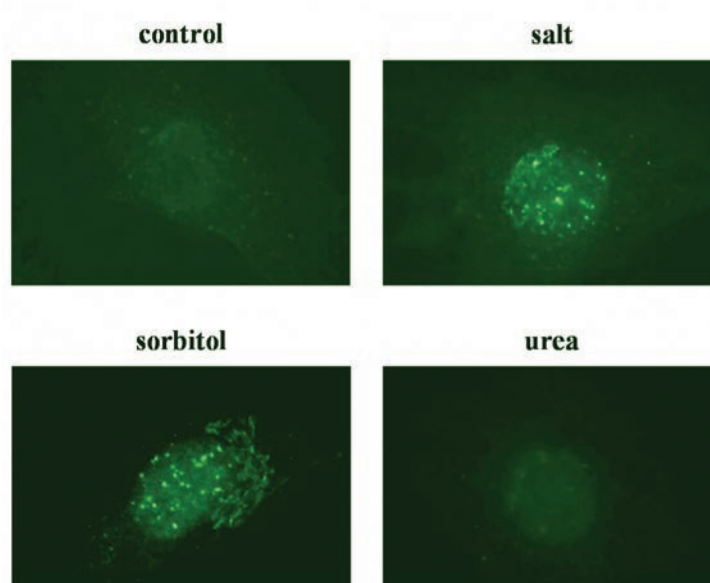


Figure 1. Study of the effect of high salinity, high sorbitol and high urea concentration on the DNA double strand breaks' formation in the nuclei of intervertebral disc cells. Our findings demonstrate that the observed DNA damage is the result of the hyperosmotic stress itself and not of the increased ionic concentration. [From E. Mavrogonatou & D. Kletsas (2012) *J. Cell. Physiol.* 227, 1179-1187].

Publications

Mavrogonatou, E., Kletsas, D. (2012). Differential response of nucleus pulposus intervertebral disc cells to high salt, sorbitol, and urea. *J. Cell. Physiol.* 227, 1179-1187

Pratsinis, H., Constantinou, V., Pavlakis, K., Sapkas, G., Kletsas, D. (2012). Exogenous and autocrine growth factors stimulate human intervertebral disc cell proliferation via the ERK and Akt pathways. *J. Orthop. Res.* 30, 958-964.

Makropoulou, M., Aligiannis, N., Gonou-Zagou, Z., Pratsinis, H., Skaltsounis, A.L., Fokialakis, N. (2012). Antioxidant and cytotoxic activity of the wild edible mushroom *Gomphus clavatus*. *J. Med. Food* 15, 216-221.

Fokialakis, N., Alexi, X., Aligiannis, N., Siriani, D., Meligova, A.K., Pratsinis, H., Mitakou, S., Alexis, M.N. (2012). Ester and carbamate ester derivatives of Biochanin A: Synthesis and in vitro evaluation of estrogenic and antiproliferative activities. *Bioorg. Med. Chem.* 20, 2962-2970.

Zampeli, D., Pratsinis, H., Eliades, T., Eliades, G., Kletsas, D., Papagiannoulis, L. (2012). In vitro estrogenicity of dental resin sealants. *Pediatr. Dent.* 34, 312-316

Anastasiadi, M., Pratsinis, H., Kletsas, D., Skaltsounis, A.L., Haroutounian, S.A. (2012) Grape stem extracts: Polyphenolic content and assessment of their in vitro antioxidant properties. *LWT-Food Sci. Technol.* 48, 316-322.

Skevaki, C.L., Psarras, S., Volonaki, E., Pratsinis, H., Spyridaki, I.S., Gaga, M., Georgiou, V., Vittorakis, S., Telcian, A.G., Maggina, P., Kletsas, D., Gourgiotis, D., Johnston, S.L., Papadopoulos, N.G. (2012) Rhinovirus-induced basic fibroblast growth factor release mediates airway remodeling features. *Clin. Transl. Allergy* 2, 14.

Dedes, P.G., Gialeli, Ch., Tsonis, A.I., Kanakis, I., Theocharis, A.D., Kletsas, D., Tzanakakis, G.N., Karamanos, N.K. (2012) Expression of matrix macromolecules and functional properties of breast cancer cells are modulated by the bisphosphonate zoledronic acid. *Biochim. Biophys. Acta* 1820, 1926-1939.

Lazáry, Á., Ito, K., Eisenstein, S., Fairbank, J., Roberts, S., Kletsas, D., Kümin, M., Brayda-Bruno, M., Varga, P.P. (2012) Surgeons and scientists: symbiosis in spinal research? *Eur. Spine J.* 21, 1681-1683

Papadimitriou, K., Ferreira, S., Papandreou, N.C., Mavrogatou, E., Supply, P., Pot, B., Tsakalidou, E. (2012) Complete genome sequence of the dairy isolate *Streptococcus macedonicus* ACA-DC 198. *J. Bacteriol.* 194, 1838-1839.

Wuertz, K., Vo, N., Kletsas, D., Boos, N. (2012) Inflammatory and catabolic signalling in intervertebral discs: The roles of NF- κ B and MAP Kinases. *Eur. Cell. Mater.* 23, 103-20.

Articles in Press

Mavrogatou, E., Kletsas, D. (2013) The effect of glucosamine sulfate on the proliferative potential and glycosaminoglycan synthesis of nucleus pulposus intervertebral disc cells. *Spine (PhilaPa 1976)* 38, 308-314. (IF: 2,078)

Georgakopoulou, E.A., Tsimaratou, K., Evangelou, K., Fernandez-Marcos, P.J., Zoumpourlis, V., Trougkos, I.P., Kletsas, D., Bartek, J., Serrano, M., Gorgoulis, V.G. (2013) Specific lipofuscin staining as a novel biomarker to detect replicative and stress-induced senescence. A method applicable in cryopreserved and archival tissues. *Aging (Albany NY)* 5, 37-50. (IF: 5,127)

Hinz, B., Ulrich, M., Beele, H., Kletsas, D. (2013) The 22nd annual meeting of the European Tissue Repair Society (ETRS) in Athens, Greece. *Fibrogenesis Tissue Repair* 6, 3. (Unofficial IF: 2,970)

Dedes, P.G., Kanakis, I., Gialeli, Ch., Theocharis, A.D., Tsegenidis, T., Kletsas, D., Tzanakakis, G.N., Karamanos, N.K. (2013) Preclinical evaluation of zoledronate using an in vitro mimetic cellular model for breast cancer metastatic bone disease. *Biochim Biophys. Acta* (in press). (IF: 5.000)

Malavaki, C.J., Roussidis, A.E., Gialeli, Ch., Kletsas, D., Tsegenidis, T., Theocharis, A.D., Tzanakakis, G.N., Karamanos, N.K. (2013) Imatinib as a key inhibitor of the platelet-derived growth factor receptor mediated expression of cell surface heparan sulfate proteoglycans and functional properties of breast cancer cells. *FEBS J.* (in press) (IF: 3,790)

Metwally, K., Khalil, A., Sallam, A., Pratsinis, H., Kletsas, D., El Sayed, K. (2013) Structure-activity relationship investigation of methoxy substitution on anticancer pyrimido[4,5-c]quinolin-1(2H)-ones. *Med. Chem. Res.* (in press) (IF: 1,271)

Kasiotis, K.M., Pratsinis, H., Kletsas, D., Haroutounian, S.A. (2013) Resveratrol and related stilbenes: Their anti-ageing and anti-angiogenic properties. *Food Chem. Toxicol.* (in press) (IF: 2,999)

Gialeli, Ch., Theocharis, A.D., Kletsas, D., Tzanakakis, G.N., Karamanos, N.K. (2013) Expression of matrix macromolecules and functional properties of EGF-responsive colon cancer cells are inhibited by panitumumab. *Invest. New Drugs* (in press) (IF: 3,357)

Neidlinger-Wilke C., Galbusera F., Pratsinis H., Mavrogonatou E., Mietsch A., Kletsas D., Wilke H.-J. (2013) Mechanical loading of the intervertebral disc –from the macroscopic to the cellular level. *Eur. Spine J* (accepted) (IF: 1,965)

Konstantonis D, Papadopoulou A, Makou M, Eliades T, Basdra EK, Kletsas D. (2013) Senescent human periodontal ligament fibroblasts after replicative exhaustion or ionizing radiation have a decreased capacity towards osteoblastic differentiation. *Biogerontology* (in press) (IF: 3,190)

Pratsinis H, Armatas A, Dimozi A, Lefaki M, Vassiliu P, Kletsas D (2013) Paracrine anti-fibrotic effects of neonatal cells and living cell constructs on young and senescent human dermal fibroblasts. *Wound Repair and Regeneration* (accepted) (IF: 2,757)

Velimezi G., Liontos M., Vougas K., Roumeliotis T., Bartkova J., Sideridou M., Dereli-Oz A., Kocylowski M., Pateras I.S., Evangelou K., Kotsinas A., Orsolic I., Bursac S., Cokaric-Brdovak M., Zoumpourlis V., Kletsas D., Papafioti G., Klinakis A., Volarevic S., Gu W., Bartek J., Halazonetis T.D., Gorgoulis V. "Functional interplay between the DNA damage response kinase ATM and ARF tumour suppressor protein in human cancer". *Nature Cell Biology* (accepted) (IF: 19,488)

Articles in Books and Conference Proceedings

Nikitovic, D., Pratsinis, H., Berdiaki, A., Gialeli, C., Kletsas, D., Tzanakakis, G.M. Growth factor signaling and extracellular matrix. In "Extracellular Matrix: Pathobiology and Signaling" (N.K. Karamanos, ed.), pp. 741-762, Walter de Gruyter (Berlin/Boston), 2012

Pratsinis, H., Mavrogonatou, E., Papadopoulou, A., Kletsas, D. In vitro assessment of biocompatibility for orthodontic materials. In "Research Methods in Orthodontics. A Guide to Understanding Orthodontic Research" (T. Eliades, ed.), pp. 61-78, Springer-Verlag Berlin Heidelberg, 2013

Pratsinis, H., Armatas, A., Kletsas, D. Response of fetal and adult cells to growth factors. In "Human fetal tissue transplantation" (N. Bhattacharya, and P. Stubblefield, eds.), pp. 65-77, Springer-Verlag London, 2013

Presentations at Scientific Conferences

A. Dimozi, E. Mavrogonatou, H. Pratsinis, D. Kletsas (2012). The Role of Exogenous Stresses on the Proliferation of Intervertebral Disk Cells. World Forum for Spine Research 2012 "The Intervertebral Disc— from Degeneration to Pain", June 18-21, 2012, Helsinki, Finland.

E. Chaita, N. Aligiannis, A. Argyropoulou, B.I. Boka, E. Kalpoutzakis, E. Liakou, H. Pratsinis, D. Kletsas, N. Gulbrandsen, M. Hamburger, V. Dumontet, O. Pamard, F. Guéritte, A.L. Skaltsounis (2012). Cosmetic properties of plant derived natural products: antioxidant and UV-protection effects. 8th Joint Meeting of AFERP, ASP, GA, PSE and SIF, July 28 – August 1, 2012, New York, NY, USA.

N. Fokialakis, N. Aligiannis, X. Alexi, M.N. Alexis, H. Pratsinis, E. Kalpoutzakis, S. Mitakou (2012). Phytoestrogens from *Genista halacsyi* (Leguminosae). 8th Joint Meeting of AFERP, ASP, GA, PSE and SIF, July 28 – August 1, 2012, New York, NY, USA.

D. Kletsas (2012). Senescence of mesenchymal stem cells. Implications in tissue regeneration. 4th Congress of the World Union of Wound Healing Societies, 2-6 September 2012, Yokohama, Japan (invited speaker).

H. Pratsinis, A. Armatas, E. Mavrogonatou, V. Gioni, M. M. Lefaki, N.K. Karamanos, K. Syrigos, D. Kletsas (2012). Proliferative Response of Human Fetal and Adult Lung Fibroblasts to TGF- β . 22nd European Tissue Repair Society Meeting, October 4-5, 2012, Athens, Greece.

H. Pratsinis, A. Armatas, A. Dimozi, M. Lefaki, P. Vassiliou, D. Kletsas (2012). Paracrine Effects of Young Neonatal Skin Cells on Senescent Fibroblasts: Implications for Cell-Based Therapeutic Approaches in Wound Repair. 22nd European Tissue Repair Society Meeting, October 4-5, 2012, Athens, Greece.

E. Mavrogonatou, H. Pratsinis, D. Kletsas (2012). The Response of Intervertebral Disc Cells to Exogenous Growth Factors Towards Tissue Repair and the Role of the Extracellular Osmolality. 22nd European Tissue Repair Society Meeting, October 4-5, 2012, Athens, Greece.

D. Kletsas (2012). The role of cellular senescence on tissue homeostasis and cell replacement therapies. 10th IQUAM Congress and Consensus Conference. 2-4 November 2012, Athens, Greece (invited speaker).

H. Pratsinis, A. Dimozi, K. Pilichos, S. Tsagarakis, A. Yiacoumettis, D. Kletsas (2012). Previous chronic exogenous glucocorticoid administration in vivo does not affect functional characteristics and cellular lifespan of human skin fibroblasts in vitro. 63rd National Conference of Biochemistry and Molecular Biology, 9-11 November 2012, Heraklion.

E. Mavrogonatou, D. Kletsas (2012). The role of glucosamine sulfate in the proliferation and glycosaminoglycan synthesis of nucleus pulposus intervertebral disc cells. 63rd National Conference of Biochemistry and Molecular Biology, 9-11 November 2012, Heraklion.

D. Kletsas (2012). Exogenous stresses and intervertebral disc homeostasis. 18th Seminar on Spinal Biomechanics and Biotechnology. 20 November-2 December 2012, Athens (invited speaker).

Educational Activities

"Cell senescence and tissue homeostasis", NCSR "Demokritos" Summer School, 1 hour, 50 students (D. Kletsas)

Supervision of the Ph.D. theses of Adamantia Papadopoulou, Dimitris Konstantonis, and Eleni Liakou (D. Kletsas)

Supervision of the theses for the acquisition of a Master's degree of Maria Angelopoulou and Aimilia Sklirou (D. Kletsas)

Participation in the internal advisory committee of the fellow A. Athanassopoulos (D. Kletsas & H. Pratsinis)

"Cell senescence and carcinogenesis" Lecture in the framework of the class entitled "Thoracic oncology", Medical School of the University of Athens, 1 hour, 50 students. (D. Kletsas)

"Cell senescence and tissue homeostasis" Post-graduate Master's Degree in Physiology, Medical School of the University of Athens, 2 hours, 30 students. (D. Kletsas)

"Cell senescence and carcinogenesis" Lecture in the framework of the class entitled "Oncogenes and growth factors in cancer biology" Medical School of the University of Athens, 2 hours, 20 students. (D. Kletsas)

"Cell Culture-Tissue Culture", Post-graduate Master's Degree "Applications of Biology in Medicine", Department of Biology of the University of Athens, 6 hours, 20 students. (D. Kletsas, H. Pratsinis and E. Mavrogonatou)

Member of three examination committees for Ph.D. theses in the University of Athens (Department of Biology, Chemistry and School of Dentistry) (D. Kletsas).

Dimitris Konstantonis concluded his Ph.D. thesis entitled "Effect of donor age on human periodontal fibroblasts' response to mechanical stimulation" in the School of Dentistry, University of Athens, which was unanimously accepted and awarded the degree "Excellent" (Scientific Supervisor D. Kletsas).

Eleni Liakou concluded her M.Sc. thesis entitled "Study of antioxidant / anti-aging action of natural products» in the framework of the Master's Degree Program of the Biology Department of the University of Athens; the thesis was unanimously accepted and awarded the degree "Excellent" (Scientific Supervisor D. Kletsas).

Other Scientific Activities

Member (Treasurer) of the Administrative Board of the Hellenic Society for Biochemistry and Molecular Biology (D. Kletsas)

Member (Secretary General) of the Research Club for Connective Tissue and Matrix Biology of the Hellenic Society for Biochemistry and Molecular Biology (D. Kletsas)

Member of the Administrative Board of the European Tissue Repair Society (D. Kletsas)

Secretary of the Biology Section (Europe) of the International Association of Gerontology and Geriatrics (IAGG) (D. Kletsas)

D. Kletsas (2012) Cellular senescence: Molecular mechanisms and role in tissue homeostasis. May 15, 2012, Institute for Biomedical Aging Research, University of Innsbruck, Austria (invited speaker).

D. Kletsas (2012) Cellular senescence: Molecular mechanisms and role in tissue homeostasis. August 30, 2012, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan (invited speaker).

Participation in editorial boards of scientific journals:

Editorial board member of the scientific journals "Biogerontology", "Mechanisms of Ageing and Development", "European Spine Journal", "PLOS One", "Fibrogenesis and TissueRepair", "Open Longevity Science", "Open Spine Journal" και "Journal of Dental Biomechanics" (D. Kletsas)

Organization of scientific conferences or participation in organizing committees of conferences:

President of the Scientific Committee of the 22nd European Tissue Repair Society (ETRS) Meeting, 4-5 October 2012, Athens (D. Kletsas) – President (D. Kletsas) and members (E. Mavrogonatou, H. Pratsinis) of the Organizing Committee of the 22nd ETRS Meeting.

Participation in committees for the reviewing of research proposals:

Netherland Organization for Scientific Research, AO Foundation, AO Spine East Asia (D. Kletsas)

Reviewing of manuscripts in scientific journals:

European Spine Journal (7), PLoS ONE (8), American Journal of Pathology, Annals of Biomedical Engineering, Cell Death & Disease, Biogerontology, Experimental Dermatology, Mechanisms of Ageing and Development, Osteoarthritis and Cartilage, Fibrogenesis & Tissue Repair, Arthritis Research & Therapy, Anticancer Agents in Medicinal Chemistry (2), Tissue Engineering, AGE, Acta Biochimica et Biophysica Sinica, Spine Journal, Anticancer Drugs, Cells Tissues & Organs, The International Journal of Lower Extremity Wounds (D. Kletsas) and

Experimental Dermatology, PLoS ONE, Journal of Molecular Biochemistry, Journal of Aging Research (H. Pratsinis)

Other Activities for the Institute of Biosciences & Applications

D. Kletsas:

Scientific Supervisor of the Experimental Animal Colony
Supervisor of the Fluorescence Activated Cell Sorting Facility
Member of the Finances Committee of NCSR "Demokritos"
Member of the Committee on Services-Providing Laboratories of NCSR "Demokritos"
Member of the Hygiene and Security Committee of NCSR "Demokritos"

H. Pratsinis:

Responsible (jointly with Dr. A. Prombona) for the conducted tours in the Institute of Biosciences and Applications

Impact Factors:

D. Kletsas (for 8 publications): 21,054
H. Pratsinis (for 6 publication): 11,516

Citations 2012 (without self-citations):

D. Kletsas: 428
H. Pratsinis: 130

Total Citations 2008-2012 (without self-citations):

D. Kletsas: 2428
H. Pratsinis: 592

h-factor:

D. Kletsas: 27
H. Pratsinis: 19

Laboratory equipment

The lab has the necessary equipment for gene and protein expression analyses, as well as for cell biology studies. In particular, a fully equipped cell culture room exists. Dr. D. Kletsas is the supervisor of the aforementioned equipment. In addition, some institutional facilities exist in the lab (under the same

supervisor), such as a flow cytometer FACSCalibur (Becton-Dickinson), a Coulter Counter cell counter and a UV-visible light microscope Axioplan (Zeiss) bearing a CCD camera and a software for image capturing and processing.

Current External Funding

Project entitled *Disc-degeneration linked pathologies: novel biomarkers and diagnostics for targeting treatment and repair (GENODISC)*, funded by EE with Coordinator Dr. J. Urban (Greek Coordinator: D. Kletsas)

Duration: 2008-2010

Total programme funding: 2.997.144€

Funding of the lab for 2012: 0 €.

Program entitled *From Biodiversity to Chemodiversity: Novel Plant Produced Compounds with Agrochemical and Cosmetic Interest (AgroCos)*, funded by EU with Coordinator Prof. A.-L. Skaltsounis, University of Athens - (Coordinator for NCSR "D": D. Kletsas)

Duration: 2010-2014

Total programme funding: 2.903.633 €

Funding of the lab for 2012: 26.350€.

Program entitled *Contribution of the intracellular cross-talk of ER α / β with EGF-R and IGF-R in the development and progress of breast cancer: Functional cell characteristics, expression of bioactive molecules and EMT induction*, funded by GSRT (THALES project) with Prof. N.K. Karamanos (Department of Chemistry, University of Patras) as the scientific supervisor - (NCSR "D" supervisor: Dr. D. Kletsas)

Duration: 2010-2014

Total funding : 600.000 €

Funding of the lab for 2012: 0€.

Research Group: **Nuclear Proteins and Chromatin Function**

Research Staff

Thomae Sourlingas, Senior Researcher

Kalliope Sekeri, Emeritus Researcher

Marios Xidous, Graduate Student – *PhD obtained in 2012*

Eleni Kostopoulou, Collaborating Graduate Student (MSc) – *MSc obtained in 2012*

Research Interests

Our research is focused on the functional role of the histone subtypes and their epigenetic post translational modifications, as well as the effects that histone deacetylase inhibitors have on gene expression and cellular function in various biological processes.

1. Ageing-Apoptosis: Investigation of the relationship of the expression profiles of the subtypes of the H1 histone family (somatic subtypes and H1^o) and their epigenetic histone modifications (phosphorylation, acetylation and methylation) during ageing and apoptosis in cell strains and cell lines. We are also studying the changes that these epigenetic modifications bring about in the expression profiles of age-dependent genes in human peripheral blood leucocytes.
2. Mammalian Biological Clock: Investigation of the role of chromatin conformational changes that are brought about by histone post translational epigenetic modifications, such as histone acetylation and methylation in the regulation of the mammalian biological clock.
3. Psychiatric Disorders: How changes in histone epigenetic modifications and in the expression profile of the H1 DNA linker histone subtypes affect chromatin remodelling (conformational reorganization) events in human peripheral blood leucocytes from individuals with psychiatric disorders.

2012 Findings

Investigation of the role of chromatin remodeling events in the regulation of circadian clock function:

We examined the effects that two histone deacetylase inhibitors (HDACs), nicotinamide and trichostatin A (TSA), have on circadian clock function in NIH3T3 cell cultures. Real-time PCR showed that TSA increases the mRNA levels of the early response clock gene *per1* and that nicotinamide inhibits this TSA-induced effect. Moreover, nicotinamide blocks the acute response of *per1* induced by dexamethasone. This response is necessary for the synchronization of circadian rhythm in cell cultures. ChIP experiments showed that this inhibition of the acute response, as well as the increase in *per1* mRNA levels induced by TSA, is associated with a decrease in the lysine 4 trimethylation levels of histone H3 (H3K4me3) in the promoter region of this gene. We conclude that the decrease in H3K4me3 levels in the *per1* promoter is the repressive mechanism by which nicotinamide inhibits the *per1* acute response induced by dexamethasone. These results reveal the existence of a novel, as yet to be reported, mechanism with which clock function is regulated.

This study was carried out by M. Xydous within the framework of his doctoral thesis which he completed.

We are also investigating the changes in histone acetylation and methylation levels in the promoter region of the circadian clock gene *per1* that are brought about during in the acute response induced by the glucocorticoid, dexamethasone. Our results showed that there is an increase in the acetylation levels of lysines 9/14 of histone H3 (H3K9/14ac) as well as the trimethylation levels of lysine 4 of histone H3 in the promoter region near the transcription start site (TSS) but not in the promoter region near

the glucocorticoid response element (GRE) in NIH3T3 mouse fibroblast cell cultures. However, these two histone modifications increase, not only in the TSS region, but also in the GRE region in the mouse cancer neuroblastoma cell line, N2A. These results indicate that the mechanism by which the acute response is induced by dexamethasone is dependent on the cell type and may possibly be a biomarker of the specific cell type or state.

The general aims of this project are being carried out within the framework of a research collaboration with the laboratory of "Chronobiology" (Group Leader, Dr. Anastasia Prombona).

Investigation of the relationship amongst aging and changes in the epigenetic profile on a whole genome basis

1. Results from next generation DNA sequencing technology showed that there were age-dependent changes in the methylation state of DNA.
2. Concomitantly, the trimethylation of lysine 27 of histone H3 (H3K27me3), which is also a modification associated with transcriptional repression, was also studied on a whole genome basis using monocytes from neonatal cord blood and monocytes from adult peripheral blood. Results from this line of work revealed that the genomic loci which showed increased trimethylation levels of H3K27 co-localized with those that showed the greatest differences in DNA methylation as a function of age.

This work was accomplished by P. Salpea in the laboratory of Dr. Bruce Howard, Head of the Laboratory of Molecular Growth Regulation, of the National Institute of Child Health and Human Development; National Institutes of Health (NIH) within the framework of a research collaboration between the two labs.

Publications

Xydous, M., Sekeri-Pataryas, K.E., Prombona, A., Sourlingas, T.G. (2012). Nicotinamide treatment reduces the levels of histone H3K4 trimethylation in the promoter of the *mper1* circadian clock gene and blocks the ability of dexamethasone to induce the acute response. *BBA-Gene Reg. Mech.* **1819**, 877-884.

Salpea, P., Russanova, V.R., Hirai, T.H., Sourlingas, T.G., Sekeri-Pataryas, K.E., Romero, R., Epstein, J., Howard, B.H. (2012). Postnatal development- and age-related changes in DNA-methylation patterns in the human genome. *Nucleic Acids Res.* **40** (14): 6477-6494, 2012.

Educational Activities

Summer School (I.B.-A/NCSR "D") "Histone Variants and Post Translational Modifications: Fundamental Factors in Chromatin Remodeling Events during Aging and Apoptosis" (1 hour).

Seminar: "Cell Cycle: Checkpoints and Consequences for Normal Cellular Function" within the framework of the course "Cell Cultures-Tissue Cultures" of the Graduate Masters' Program: Applications of Biology in Medicine, Dept. of Biology, University of Athens (6 hours, 20 students).

Supervision of the doctoral thesis work of Marios Xydous, biologist, recipient of a 4-year scholarship from NCSR "D". Title of thesis work: "The effect of histone acetylation and methylation levels in the regulation of the mammalian biological clock". **This thesis work has been completed and presented** in the Dept. of Biological Chemistry, School of Medicine, Athens University in July, 2012. Part of this work has been published in *BBA: Gene Regulatory Mechanisms* (March, 2012).

Supervision of the Masters' research thesis work of Elena Kostopoulou. Title of thesis work: "Histone modifications and regulation of the circadian clock in neuroblastoma and fibroblast cell cultures". **This**

thesis work has been completed and presented in the Dept. of Pathological Anatomy, School of Medicine, Athens University in October, 2012.

Member of the Internal Advisory Committee for the doctoral thesis work of A. Galeou and M. Xydous.

Other Activities for the Institute of Biosciences & Applications

Member of the IB-A Committee for the receipt of new materials and services and for the examination and characterization of materials unsuitable for use and to be destroyed that have been acquired by funds from the Demokritos budget.

Other Scientific Activities

Reviewer of scientific publications:

Apoptosis, Leukemia Research, Life Sciences

Scientific collaborations:

- With Dr. A. Prombona (Laboratory of Chronobiology), Institute of Biosciences and Applications, NCSR "D". This research collaboration involves the study of the effects of histone modifications on genes that regulate the mammalian biological clock (circadian rhythm) and the potential consequences to cellular function and carcinogenesis.
- With the laboratory of Dr. Bruce Howard, Head of the Laboratory of Molecular Growth Regulation of the National Institute of Child Health and Human Development; National Institutes of Health (NIH). Within the framework of this collaboration, P. Salpea, biologist, who completed her doctoral thesis work in our lab, also worked in the lab of Dr. Howard on a project that was part of her thesis work.
- With the Dept. of Biological Chemistry of the Medical School, University of Athens (Associate Prof. P. Moutsatsou). The collaboration involves "The study of the induction of apoptosis by ursolic acid in the MCF-7 breast cancer cell line.
- With the Neurobiology Research Institute of the Th. Th. Cozzika Foundation. Within the framework of this program we are studying changes in the expression levels of histone genes in leucocytes of patients with bipolar disorder and schizophrenia.

Impact factor (for 2 publications): 12,431

Citations 2012 (without self-citations): 18

Total Citations 2008-2012 (without self-citations): 78

h-factor: 6

Laboratory Equipment and Common Usage Equipment (IB-A) (Allocated to T. Sourlingas)

- Cell cultures: Laminar flow, cell culture incubator (IB-A), cell culture incubator, light microscope, phase contrast microscope, 2 CO₂ tanks, humidity (liquid) sterilizer incubator (IB-A), dry sterilizer incubator (IB-A), nitrogen tank for storage of cell cultures, nitrogen tank for storage of cell cultures (IB-A), tabletop refrigerated centrifuge with three heads (IB-A), 2 glass apparatus for filtering cell culture material.
- Protein analysis, etc.: Floor standing refrigerated centrifuge Sorvall with three heads (IB-A), water circulator, 2 powersupplies, 2 electrophoresis apparatus, 2 protein transfer apparatus (Westerns), shaker/waterbath, 2 scales (accuracy and standard), pH-meter (IB-A), 2 stirrer/hotplates, 2 vortexers, eppendorf tube shaker, refractometer (densitometer for solutions), H₂O distillation apparatus.
- Standard refrigerator for reagents, small refrigerator for food.

IB-A Common Usage Equipment Outside of the Lab that T. Sourlingas is responsible for

B-counter

Deep freezer (-80°C)

-40°C freezer

Note: The following project has been submitted and is currently under review:

We are partners (participants) in a program within the framework of "ARISTEIA II" with the following title: The role of the circadian biological clock and chromatin remodeling in mood disorders (Acronym: MOODCLOCK)

Research Group: **Cell & Matrix Biochemistry/Pathobiology**

Research Staff

Fotini-Effie Tsilibary, Research Director
Athina Tzinia, Senior Researcher
Angelika Chroni, Senior Researcher
Paraskevi Kitsiou, Senior Researcher
Garyfalia Drossopoulou, Researcher
 Ioannis Dafnis, Postdoctoral Fellow
 Katerina Kapodistria, Graduate Student
 Georgios Daniil, Graduate Student
 Letta Argyri, Collaborating Graduate Student
 Nefeli Lagopati, Collaborating Graduate Student
 Archontia Kaminari, Collaborating Graduate Student
 Sofia Verouti, Collaborating Graduate Student
 Christina Golfinopoulou, Collaborating Graduate Student (*MSc*)
 Chrisoula Nikolakopoulou, Undergraduate Student
 Dionyssi Giannimaris, Undergraduate Student
 Ioanna Tiniakou, Training Student
 Enaggelia Zvintzou, Training Student
 Alexia Kafkoutsou, Training Student
 Eleni Kotsopoulou, Technical Specialist
 Nikolaos Giannakas, Research Technician

Research Interests

1. Diabetes mellitus **A)** Regulation of gene expression and epigenetic mechanisms in glomerular podocytes in physiological and diabetic conditions, **B)** Renoprotective role of Vitamin D3 on glomerular podocytes. **C)** Nephron signalling in pancreatic β -cells: Cross talk between nephron signalling and insulin survival signalling.
2. Neurodegenerative Disorders: **A)** *In vivo* effect of collagenase B (MMP-9) in the brain of transgenic mice (TgMMP9). **B)** The protective role of MMP9 overexpressed in the brain of 5XFAD mice, a model of Alzheimer's disease (TgMMP9 x Tg5XFAD). **C)** Studies of insulin-dependent survival pathways in primary neuronal cells isolated from the above transgenic animals. **D)** Elucidation of the role of apoE4, a major risk factor for Alzheimer's disease, in the pathogenesis of the disease
3. Biological activities of TiO₂ innovative nanoparticles for diagnostic and therapeutic use. Mechanisms and applications.
4. Atherosclerotic disease: **A)** Elucidation of the interactions among the various proteins (apolipoprotein A-I, apolipoprotein E, cholesterol transporters, lipoprotein receptors, enzymes, lipid transport proteins) of the high-density lipoprotein (HDL) pathway. Evaluation of the significance of these interactions in the formation, remodeling and functions of HDL that are important for atheroprotection, as well as of the contribution of these interactions in plasma lipid homeostasis and induction of dyslipidemia. **B)** Elucidation of the structure-function relationship of apolipoprotein E (apoE) and its role to various dyslipidemias. **C)** Compositional and functional characterization of HDL obtained from individuals with distinct disturbances of HDL metabolism, cardiovascular events, chronic inflammatory diseases and from animal models.

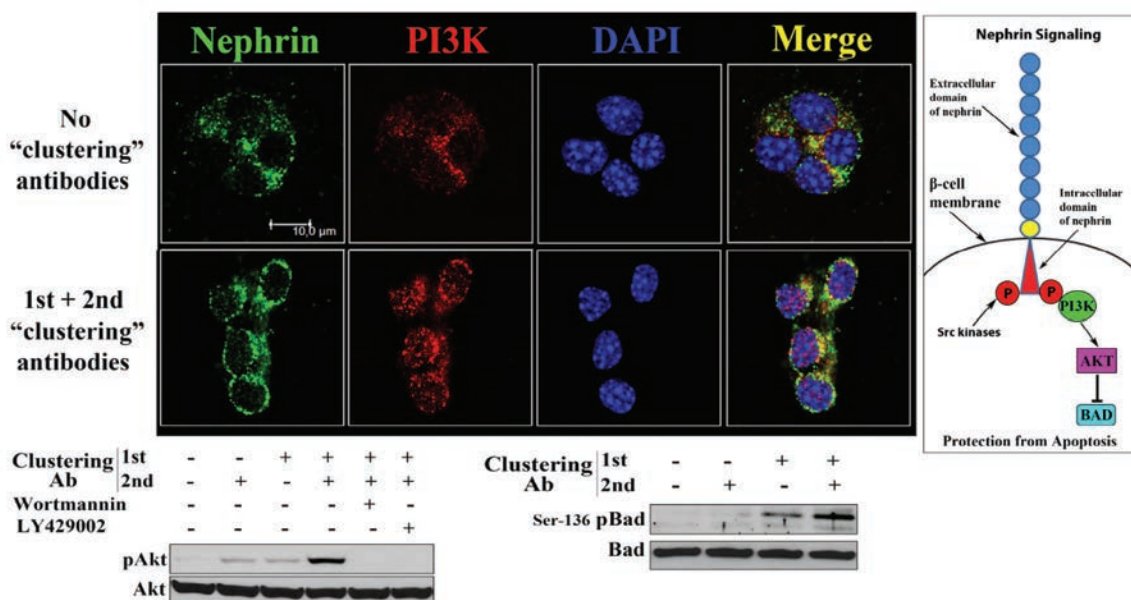
2012 Findings

1. Diabetes Mellitus

Nephrin signalling in pancreatic β -cells: Cross talk between nephrin signalling and insulin survival signalling.

We investigated the role of nephrin and nephrin-interacting proteins in cellular survival signaling and function of pancreatic insulin-producing β -cells. We demonstrated that cultured mouse pancreatic BTC-6 cells express nephrin, CD2AP and podocin. *In vivo*, in mouse pancreatic islets, nephrin is expressed by β -cells. Our results indicated that nephrin is associated and co-localized with PI3K and CD2AP. Incubation of BTC-6 cells with functional anti-nephrin antibodies induced nephrin clustering and recruitment of PI3K to clustered nephrin at the plasma membrane. This process led to activation of PI3K, since it was associated with increased phosphorylation of Akt; this effect was inhibited by wortmannin and LY294002, indicating that Akt activation was PI3K-dependent (*see figure*). Pre-treatment of cells with PP1, a selective inhibitor of Src kinases, inhibited the interaction of nephrin with PI3K as well as Akt activation, demonstrating that Src kinases mediate signaling downstream of nephrin. Nephrin-induced Akt activation resulted in increased phosphorylation/inhibition of pro-apoptotic Bad protein (*see figure*); hence nephrin-mediated PI3K-Akt activity can trigger anti-apoptotic signaling. Silencing of nephrin expression by nephrin-siRNA abolished nephrin-mediated Akt activation, suggesting a key role for nephrin in this process. Moreover high glucose impaired nephrin-mediated Akt activation without affecting nephrin expression; this effect was associated with increased nephrin endocytosis and upregulation of PKC α expression.

Our findings revealed that nephrin is involved in pancreatic β -cell survival signaling and demonstrated that glucose-induced changes in nephrin signaling could lead to β -cell apoptosis in pathological conditions such as type 2 diabetes.



Regulation of gene expression and the renoprotective role of Vitamin D3 on glomerular podocytes.

Renal podocytes form the main filtration barrier possessing a unique phenotype maintained by proteins including podocalyxin (PC) and nephrin, the expression of which is suppressed in pathological conditions. We use an *in vitro* model of human glomerular epithelial cells (HGEC) to investigate the role of high glucose in dysregulating the podocytic epithelial phenotype. Chronic exposure to high glucose induced

a phenotypic conversion of cultured podocytes resembling dedifferentiation. This dedifferentiation process was gradual and progressive, first started with loss of the differentiation markers CD10/CALLA and PC, and was followed by enhanced vimentin and markedly reduced nephrin expression. Reversible upregulation of vimentin expression was associated with restoration of normal nephrin expression. However, PC downregulation was irreversible when maximal loss of PC had been established. These observations indicate that attenuation of PC expression was mainly glucose dependent and persisted in HGEC possessing podocytic characteristics. Dumpening of PC expression could be thus considered a reliable marker of podocytic injury and partial dedifferentiation. Therefore, rescuing PC expression may be pivotal in hyperglycemic conditions such as diabetic nephropathy. However this needs to be verified by in vivo studies. Finally, our data suggested that maintenance of the previously established, differentiated podocytic phenotype does not necessarily involve WT1, which however is crucial for the process of differentiation of podocytic presursors to podocytes. Hence, investigating the role of other transcription factors in preserving and restoring structural and functional integrity of the podocytes is of paramount importance.

Vitamin D is beneficial in human and experimental chronic kidney disease (CKD). In view of the fact that suppressed PC expression, in podocytes, is partly irreversible we investigated the effects of activated vitamin D3 (calcitriol) and its analogue (paricalcitol) on high glucose induced reduction of nephrin and PC expression in podocytes and assessed the role of their receptor VDR. Calcitriol and paricalcitol reversed high glucose induced decrease of nephrin and significantly enhanced PC expression in podocytes cultured in high glucose. Podocytes express VDR and retinoid X receptor (RXR). In the presence of calcitriol and paricalcitol VDR expression was up-regulated and VDR co-localized with RXR in the nucleus. VDR knock-down abolished the protective action of calcitriol and paricalcitol on PC expression indicating that PC activation of expression is partly mediated by VDR. Furthermore VDR specifically regulates PC expression by bounding to a site upstream of the podocalyxin promoter. Therefore, Vitamin D analogues maintain and furthermore, re-activate the expression of specialized components of podocytes including PC, hence they provide protection against loss of the permselective renal barrier.

2. Neurodegenerative Disorders

The neuroprotective role of MMP-9 in AD

For the in vivo study of MMP9 we have generated transgenic mice overexpressing MMP9 in the brain (TgMMP9). These animals were crossed with animal models for AD (5XFAD) to produce double transgenic animals (TgMMP9/5XFAD). Double transgenic animals exhibit improved cognitive abilities compared to Tg5XFAD animals. This might be due to increased sAPP α and decreased AB oligomers and to the preservation of synaptic functions; increased levels of the synaptic protein synaptophysin and mature brain-derived neurotrophic factor in the brain were observed.

Insights on the relationship between apolipoprotein E4 and inflammation in brain

ApoE4 isoform, a major risk factor for Alzheimer disease (AD), is susceptible to proteolysis. ApoE4 fragments have been found in AD patients' brain. Neuroinflammation has been suggested to play an important role in the initiation or progression of AD. We studied whether truncated apoE4 forms have an effect on neuroinflammatory response. We found that apoE4 truncated form apoE4[Δ (186-299)], but not full-length apoE4 or shorter form apoE4[Δ (166-299)], increases the expression of IL-1 β , which possibly promotes MMP9/TIMP1 imbalance, and also decreases IL-10 expression in SK-N-SH neuroblastoma cells. In addition, apoE4[Δ (186-299)] also induces MMP9/TIMP1 imbalance in SW-1783 astrocytoma cells. We therefore show an association between two molecular events, the proteolysis of apoE4 and neuroinflammation, both of which have been correlated with AD pathogenesis. We propose that apoE4 proteolysis and specific short apoE4 proteolytic fragments produced in the brain of AD patients may induce or sustain inflammatory responses in brain cells.

3. Biological activities of TiO₂ innovative nanoparticles for diagnostic and therapeutic use: Mechanisms and applications.

We investigated the effect of the effect of crystal phase of nanostructured titanium dioxide particles on their bioactivity in breast cancer epithelial cells. Cultured MCF-7 and MDA-MB-468 breast cancer epithelial cells were irradiated, using UV-A light (wavelength 350 nm) for 20 minutes, in the presence of aqueous dispersions of two different nanostructured titania (TiO₂) crystal phases, anatase and anatase/rutile mixture. We demonstrated that both TiO₂ nanoparticles and UVA-photoexcited TiO₂ nanoparticles exerted a significant cytotoxic effect specifically in the highly malignant MDA-MB-468 cancer cells, whereas MCF-7 cells were not considerably affected. Our results documented that 100% anatase TiO₂ particles (110-130 nm) exhibited higher cytotoxic activity than anatase/rutile mixtures (75%/25%) with the same sizes. TiO₂-nanotoxicity in breast cancer epithelial cells was associated with increased pro-apoptotic Bax expression and caspase-mediated poly (ADP-ribose) polymerase (PARP) cleavage, thus leading to DNA fragmentation and programmed cell death-apoptosis.

4. Research related to atherosclerotic disease:

Functional characterization of HDL from individuals with genetic disorders of HDL metabolism

HDL can exert atheroprotective properties and protect against oxidation through actions of its associated enzymes (paraoxonase-1 (PON1), platelet-activating factor-acetylhydrolase (PAF-AH), lecithin:cholesterol acyltransferase (LCAT)). Whether LCAT plays a role in the depletion of lipid oxidation products has not been studied in humans. We investigated parameters relevant to lipid oxidation in carriers of *LCAT* mutations (4 carriers of 2 mutant *LCAT* alleles, 63 heterozygotes, 63 family controls). In patients with *LCAT* mutations, HDL-cholesterol, apoA-I, HDL-associated PON1 and PAF-AH activities, and HDL antioxidative capacity were gene-dose-dependently decreased. The OxPL/apoB ratio was increased by 17% in heterozygotes ($p < 0.001$) but not in carriers of two defective *LCAT* alleles. The reduced anti-oxidative potential of HDL and the increased oxPL/apoB suggest increased lipid oxidation in (partial) *LCAT* deficiency, but this is not supported by other parameters of lipid oxidation.

Publications

Kostomoiri M, Fragkouli A, Sagnou M, Skaltsounis LA, Pelecanou M, Tsilibary EC, Tzinia AK. *Cell Mol Neurobiol.* 2013 Jan; 33(1):147-54. Epub 2012 Oct 7

I. Dafnis, A. Tzinia, E. C. Tsilibary, V. I. Zannis and A. Chroni. *Neuroscience* 2012; 210 21–32.

Fragkouli A, Papatheodoropoulos C, Georgopoulos S, Stamatakis A, Stylianopoulou F, Tsilibary EC and Tzinia AK. *J. Neurochem.* 2012; 121, 239–251.

V.Ch. Fotopoulos, A. Tzinia, M. Tzurbakis, V. Kalfakakou, S. Levidiotou-Stefanou, A. Georgoulis. *Knee Surgery, Sports Traumatology, Arthroscopy* 2012; 20: 1159-1167.

Holleboom A. G., Daniil G., Fu X., Zhang R., Hovingh G. K., Schimmel A. W., Kastelein J. J. P., Stroes E. S. G., Witztum J. L., Hutten B. A., Tsimikas S., Hazen S. L., Chroni A. and Kuivenhoven J. A. Lipid Oxidation in Carriers of Lecithin:Cholesterol Acyltransferase Gene Mutations. *Arterioscler Thromb Vasc Biol*, 32, 3066-3075 (2012).

Articles in Press

Verouti S.N., Tsilibary E.C., Fragopoulou E., Iatrou C., Demopoulos C.A., Charonis A.S., Charonis S.A., Drossopoulou G.I. Vitamin D receptor activators upregulate and rescue podocalyxin expression in high glucose-treated human podocytes. *Nephron-Experimental Nephrology* 122(1-2):36-50. (IF 1.83).

Tsotakos N., Sagnou M., Kotsopoulou E., Tsilibary E., Drossopoulou G. Glucose-induced phenotypic changes independent of Wilms' tumor 1 (WT1) in cultured human podocytes. *BMC Cell Biology* 14(1):28 (IF 2.57).

Presentations at Scientific Conferences

Fragkouli, A., Tzinia A., Tsilibary E. (*E. Tsilibary: invited speaker*). Over-expression of MMP9 leads to increased sAPP α and restores cognitive abilities in a mouse model of Alzheimer's disease, COST 1001: Brain Extracellular Matrix in Health & Disease, July 12-13, 2012, Barcelona, ES

Fragkouli A., Stamatakis A., Stylianopoulou F., Tsilibary F. and Tzinia A overexpression of matrix metalloproteinase 9 (MMP9) leads to increased sAPP α and restores cognitive abilities in a mouse model of Alzheimer's disease. 8th FENS Forum 2012 Barcelona

V. C. Fotopoulos, A. Tzinia, M. Tzurbakis, V. Kalfakakou, S. Levidiotou-Stefanou, A. Georgoulis Expression levels of matrix metalloproteinase (MMP)-9 and its specific inhibitor TIMP-1 in septic and aseptic arthritis of the knee. EFORT 2012, 23 - 25 May 2012, Germany

Georgiadou D., Chroni A., Drosatos K., Kypreos K. E., Zannis V. I. and Stratikos E. Biophysical properties of apoE variants associated with the correction of dyslipidemia and formation of HDL particles. 80th European Atherosclerosis Society Congress Satellite Symposium, High Density Lipoproteins: From Basic Science to Therapeutic Advances. 28-29 May, 2012, Milan, Italy

Dafnis I., Tzinia A., Tsilibary E. C., Zannis V. I. and Chroni A. An apolipoprotein E4 fragment is involved in inflammatory responses in brain cell lines. 35th European Lipoprotein Club meeting, 10-13 September 2012, Tutzing, Germany

Verouti SN, Tsilibary EC, Fragopoulou E, Iatrou C, Demopoulos C, Charonis AS, Kotsopoulou E, Drossopoulou G (*E. Tsilibary: Invited speaker*). Vitamin D receptor mediates vitamin D- and paricalcitol-induced up-regulation of podocalyxin and nephrin expression in human podocytes cultured in high glucose, 24th Annual Meeting of the European Renal Cell Study Group, 22-15 March 2012, Arnhem, The Netherlands

Daniil G., Zannis V. I. and Chroni A. The carboxyl terminus of apolipoprotein A-I (ApoA-I) is necessary for rHDL to promote ABCG1-dependent cholesterol efflux. 15th Meeting of Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease, 18-20 October 2012, Athens

Daniil G. and Chroni A. The cholesterol transporter ABCG1 is involved in inflammatory processes. 63rd Conference of the Hellenic Society for Biochemistry and Molecular Biology, 9-11 November 2012, Heraklion, Crete

Argyri L., Stratikos E. and Chroni A. Biophysical analysis of an apolipoprotein E4 variant associated with increased risk of late-onset Alzheimer's disease. 63rd Conference of the Hellenic Society for Biochemistry and Molecular Biology, 9-11 November 2012, Heraklion, Crete

Kateifides A.K., Fotakis P., Georgiadou G., Beck M., Chroni A., Stratikos E., Zannis V.I., Kardassis D. Role of the hydrophobic and charged residues in the 218 to 226 region of apoA-I in the biogenesis of HDL. 63rd Conference of the Hellenic Society for Biochemistry and Molecular Biology, 9-11 November 2012, Heraklion, Crete

Argyri L., Stratikos E. and Chroni A. Biophysical analysis of a frequent apoE4 variant, apoE4Freiburg (Leu28Pro) that has been associated with the prevalence of Coronary Artery Disease. 5th Panhellenic Atherosclerosis Meeting of Hellenic Atherosclerosis Society, 29 November - 1 December 2012, Athens

Georgiadou D., Chroni A., and Stratikos E. Thermodynamic and structural destabilization of apolipoprotein E3 by hereditary mutations associated with the development of Lipoprotein Glomerulopathy. 5th *Panhellenic Atherosclerosis Meeting of Hellenic Atherosclerosis Society*, 29 November - 1 December 2012, Athens

Other Scientific Activities

E.Tsilibary: 1) Member, COST BM 1001: Brain Extracellular Matrix in Health and Disease (2011-2014) (representative for Greece), 2) member COST "EUROKUP" (2008-2012), 3) Scientific Editor for the Journal: PLoSOne, 4) Abstract evaluator for the 49th Annual Meeting of the European Renal Association- European Dialysis Transplant Association (ERA-EDTA), 5) proposal evaluator for ANR, (Agence National de Recherche), FR, 6-7) Invited Speaker for the 24th Annual meeting of the European Renal Cell Study Group (ERCSG) Doorwerth, The Netherlands, and the Department of Chemistry, Athens Capodistrian University,, 8) 2^o Annual Meeting of COST B1001-ECMNet-Scientific Collaboration with collaborators of the European COST program ECMNet- Brain Extracellular Matrix in Health and Disease" (Working Group and Manager Committee meeting)" Barcelona, ES July 12, 2013, 9-10) invited speaker for two workshops organized by the Medical School, Athens Capodistrian University 11) invited speaker for workshop organized by NOVONORDISC, 12) evaluator for Prize organized by Program : "Development of research & Innovative Culture", Cyprus, 13) Reviewer for: PLoSOne, Photodiagnosis and Photodynamic Therapy, Microfluidics, Cells/Tissues/Organs, Kidney International, Am. J. Physiology Renal, Brain Research, BBA, J. Biol. Chemistry, Environmental Science & Technology), BMC Molecular Biology, Current Pharmacology Design Journal

A. Chroni: 1) Reviewer for scientific journals Atherosclerosis, Journal of Biomedicine and Biotechnology, Annals of Rheumatic Diseases, Clinica Chimica Acta, Biochimica et Biophysica Acta- Molecular and Cell Biology of Lipids, PLOS ONE, Hellenic Journal of Atherosclerosis, 2) Member of the Core Group of COST Action BM0904 ("HDL - From Biological Understanding to Clinical Exploitation"). Participation in 2nd joint WG Meeting / 3rd MC Meeting of COST Action BM0904, Barcelona, January 26-27, 2012, 3) Participation in Core Group meeting of COST Action BM0904 in Tutzing, Germany, 10 September 2012.

P. Kitsiou: Reviewer for scientific journals *Current Diabetes Reviews*, *Current HIV Research*, *PLoS ONE*, *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery* Kidney and Blood Pressure Research

A. Tzinia: Member (Representative from Greece) of COST Action BM1001/ECM Net: Brain Extracellular Matrix in Health and Disease (2011-2014)

Reviewer for scientific journals: *PLoS ONE*

Reviewer for grand proposals: Alzheimer's Association

G. Drossopoulou: Academic Editor for scientific journal PLoS ONE.

Reviewer for scientific journals: *Current Diabetes Reviews*, *Journal of Nephrology*, *PLoS ONE*, *Nutrients*.

Other scientific presentations

Tsilibary, EC 1). « Opportunities for translational research for young physicians in Greece and the USA", Hellenic Association of young physicians workshop on: "Medical Education and Biomedical Research in Greece, Opportunities, Challenges and Problems", March 22nd, 2012, National Capodistrian University; 2) « Stem cell use in chronic and incurable diseases: How close is medical practice to therapeutic applications of cell therapy?", February 22nd, 2012, Department of Chemistry, National Capodistrian University 3) Participation to round table discussion: "Research and Biosciences in the era of the Greek financial crisis", March 2nd, 2012, Department of Biology, National Capodistrian University, 4) Pancreatic, insulin-producing, β -cell survival" Workshop entitled: "Re-defining therapy for Diabetes Mellitus" by Novonor-disc, Biomedical research Foundation of the Academy of Athens (BRFAA), October 20th, 2012; 5) Tsilibary

EC, A.Fragkouli, A. Stamatakis, F. Stylianopoulou, A. Tzinia. "Over-expression of MMP9 in the CNS leads to increased sAPP α and restores cognitive abilities in a mouse model of Alzheimer's disease", NEURO-SCIENCES ADVOCACY MEETING, November 3rd, 2012, BRFAA, Athens

A. Chroni: 1) *Molecular basis of the role of apolipoprotein E in the pathogenesis of Alzheimer's disease*. Invited speaker seminar series, University of Patras Medical School, April 4, 2012, Patra. 2) *Unraveling the connection between apolipoprotein E and Alzheimer's disease*. 63rd Panhellenic Congress of Hellenic Society of Biochemistry and Molecular Biology, November 9, 2012, Heraklio, Crete.

G. Drossopoulou: Genes and Disease: Connecting Molecular Biology to Nephropathy, "Researcher's Night 2012" September 28, National Hellenic Research Foundation, Athens

Other Distinctions and Awards

A. Chroni: Research proposal award from the Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease

Educational Activities

E. Tsilibary: i) Member of the committee for follow-up of doctoral studies of G. Daniel and M. Kostomiri (Institute of Biosciences & Applications, NCSR Demokritos); ii) Lecture: "Molecular Mechanisms and Therapeutic Approaches of Diabetes Mellitus": Three-hour lecture for post-graduate course "Pathobiochemistry", Department of Biology, National Capodistrian University, March 27th, 2012 (Number of graduate students: 12); iii) "The pros and cons of the process of cell apoptosis in diseases", October 4, 2012; three-hour lecture for post-graduate course "Molecular and Applied Physiology, Medical School, National Capodistrian University 2012 (Number of graduate students: 23)

A. Chroni, i) Member of the IBE Advisory Committee of PhD students G. Daniil and M. Kostomoiri, ii) "Alzheimer's Disease: Is it in our genes?" Summer School, NCSR "demokritos", 18 July 2012 (1h – 70 students), iii) "Lipoprotein metabolism pathways and atherosclerosis. The association between atherosclerosis and Alzheimer's disease." Guest lecturer in graduate course "Human Biochemistry", Biochemistry Graduate Program, Department of Chemistry, University of Athens. 9 May 2012 (3 hours- 10 students), iv) "Lipids and apolipoproteins: From cardiovascular disease to Alzheimer's disease". Guest lecturer in graduate course "Clinical Chemistry II", Clinical Chemistry Graduate Program, Department of Chemistry, University of Athens. 31 May 2012 (2h -5 students).

P. Kitsiou: Member of the Institute of Biosciences & Applications Advisory Committee of Ph.D. students N. Tsoakos and K. Kapodistria.

G. Drossopoulou: i) Basic Research: From bench to patient-Nephropathies; NCSR "Demokritos" Summer School, July 16 2012. ii) Genes and Disease: Connecting Molecular Biology to Nephropathy, "Researcher's Night 2012" September 28, National Hellenic Research Foundation, Athens

Other Activities for the Institute of Biosciences & Applications

A. Chroni:

- 1) Person in charge for education issues in IBE. Representative of IBE in the Education Committee of NCSR Demokritos
- 2) Member of the Scientific Advisory Committee of IBE.
- 3) Person in charge for the operation of FPLC

E.F.C. Tsilibary:

Director, Institute of Biosciences & Applications, and Member of the Executive Committee, NCSR Demokritos

Member of the committee for anonymous complaints

Impact Factors (for 5 publications): 18,075

Citations 2012 (without self-citations): E.F.C. Tsilibary: 123, A. Tzinia: 29, P. Kitsiou: 17, A. Chroni: 92, G. Drossopoulou: 49. Total: 310

Total Citations 2008-2012 (without self-citations): E.F.C. Tsilibary: 455, A. Tzinia: 103, P. Kitsiou: 66, A. Chroni: 321, G. Drossopoulou: 294. Total: 1239

h-factor: F. Tsilibary: 30, A. Tzinia: 10, P. Kitsiou: 7, A. Chroni: 14, G Drossopoulou: 10

Laboratory Equipment and Common Usage Equipment (IB-A)

FPLC (common equipment)

Refrigerated shaking incubator for cells (equipment purchased with grant funds, A. Chroni)

Tabletop refrigerated microcentrifuge Heraeus (equipment purchased with grant funds, A. Chroni)

Waterbath (equipment purchased with grant funds, A. Chroni)

2 Magnetic stirrers (equipment purchased with grant funds, A. Chroni)

Eppendorf microcentrifuge

Water-bath Heto

Ultrasound-Sonics (equipment purchased with grant funds, E. Tsilibary)

PCR apparatus

2 Digital balances (one was purchased with grant funds, A. Chroni)

3 Freezers -20°C (one was purchased with grant funds, A. Chroni)

3 Refrigerators

2 CO₂ incubators (equipment purchased with grant funds, E. Tsilibary)

2 Laminar Flow (one was purchased with grant funds, E. Tsilibary)

Table top centrifuge (equipment purchased with grant funds, E. Tsilibary)

Microwave oven

pH meter

Incubator

5 Gel electrophoresis apparatuses (two were purchased with grant funds, A. Chroni)

5 Power supplies (two were purchased with grant funds, A. Chroni)

4 Vortex (one was purchased with grant funds, A. Chroni)

3 Shaking platforms (one was purchased with grant funds, A. Chroni)

Current External Funding

Program entitled *Common pathogenetic mechanisms and pathways of the matrix-related diseases of Diabetes and Alzheimer's disease resulting in apoptotic cell death (DIABET-AL)* funded by GSRT (EXCELLENCE) with Project Coordinator Dr. E. Tsilibary.

Duration: 6/9/2012-6/9/2015

Total funding (lab): €360.000

Funding of the lab for 2012: 72.000.

Program entitled *Targeted strategies for new treatment approaches for cardiovascular and inflammatory diseases based on the protective functions of high density lipoprotein (HDL)* funded by GSRT (COOPERATION 2009) with Principal Investigator for NCSR "Demokritos" Dr. A. Chroni.

Duration: 18/2/2011-17/2/2014
Total funding (lab): €100.400
Funding of the lab for 2012: €0.

Program entitled *Apolipoprotein E mutations and hereditary Lipoprotein Glomerulopathy: mechanism of pathogenesis and diagnostic value* funded by the Hellenic Atherosclerosis Society with the Principal Investigator for NCSR "Demokritos": A. Chroni,

Duration: 2011-2012
Total funding (lab): €5.000
Funding of the lab for 2012: €2.500

Program entitled *Structure-function relationship, regulation and genetic variation in High Density Lipoproteins: Prospects for the prevention and therapy of Coronary Artery Disease (HDLbio-therapies)* funded by Ministry of Education, Lifelong Learning and Religious Affairs of Greece, Thalis grant with Principal Investigator for NCSR "Demokritos": A. Chroni,

Duration: 1/11/2012-31/10/2015
Total funding (lab): €125.000
Funding of the lab for 2012: €0

Program entitled *Role of apolipoprotein E mutants in the pathogenesis of hereditary Lipoprotein Glomerulopathy* funded by the Hellenic Atherosclerosis Society with the Principal Investigator for NCSR "Demokritos": A. Chroni,

Duration: 2012-2013
Total funding (lab): €5.000
Funding of the lab for 2012: €2.500

Abbot Hellas (Donation for Research and Development)

Title: "Renoprotective role of vitamin D", Principal Investigators for NCSR: Dr. E. Tsilibary, Dr. G. Drossopoulou

Duration: 2010-2013
Total funding: € 52.000
2012 funding: €2000

Program entitled: ECMNET "*Brain Extracellular Matrix in Health and Disease*", funded by the European Science Foundation - COST Action (Dr. Tsilibary and Dr. Tzinia: representatives from Greece; Dr. Tsilibary: member of the Management Committee).

Program Duration: 15/12/2010 – 14/12/2014

European Science Foundation - COST Action BM0904

Title: "HDL - From Biological Understanding to Clinical Exploitation."

A. Chroni: Member of the Management Committee and the 6 member Core Group Duration: 8/6/2010 – 7/6/2014

Note: Submitted and funded

E. Tsilibary:

1. Program «KRHPIS» Identification of targets for disease diagnosis and treatment (ΔIAS) (1.039.000€) (E. Tsilibary, scientific co-ordinator)
2. Program: Collaboration II: "Studies for pancreatic β-cell survival for interfering with type 2 diabetes: the role liraglutide": (E. Tsilibary, (1.335.000€)
3. Program "BIONIAN CLUSTER" Approval of the first phase

Research Group: **Environmental Mutagenesis -Carcinogenesis**

Research Staff

Gerassimos Voutsinas, Senior Researcher

Antonis Lampidonis, Postdoctoral Fellow (IKY Fellowship)

Eumorphia Konstantatou, Collaborating Graduate Student

Eleutheria Peristeri, Collaborating Graduate Student (*MSc*)- *MSc obtained in 2012*

Angeliki Delimitsou, Collaborating Graduate Student (*MSc*)

Angeliki-Stephania Bassoyianni, Undergraduate Student - *Undergraduate dissertation completed in 2012*

Michaela Diakatou, Undergraduate Student

Dionysis Iglezos, Training Student

Dimitris Androulakis, Training Student

Sokratis Avgeris, Research Technician

Research Interests

1. Identification and validation of drug targets for cancer therapy
2. Development and evaluation of biomarkers for diagnosis, prognosis and response to treatment in human diseases
3. Development of genetic testing protocols for molecular diagnosis of human genetic diseases

2012 Findings

1. Correlation of CYP1A1, GSTP1 and GSTM1 gene polymorphisms and lung cancer risk among smokers

Lung cancer is the leading cause of cancer mortality worldwide and tobacco smoking has been established as its biggest risk factor. Cigarette smoke contains several carcinogens. Most of them need to be activated by phase I enzymes, such as cytochrome P450 (CYP), while phase II enzymes, such as glutathione S-transferases are responsible for the detoxification of activated forms. The present study aimed to determine the role of CYP1A1, GSTP1 and GSTM1 gene polymorphisms in smoking-related lung cancer risk. It also aimed to investigate the association of the above polymorphisms with clinicopathological parameters, as well as their effect on survival. One hundred newly diagnosed lung cancer patients with advanced disease and 125 healthy controls with a smoking history participated in the study. The participants were screened for the presence of the following polymorphisms: MspI (CYP1A1), Ile105Val (GSTP1) and GSTM1 deletion. The above polymorphisms were also examined with regards to gender, age, histological type and survival. GSTP1 Ile/Val and GSTM1-null genotypes were associated with increased lung cancer risk and the presence of the combination of the three non-wild-type genotypes increases susceptibility to lung cancer (OR 3.328, 95% CI=1.681-6.587, p=0.001). In the non-small cell lung cancer group, the GSTP1 homozygous variant was significantly associated with increased lung cancer risk (p=0.008) and shorter survival. The results of this study suggest that the GSTP1 Ile/Val genotype and GSTM1 deletion contribute to increased lung cancer susceptibility. Moreover, GSTP1 Val/Val genotype is associated with increased lung cancer risk and shorter survival in non-small cell lung cancer patients.

Publications

Pliarchopoulou, K., G. Voutsinas, G. Papaxoinis, K. Florou, M. Skondra, K. Kostaki, P. Roussou, K. Syrigos and D. Pectasides (2012) Correlation of CYP1A1, GSTP1 and GSTM1 gene polymorphisms and lung cancer risk among smokers, *Oncol Lett* 3, 1301-1306.)

Educational Activities

E. Peristeri successfully defended her M.Sc. thesis entitled «*Molecular diagnosis of Neurofibromatosis type I*», in June 2012, at the Department of Biology of NKUA.

Lecture (2 hours) and practical laboratory exercise (3 hours): "Cytotoxicity study on conventional and targeted chemotherapeutic drugs" included in the course for "Cell and tissue cultures", in the frame of the Post-Graduate Specialization Diploma "Biological Applications in Medicine" of the Departments of Biology and Medicine of the National Kapodistrian University of Athens (NKUA), Athens.

Lectures (2 x 3 hours): "Pharmacological targeting of Hsp90" and "Molecular diagnosis of genetic diseases" included in the course for "Molecular Biology – Systemic and in silico approaches", in the frame of the Post-Graduate Specialization Diploma "Biological Applications in Medicine" of the Departments of Biology and Medicine of the National Kapodistrian University of Athens (NKUA), Athens.

Course: "Introduction to Molecular Biology", including 16 hours of practical laboratory exercises, in the American College of Greece (Deree College), Aghia Paraskevi Attikis, Greece.

Lectures (3x3 hours): with the general title "Biology: the Science of Life", in the frame of the Open Cycle of Academic Lectures of the Municipality of New Heracleion Attikis, Greece, April – June 2012.

Lecture entitled "Molecular Diagnosis of *Neurofibromatosis type I*", American College of Greece (Deree College), Aghia Paraskevi Attikis, Greece, October 30, 2012.

Other Scientific Activities

Participation in Greek and International scientific bodies and organizations:

1. Reviewer for International Journal of Cancer and Nature Reviews Urology.
2. Reviewer for the Union for International Cancer Control (International Union Against Cancer).
3. Association of Medical Geneticists of Greece (SIGE) (Treasurer).
4. Greek Alliance for Rare Diseases (PESPA) (Treasurer).
5. Greek Alliance for Rare Diseases (Member of the Scientific Committee).
6. Tuberous Sclerosis Association of Greece (EEOS) (Member of the Scientific Committee).
7. 6th European Conference on Rare Diseases and Orphan Products, 23-25 May 2012, Brussels, Belgium.
8. International TSC Congress 2012, 6-9 September 2012, Napoli, Italy
9. Member of the Organizing Committee and Facilitator of the Greek Europlan II Conference of EU-Eurordis, 1 December 2012, Athens, Greece.

Other Activities for the Institute of Biosciences and Applications

Head of the Laboratory for "Molecular Diagnosis of Genetic Diseases" (E1609), rendering genetic testing services for Tuberous Sclerosis Complex and Neurofibromatosis type I.

In charge for the operation of ABI Prism 310 Genetic Analyzer (Applied Biosystems), Mx3000P QPCR system (Stratagene), Image Analysis System (Vilber Lourmat), LAS-4000 Luminescent Image Analyzer (Fuji-Film) and FLA-7000 Fluorescent Image Analyzing System (Fuji-Film) of the Institute of Biosciences and Applications, NCSR "Demokritos".

Impact factors (for 1 publication): 0,237

Number of citations for 2012 (without self-citations): 83

Number of citations 2008-2012 (without self-citations): 330

h-factor: 13

Laboratory Equipment and Common Usage Equipment (IB-A)

Old equipment charged to G. Voutsinas or equipment acquired through research program funding: Laminar flow cabinets for cell cultures, CO₂ incubator, refrigerated centrifuge, bench-top microcentrifuge, waterbath, refrigerator, freezers, deep-freezer, agarose and polyacrylamide gel electrophoresis apparatuses, computer, laser printer.

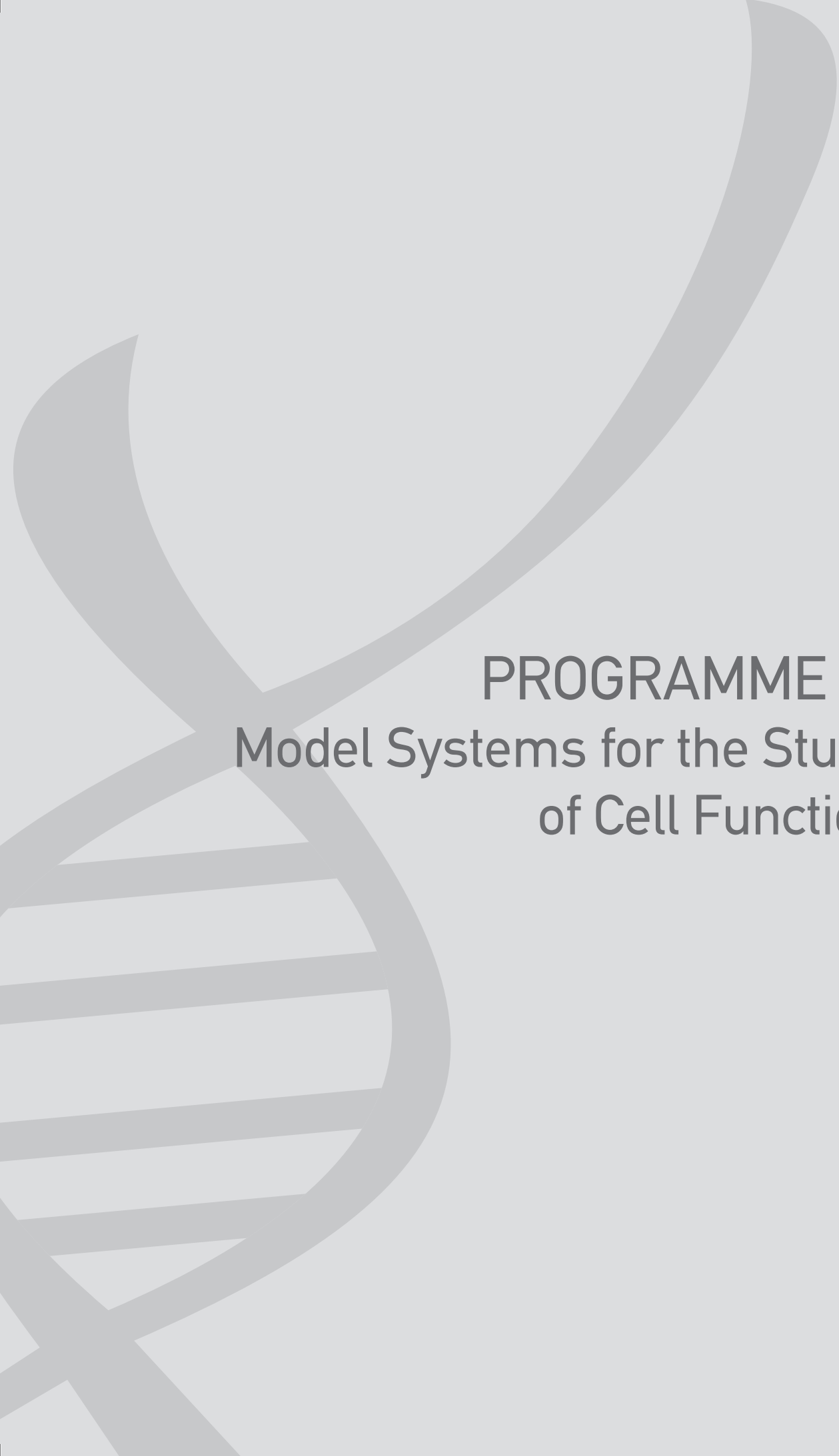
Common equipment of the Institute of Biosciences and Applications in the responsibility of G. Voutsinas: Thermal cycler, ABI Prism 310 Genetic Analyzer (Applied Biosystems), QPCR Mx3000P system (Stratagene), Image Analysis System (Vilber Lourmat), LAS-4000 Luminescent Image Analyzer (Fuji-Film), FLA-7000 Fluorescent Image Analyzing System (Fuji-Film).

Current External Funding

«Sequencing and genome characterization of lactic acid bacteria *Streptococcus macedonicus*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactobacillus acidipiscis*. Physiological, evolutionary and technological extensions», Research proposal funded by the General Secretariat for Science and Technology, Ministry of Education, Life-long Learning and Religion.

Note: Three (3) research proposals have been approved for funding:

1. «Detection of New Breast and Ovarian Cancer predisposing Alleles, using whole genome next generation sequencing», Research proposal funded by the General Secretariat for Science and Technology, Ministry of Education, Life-long Learning and Religion.
2. «Non-ionizing electromagnetic radiations: biologic effects», Research proposal to be funded by the General Secretariat for Science and Technology, Ministry of Education, Life-long Learning and Religion.
3. «Contribution of intracellular communication of ER α / β with EGR-R and IGF-R in the development and progression of breast cancer: functional properties of cells, expression of bio-active molecules and induction of EMT», Research proposal to be funded by the General Secretariat for Science and Technology, Ministry of Education, Life-long Learning and Religion.



PROGRAMME B:
Model Systems for the Study
of Cell Function

Research Group: **Molecular Genetics of Insects and Biotechnology**

Research Staff

Kostas Iatrou, Research Director

Luc Swevers, Research Director

Vassiliki Labropoulou, Senior Researcher

Lydia Ignatiadou, Emeritus Scientist

Panagiota Tsitoura, Postdoctoral Fellow

Georgia Kythraioti, Postdoctoral Fellow

Theodoros Georgomanolis, Graduate Student – *PhD obtained in 2012*

Konstantinos Ioannidis, Graduate Student

Anna Kolliopoulou, Graduate Student

Jisheng Liu, Visiting Graduate Student

Mariana Kolovou, Training Student

Dimitris Raptopoulos, Special Collaborator

Dimitra Stefanou, Technical Specialist

Alexandra Amaral, Research Technician

Dimitris Kopanelis, Research Technician (*retired*)

Research Interests

1. Regulatory mechanisms controlling insect physiological functions.

- (i) Oogenesis in lepidopteran insects: a model differentiation program induced by ecdysteroid hormones.
- (ii) Mechanisms of immunosuppression in lepidopteran insects following parasitization by hymenopteran endoparasitoids: the role of interactions between proteins of hymenopteran endosymbiotic viruses and proteins of the hemocytes of the lepidopteran hosts.
- (iii) Mechanisms controlling olfactory function in the malaria mosquito vector *Anopheles gambiae*.
- (iv) Analysis of small RNA (miRNA, siRNA) pathways in lepidopteran insects.

2. Molecular biology and genetic manipulation of insect nuclear polyhedrosis viruses.

- (i) Viruses that express proteins toxic for the insect hosts.
- (ii) Genetically modified nuclear polyhedrosis viruses as vectors for insect genetic transformation.
- (iii) Genetically modified nuclear polyhedrosis viruses as vectors for gene therapy and cellular immunization applications.

3. Functional genomics.

- (i) Systems for production of proteins of economic importance in lepidopteran insect and mammalian cell lines.
- (ii) High throughput screening systems for detection of bioactive substances (activators and inhibitors of pharmacological targets) in chemical libraries and collections of natural products (plants and microorganisms).

2012 Findings

Analysis of small RNA pathways in the silkworm, *Bombyx mori*.

The contribution of the basic machinery of the different pathways of small RNAs to the process of dsRNA-mediated gene silencing was determined by “RNAi-of-the-RNAi” experiments in Bm5 cells. The results show contribution of components of the siRNA but also of the piRNA pathway in the silencing process. On the other hand, experiments of over-expression of the basic siRNA factors showed only minimal increase in the efficiency of RNAi. In vivo experiments showed that injection of dsRNA in

the hemolymph of silkworm larvae induces the expression of the mRNA of two basic enzymes in the RNAi pathway, Dicer-2 and Argonaute-2. The results show that dsRNA can function as a pathogen-associated molecular pattern that can activate the innate immune system in the silkworm *Bombyx mori* (collaboration with Dr. Guy Smagghe, Ghent University, Belgium).

Genetic modification of insect virus vectors for transduction of mammalian cells and insect transformation

Further studies were carried out that investigate the functional role of the gene *lef8* of baculovirus, which encodes a component of the viral RNA polymerase, using a knockout mutant virus deficient for *lef8*. Rescue of the deficient virus is not possible by co-transfection with expression constructs for Lef8 protein or in transformed cell lines that permanently express Lef8. Instead, successful rescue is only possible after the re-introduction of the *lef8* gene into its original site in the deficient virus. Those findings indicate a previously unknown function of the LEF8 ORF, which is related to its position in the viral genome and its regulatory effects on adjacent genes.

Parasitism of lepidopteran insects by parasitoid hymenopterans

Further studies were carried out to characterize the role of Ank proteins, because of their interaction with the active form of the transcription factor BmRelishd2, and especially Ank2, due to its likely association with microtubules. Further studies were also carried out in relation to the functional role of hemolin, a member of the family of IgG proteins, in the immune response of the host; more specifically, interactions between mutated forms of hemolin with the CcV1 protein of polydnavirus were investigated. Finally, preliminary experiments indicate that the "horseshoe"-like structure of hemolin is not maintained in aqueous solution, a finding suggestive of a possible function of the protein as opsonin, which may enhance phagocytosis (collaboration with Olle Terenius, University of Agricultural Sciences, Uppsala, Sweden).

Mechanisms of olfactory function in the insect vector for malaria *Anopheles gambiae*

Following successful testing in experimental huts in Nigeria, 4 repellent compounds isolated from essential oils by virtue of binding to recombinant odorant binding proteins (OBPs) and induction of behavioral effects on experimental mosquito populations, were tested in a Nigerian village where *A. gambiae* mosquito vectors of the malaria parasite are endemic. Their bioactivity in terms of mosquito space-repellency in village dwellings was found to be slightly better than that of DEET, the mosquito repellent most widely used for human protection from mosquitoes at present (collaboration with Dr. Samson Awolola, Nigerian Institute of Medical Research, Lagos, Nigeria). Moreover, to identify ligands of mosquito olfactory receptors (ORs), the functional characterization of the ORs was extended to another 6 members of the family. Five of these receptors were orphan while the last one was the common subunit Orco, which also has the capacity to form or homomeric ion channels (as opposed to the heterodimeric receptors that were investigated so far). For the detection of compounds with specific agonist or antagonist action, eighteen synthetic compounds were tested. Compounds with known insect repellent activity were also tested in parallel for possible effects on the olfactory receptor function, in an attempt to deduce the molecular basis of their activity.

Functional genomics

Detection systems for compounds with moult-accelerating activity

Dibenzoyl-hydrazine compounds with high activity previously identified with *in vitro* tests were tested for toxicity against mosquito (*Anopheles gambiae*) larvae. All the compounds that were tested proved to be active, therefore inviting the execution of more systematic toxicity tests (collaboration with Dr. J. Vontas, University of Crete).

A new expression vector for DNA vaccination was tested, which directs the secretion of intracellular

proteins in the mammalian cell culture medium through their fusion with the mouse granulocyte macrophage colony-stimulating factor (mGMCSF). The aim has been to produce antibodies against intracellular proteins in mice by simple injections of expression plasmid DNA rather than purified protein. The technique was used successfully for protein, Ank2 of the insect bracovirus CcBV and the production of antibodies is in progress.

Moreover, we carried out the cloning of wild type and mutant esterase genes related to insecticide resistance and their expression in insect cell lines. Preliminary results regarding the enzymatic activity of the recombinant esterases showed that the proteins that are expressed by insect cell lines are active and can be used for the analysis of the mechanism of resistance and the identification of inhibitors, which is the ultimate goal of the study (collaboration with Dr. J. Vontas, University of Crete).

Publications

De Geyter, E., Swevers, L., Soin, T., Geelen, D., and Smagghe, G. (2012). Saponins do not affect the ecdysteroid receptor complex but cause membrane permeation in insect culture cell lines. *J. Insect Physiol.* 58, 18-23.

De Geyter, E., Swevers, L., Caccia, S., Geelen, D., and Smagghe, G. (2012). Saponins show high entomotoxicity by cell membrane permeation in Lepidoptera. *Pest Manag. Sci.* 68, 1199-205

Liu, J., Swevers, L., Iatrou, K., Huvenne, H., and Smagghe, G. (2012). Bombyx mori DNA/RNA non-specific nuclease isoforms: expression in insect culture cells, subcellular localization and functional assays. *J. Insect Physiol.* 58, 1166-1176.

Ogura, T., Nakagawa, Y., Swevers, L., Smagghe, G., and Miyagawa, H. (2012). Quantitative evaluation of the molting hormone activity in coleopteran cells established from the Colorado potato beetle, *Leptinotarsa decemlineata*. *Pesticide Biochemistry and Physiology* 104, 1-8.

Sdralia, N., Swevers, L., and Iatrou, K. (2012). BmVMP90, a large vitelline membrane protein of the domesticated silkworm Bombyx mori, is an essential component of the developing ovarian follicle. *Insect Biochem. Mol. Biol.* 42, 717-27.

Ignatiades L. 2012. Mixotrophic and heterotrophic dinoflagellates in the eutrophic coastal waters of the Aegean Sea (eastern Mediterranean Sea). *Botanica Marina*, 55: 39-48.

Articles in press

Morou, E., Lirakis, M., Pavlidi, N., Zotti, M., Nakagawa, Y., Smagghe, G., Vontas, J., and Swevers, L. (2013). A new dibenzoylhydrazine with insecticidal activity against Anopheles mosquito larvae. *Pest Manag. Sci.* (In Press). (IF = 2.251)

De Wilde, R., Swevers, L., Soin, T., Christiaens, O., Rougé, P., Cooreman, K., Janssen, C.R., and Smagghe, G. (2013). Cloning and functional analysis of the ecdysteroid receptor complex in the opossum shrimp Neomysis integer (Leach, 1814). *Aquat. Toxicol.* (In Press). (IF = 3.761)

Farrell, P., Kucknoor, A.S., Iatrou, K., Gedamu, L. (2013). A simple technique to enhance the humoral immune response to intracellular protein antigens in genetic immunizations. *J. Immunol. Meth.* 387, 308-311. (IF=2,203)

Ignatiades, L. and Gotsis-Skretas, O. (2013). The contribution of rare species to coastal phytoplankton associations. *Marine Ecology*, In Press (IF=1,836)

Presentations at Scientific Conferences

A. Kolliopoulou, K. Iatrou, and L. Swevers (2012). Functional Genomics of dsRNA-Mediated Gene Silencing in Transfected Silkmoth-derived Bm5 Cells. 6th Annual Arthropod Genomics Symposium: Arthropod Genomics 2012: Taking Center Stage and i5k Community Workshop, Kansas City, Missouri, USA, May 30 - June 2, 2012.

A. Kolliopoulou, J. Liu, H. Huvenne, K. Iatrou, G. Smaghe and L. Swevers (2012). Dissection of the function of the RNAi response in the silkmoth and the silkmoth-derived Bm5 cell line. 13th International Conference on Invertebrate and Fish Cell Culture, 2012 World Congress on In Vitro Biology, Bellevue, Washington, USA, June 3-7 2012.

A. Kolliopoulou, K. Iatrou, J. Sun and L. Swevers (2012). Interactions between cypovirus infection and the RNAi machinery in silkmoth and silkmoth-derived Bm5 cells. 22nd IUBMB & 37th FEBS Congress, Sevilla (Spain), September 4th-9th.

A. Kolliopoulou, K. Iatrou, and L. Swevers (2012). Small RNA pathways in *Bombyx mori*: an *in vitro* and *in vivo* approach. 22nd IUBMB & 37th FEBS Congress, Sevilla (Spain), September 4th-9th.

Ferreira, J.G., Andersen, J.H., Borja, A., Bricker, S.B., Camp, J., Cardoso da Silva, M., Garses, E., Heiskanen, A.S., Humborg, C., Ignatiades, L., Lancelot, C., Menesquen, A., Tett, P., Hoepffner, N. and Claussen, U., 2012. Is there life after guidance? Developments on the Marine Strategy Framework Directive Eutrophication Descriptor. Symposium on Research and Ecosystem-Based Management Strategies in Support of the Marine Strategy Framework Directive. Copenhagen, Denmark, 14-16 May, 2012.

P. Tsitoura and K. Iatrou (2012). In search of new repellents for reducing the spread of mosquito-borne infectious diseases: the case of Anopheline odorant receptors and malaria. International Conference on Chemistry for Health, Athens, Greece, September 9-14, 2012.

Swevers, L., and Iatrou, K. Insect cell-based expression technologies as tools for structural and functional analysis of eukaryotic proteins. 1^o FP7-SEE-DRUG Workshop. From chemical to systems biology: Peptide Synthesis and Protein Production. University of Patras, 9-10 May.

Ioannidis K., Swevers L., Iatrou K. Characterization of a *lef8* knock-out BmNPV: functional elements and "rescue" implications. 63^o Panhellenic Conference HSBMB, 9-11 November, Herakleion, Crete.

Kolliopoulou A., Iatrou K., Swevers L. Functional analysis of the RNAi response in transfected silkmoth-derived Bm5 cells. 63^o Panhellenic Conference HSBMB, 9-11 November, Herakleion, Crete. (oral presentation).

Kolliopoulou A., Iatrou K., Sun J., Swevers L. Persistent cypovirus infection in silkmoth larvae: Interactions with the RNAi machinery. 63^o Panhellenic Conference HSBMB, 9-11 November, Herakleion, Crete.

Kontogiannatos D., Swevers L., Iatrou K., Kourti A. Evaluation of transient gene knockdown techniques in the Lepidopteran non model insect *Sesamia nonagrioides*. 63^o Panhellenic Conference HSBMB, 9-11 November, Herakleion, Crete.

Tsitoura P., Koussis K., Amaral Psarris A., Iatrou K. (2012). On the hunt for new insect repellents: the development of a cell-based screening platform with mosquito odorant receptors as molecular targets. 63rd Congress of the Hellenic Society for Biochemistry and Molecular Biology, October 9-11, 2012, Heraklion, Greece.

Kythreoti G., Koussis K., Tsitoura P., Amaral-Psarris A., Tsitsanou K.E., Drakou C.E., Zographos S.E., Thireou T., Eliopoulos E., Kröber T., Guerin P., Iatrou K. (2012). Structure-based design for novel mosquito repellents: correlations between ligand affinities for *Anopheles gambiae* odorant binding proteins, olfactory receptor signaling and *in vivo* behavioral responses. 63rd Congress of the Hellenic Society for Biochemistry and Molecular Biology, October 9-11, 2012, Heraklion, Greece.

Other Scientific Activities

Invited speaker, Department of Biochemistry and Molecular Biology, University of Calgary, Canada "Mosquito olfaction as target for malaria transmission control" 17 May 2012. (K. Iatrou)

Invited speaker, 1st FP7-SEE-DRUG Workshop. From chemical to systems biology: Peptide Synthesis and Protein Production. University of Patras, Departments of Chemistry and Pharmaceutical Medicine. 9-10 May. Title: "Insect cell-based expression technologies as tools for structural and functional analysis of eukaryotic proteins". (L. Swevers)

Invited speaker, 13th International Conference on Invertebrate and Fish Cell Culture, 2012 World Congress on In Vitro Biology, Bellevue, Washington, USA, June 3-7 2012. Title: "Dissection of the function of the RNAi response in the silkworm and the silkworm-derived Bm5 cell line" (L. Swevers).

Member, Steering Committee, International Lepidopteran Genome Project Consortium (K. Iatrou).

Editor of "The Journal of Insect Science" (K. Iatrou)

Member of the Editorial Board of the Scientific Journals: "Sericologia", "Insect Biochemistry and Molecular Biology", "Archives of Insect Biochemistry and Physiology", "Open Biotechnology Journal", "Current Biotechnology" and "BioMed Research International" (formerly Journal of Biomedicine and Biotechnology) (K. Iatrou)

Member of the Editorial Board of the Scientific Journals: «Archives of Insect Biochemistry and Molecular Biology» and «Journal of Insect Science». (L. Swevers)

Member of the Editorial Board of the Scientific Journal: Mediterranean Marine Science Journal. (L. Ignatiadou)

Reviewer of articles for the scientific journals: "Insect Biochemistry and Molecular Biology", "Insect Molecular Biology", "Journal of Insect Science", "Journal of Insect Physiology", "Journal of Biomedicine and Biotechnology", "Molecular Biology Reports", "Journal of Virological Methods", "Current Biotechnology", "Pakistan Journal of Scientific & Industrial Research", "PLoS ONE", "Acta Biochimica Biophysica Sinica", "BioMed Research International", "Applied Environmental Microbiology", "Comparative Biochemistry and Physiology" "Biological Chemistry". (K. Iatrou)

Reviewer of articles for the scientific journals: "Pest Management Science" (6x), "PLoS ONE" (6x), "Journal of Insect Physiology" (3x), "Archives of Insect Biochemistry and Physiology" (2x), "Journal of Insect Science" (2x), "Comparative Biochemistry and Physiology B", "In Vitro Cellular and Developmental Biology - Animal", "Insect Molecular Biology", "Insect Science", "Molecular Biology Reports". (L. Swevers)

Reviewer of articles for the scientific journals: "Journal of Insect Biochemistry and Molecular Biology", "Journal of Insect Science" (2X), "Journal of Insect Physiology", "Insect Molecular Biology". (V. Labropoulou)

Reviewer of articles for the scientific journals: Mediterranean Marine Science Journal, Chinese Journal of Oceanography, Journal of the Marine Biological Association of U.K., Estuarine Coastal and Shelf Science, Journal of Marine Systems (L. Ignatiadou)

Member of the Committee for Recruitment of Two External Collaborators for the Scientific Program «RASTAS-SPEAR: RADIation-Shapes Thermal protection investigAtionS for high-SPEed EArth Re-en-try» (Institute of Material Sciences, NCSR "Demokritos") (L. Swevers).

Establishment of a laboratory for the provision of specialized scientific and technological services and products with title: "Biotechnological Products and Services". (K. Iatrou)

Visiting Scientist at the Faculty of Bioscience Engineering, Department Crop Protection, Ghent University, Ghent, Belgium. Period: December 2012. Participation in the Research Project: "Virus-induced mechanisms regulating RNAi in insects" (FWO – Vlaanderen G028013N). Scholarship Foreign Researcher, Special Research Fund, University of Ghent (L. Swevers)

Member of the promotions committee for research personnel of the BSRC "Alexander Fleming", 28/5/2012 (K. Iatrou)

External Examiner of PhD Thesis in Biological Sciences "Insights into the Structure and Function of an Insect Olfactory Receptor Using FRET" by Pablo German, School of Graduate Studies, The University of Auckland, New Zealand (thesis defence examination June 15, 2012) (K. Iatrou).

Member of PhD evaluation committee (External Examiner) June 2012: Faculty of Bioscience Engineering, Department Crop Protection, Ghent University, Ghent, Belgium. (L. Swevers): PhD thesis of Ellen De Geyter: "Toxicity and mode of action of steroid and terpenoid secondary plant metabolites against economically important pest insects in agriculture" (Applied Biological Sciences). And PhD thesis of Yves Verhaegen: "Mode of action, concentrations and effects of tributyltin in common shrimp *Crangon crangon*" (Applied Biological Sciences).

Scholarship (A. Koliopoulou): from the Hellenic Society for Biochemistry and Molecular Biology (HS-BMB) for participation at the 63^o Panhellenic Conference HSBMB, 9-11 November, Herakleion, Crete (travel expenses) (L. Swevers).

Scholarship (A. Koliopoulou): from the Federation of European Biochemical Societies (FEBS) for participation at the 12^o Young Scientists Forum and the 22^o IUBMB/ 37^o FEBS conference (travel and board expenses and registration at the conference) (L. Swevers).

Educational Activities

Ph.D. thesis defence of T. Georgomanolis at the Department of Biology of the National University of Athens. Thesis title: "Characterization of protein interactions and functional role of the SH3 protein of *Bombyx mori* (supervisors K. Iatrou and L. Swevers).

One hour lecture entitled "The olfactory process of mosquitoes as target for the control of the spread of malaria and other infectious diseases" at the summer school of NCSR "Demokritos" (K. Iatrou).

Aromatic plants: sources for new natural insect repellents for control of infectious diseases. Poster at the "Researcher's Night 2012", National Hellenic Research Foundation, 28 September 2012. (K. Iatrou)

One hour lecture entitled "The RNAi mechanism in lepidopteran insects: can it be exploited for insect pest control" at the summer school of NCSR "Demokritos" (L. Swevers).

Other activities for the Institute of Biosciences & Applications

Supervision of Ph.D. thesis of Konstantinos Ioannidis, IB graduate student (University of Athens) (K. Iatrou, L. Swevers)

Supervision of Ph.D. thesis of Anna Kolliopoulou, IB graduate student (University of Athens) (L. Swevers)

Member of the Internal Committee for supervision of graduate students with scholarship from NCSR "Demokritos" at the IB-A: Konstantinos Ioannidis, Anna Kolliopoulou, Maria Papakonstandinou (K. Iatrou/L. Swevers/V. Labropoulou).

Responsible for the functioning of the following instruments: Fluostar Microplate Fluorometer, HPLC Hewlett Packard, microplate luminometer TECAN InfiniTE M-200 (L. Swevers).

Impact Factor (for 6 publications): 13,731

Citations for 2012 (without self-citations): 357

Iatrou K.: 134

Swevers L.: 114

Labropoulou V.: 37

Ignatiadou, L.: 72

Total Citations 2008-2012 (without self-citations): 1359

Iatrou K.: 475

Swevers L.: 325

Labropoulou V.: 231

Ignatiadou L.: 328

h-factor:

26 (K. Iatrou)

17 (L. Swevers)

9 (V. Labropoulou)

17 (L. Ignatiadou)

Laboratory and Common Equipment (IB-A)

Cell culture: incubators, bio-reactors, laminar flow, inverted fluorescence microscope, microcentrifuges with cooling.

Incubators for rearing of insects (silkworm) in controlled environment.

Production of proteins: affinity chromatography, purification of antibodies

Biochemistry and molecular biology: DNA, RNA and protein electrophoresis, microcentrifuges, DNA sequencer (IB-A), ultracentrifuges (IB-A), Nuclear Magnetic Resonance (NMR) spectrometer (IB-A), sonicator (IB-A), micro-spectrometer, speedvac concentrator (IB-A), scintillation counter (IB-A), HPLC.

Cell Biology: automatic cell sorter (IB-A), fluorescence microscope, confocal microscope (IB-A), high-throughput screening for bio-active substances and protein interactions: microplate reader for fluorescence and luminescence (for GFP, rhodamin, fluorescein, β -galactosidase, luciferase and other photo-proteins) and studies of protein interactions by fluorescence resonance energy transfer (FRET) (IB-A).

Current External Funding

Research Program "Thalis", Strengthening of inter-scientific and inter-institutional research and innovation with possibility of attraction of high quality scientists from abroad through conduction of basic and applied research of excellence entitled *Genomic and functional approach to understand the*

resistance of insects and mites against insecticides and development of applications for its management, funded by GSRT with Scientific Coordinator Prof J. Vontas, University of Crete. Coordinating Scientist for research team at NCSR "Demokritos" [K. Iatrou (team coordinator), L. Swevers, V. Labropoulou, M. Konstantopoulou].

Duration: 1/9/2012-31/8/2015

Total funding (laboratory): 90.000 €

Laboratory funding for 2012: 10.000 €.

Program FWO – Vlaanderen G028013N (Belgium) entitled *Virus-induced mechanisms regulating RNAi in insects* and coordinator for the Greek participation Dr. L. Swevers.

Duration: 1/2013-12/2016

Coordinator: G. Smagghe (Belgium)

Total funding: 300.000 €

Total laboratory funding: 0 €

Bilateral S & T Cooperation (Greece-Slovakia) entitled *New Approaches for Insect Transformation*, funded by GSRT with Coordinator Dr. L. Swevers.

Duration: 1/2013-12/2014

Total funding (laboratory): 15.000 €

Laboratory funding for 2012: 0 €.

Project entitled *ENAROMaTIC - European Network for Advanced Research on Olfaction for Malaria Transmitting Insect Control*, funded by the European Union with Scientific Coordinator K. Iatrou.

Duration: 12/2008-12/2012

Total funding (consortium): 2.500.000 €

Total funding (lab): 563.000 €

Funding of the lab for 2011-12: 219.217 €

Note: The following proposal has also been submitted – decision pending:

Operational Program "Training and Life-Long Learning", Action "Excellence": "Cryptic dsRNA virus infection in the silkworm: implications for RNAi efficiency". Coordinator: L. Swevers. Requested laboratory funding: 361.600€.

Research Group: **Chemical Ecology and Natural Products, in collaboration with the Laboratory of "Insect Molecular Genetics and Biotechnology", Prof. K. Iatrou**

Research Staff

Maria Konstantopoulou, Senior Researcher

Rafaela Panteleri, Undergraduate Student

Anastasia Pantazi – Mazomenou, Research Technician (*retired*)

Research Interests

- Chemical ecology: isolation and identification of biologically active compounds, relating to insect chemical communication and plant – insect interactions (pheromones, volatile compounds of plant origin etc.) that may be used in integrated pest management programs.
- Isolation and identification of secondary metabolites (mainly of plant origin) acting on insect physiology and/or behavior (behavior modifying agents - infochemicals). Laboratory and field evaluation of bioactivity of the isolated metabolites; study of their mode of action.
- Development of specialized dispensers for chemical attractants/repellants for insects and technologies for their application in pest population control. Biodegradable materials endowed with controlled-release rate and UV protection properties.
- Biochemistry of insect olfactory receptors with emphasis on the localization and isolation of protein receptors for semiochemicals.
- Endosymbiotic bacteria of insects: Isolation and studies on their mutualistic relations with host insects.
- Microorganisms and Biotechnology: Isolation of naturally occurring microorganisms and their biologically active secondary metabolites (toxins) aiming to their incorporation in insect population management.

2012 Findings

- In the context of the EC funded project ENAROMaTIC (collaboration with the Laboratory of "Insect Molecular Genetics and Biotechnology", Prof. K. Iatrou), the collection of endemic botanical species was continued and our collection comprises of plant tissues (stems, leaves, flowers) from over 300 species belonging to 68 plant families. Samples were steam distilled in a modified Clevenger apparatus and their essential oils were extracted. All crude extracts (320) were tested on a high throughput screening assay for *Anopheles gambiae* OBPs ligand identification and olfactory receptors. Extracts exhibiting positive response were fractionated by means of solid phase extraction using increasing polarity solvents. Fractions were evaluated for biological activity. Gas chromatography was employed to identify differences in eluates between crude extracts and their fractions. Tentative identification of biologically active compounds was performed using Mass Spectroscopy. Four compounds positively identified have shown biological activity both in laboratory and in small scale field experiments. The same compounds and their binary and ternary combinations were evaluated under field conditions in Nigeria and exhibited biological activity superior to DEET (N,N-Diethyl-meta-toluamide), a widely used insect repellent.
- Participation in the research project "Thales" (collaboration with the Laboratory of "Insect Molecular Genetics and Biotechnology", Prof. K. Iatrou). The project aims to study the induced resistance to insecticides of two major pests of plants of economic importance, the white fly *Bemisia tabaci* and the rust mite *Tetranychus urticae*. The interactions of proteins responsible for the development of resistance in these two pests with chemical compounds of plant origin will be evaluated.
- Development of methods for the encapsulation and microencapsulation of semiochemicals in new materials and matrixes, non-toxic and environmentally friendly, endowed with improved release rate

and UV protection properties for the improvement of the efficiency and longevity of semiochemical dispensers in the context of biotechnical methods for the control of pests of economic importance.

- Development of Integrated Pest Management methods against pests of major economic importance as the extremely polyphagous *Metcalfa pruinosa*, the cryptic wood-borer moth *Zeuzera pyrina* in olive orchards in Egypt, the tomato leaf miner *Tuta absoluta* and the red palm weevil *Rhynchophorus ferrugineus*. These methods depend on the use of biorational insecticides, entomopathogenic nematodes, entomopathogenic fungi and the use of semiochemicals. In this framework the use of new kairomones as attractants and the amelioration of already used dispensers are also evaluated.
- In addition, the insecticidal and mutational properties of chemicals of plant origin on Diptera were studied.

Publications

Hegazi E., W. Khafagi, A. Herz, M. Konstantopoulou, S. Hassan, E. Agamy, G. Abd El-Aziz, S. Ali, S. Showeil (2012). Dispersal and field progeny production of *Trichogramma* species released in olive orchard in Egypt. *Biocontrol* 57: 481-492.

Hegazi E.M., M.A. Konstantopoulou, W.E. Khafagi A. Herz, D.G. Raptopoulos, E. Agamy, G. Abd El-Aziz, S. E. Ali, S. Abdel-Rahman (2012). Oviposition behavior of *Palpita unionalis* in different olive varieties. *Phytoparasitica* 40:451-459.

Kimbaris A.C., G Koliopoulos, A. Michaelakis, and M.A. Konstantopoulou (2012). Bioactivity of *Dianthus caryophyllus*, *Lepidium sativum*, *Pimpinella anisum*, and *Illicium verum* essential oils and their 6 major components against the West Nile vector *Culex pipiens*. *Parasitology Research* 111:2403-2410.

Presentations at Scientific Conferences

E. Hegazi, F. Schlyter, W. Khafagi, A. Atwa, M. Konstantopoulou, D. Raptopoulos (2012). The leopard moth borer, *Zeuzera pyrina* and olive varieties in Egypt. 42nd annual meeting of the Ecological Society of Germany, Austria and Switzerland, 10-14 September, Luneburg University, Germany.

E. Hegazi, F. Schlyter, W. Khafagi, A. Atwa, M. Konstantopoulou, D. Raptopoulos (2012). Interaction between the leopard moth borer and olive varieties: Associational Resistance at work. Meeting of the IOBC-WPRS Group "Pheromones and Other Semiochemicals in Integrated production": Bursa, Turkey October 1-5, 2012. Abstract: 93

Panteleri R., Lioulia E., Koutsonikou C., Mademtzoglu D., Mavragani-Tsipidou P. and Konstantopoulou M. 2012. Evaluation of the insecticidal and genotoxic activity of the essential oil of the plant *Pimpinella anisum* and its major constituent trans-anethole. 34ou Meeting of Hellenic Society for Biological sciences, Trikala 17-19 May. Abstract: 188-189.

Educational activities

M. Konstantopoulou, K. Koussis, P. Tsitoura, G. Kythreoti, A. Amaral, L. Swevers, V. Labropoulou, K. Iatrou (2012). Aromatic plants: sources for new natural insect repellents for control of infectious diseases. Poster at the "Researcher's Night 2012", National Hellenic Research Foundation, 28 September 2012.

Other Scientific Activities

Member of the supervising committee for Diploma of undergraduate student in the Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki (Assoc. Prof. P. Tsipidou-Mavragani).

Reviewer of the following international scientific journals: Chemosphere, Journal of Agricultural and Food chemistry, Entomologia Experimentalis et Applicata, Bulletin of Insectology, Journal of Applied Entomology, Crop Protection, Insect Science, Psyche: A Journal of Entomology Journal of Pest Science, Bulletin of Entomological Research, Pest Management Science, and Journal of Chromatography B.

Member of the editorial board of the scientific journal "*Tunisien Journal of Plant Protection*" specialist for the Chemical Ecology issues.

Member of the Cultural and Environmental Committee of the Board of NCSR "Demokritos".

Member of the Administrative and Personnel Management Committee of the Board of NCSR "Demokritos".

Member of the evaluation committee for recruitment of three external co-workers for the Institute of Nuclear and Particle Physics in the framework of an international project funded by the European Commission.

Member of the evaluation committee for the open public tender, to award the most financially advantageous offer for the procurement of a customized Ultra High Vacuum Scanning Probe Microscope (SPM), at the Institute for Advanced Materials, Physicochemical Processes, Nanotechnology & Microsystems of the NCSR "Demokritos".

Member of the acceptance committee for the contract on the «Supply of licensing of electromagnetic simulation software CST STUDIO SUITE» at the Institute for Informatics and Telecommunications of the NCSR "Demokritos".

Other activities for the Institute of Biosciences & Applications

Responsible for radioprotection of the radioactive source Co-60, with activity 5470 Ci (March 2004-).

Impact Factors (for 3 publications): 4,963

Citations 2012 (without self-citations): 28

Total Citations 2008- 2012 (without self-citations): 115

h-factor: 8

Laboratory Equipment

The laboratory is adequately equipped for analysis and identification of chemical compounds: gas chromatograph (GC), high pressure liquid chromatography (HPLC) fitted with an up to four solvent mixing system and supported by the appropriate software for recording and processing results. In addition various distillation devices, chromatography columns, a UV apparatus, an ultrasound apparatus and a solid phase microextraction (SPME) system are available.

Insect rearing units (including CT room).

Wind tunnel for studies of insect behavior under the effect of various chemical *stimuli*.

Stereoscope equipped with a camera, Incubators, laminar flow hood.

Protein purification and isolation: protein electrophoresis, western blot, centrifuge.

Current External Funding

Participation in the EU Research project entitled *ENAROMaTIC - European Network for Advanced Research on Olfaction for Malaria Transmitting Insect Control* (Coordinator: Prof. K. Iatrou).

Duration: 12/2008-12/2012

Total project funds: 2.500.000 €

Total funds for the Coordinator's laboratory: 563.000 €

Funds (2012) for the laboratory derive through the total Coordinator's funds

Participation in the action "Innovation Vouchers for Small and Medium Enterprises", Secretariat for Research and Technology (GSRT) of the Ministry for Education Lifelong Learning and Religion: "*Development of ecological and innovative technologies for the population management of pine processionary moth, *Thaumetopoea pityocampa*, in urban and suburban environments*" (Coordinator Dr. M. Konstantopoulou).

Duration: 4/2011-8/2011

Total funding (lab): 7.000 €

Funding of the lab for 2012: 7.000 €

Participation in the action "Innovation Vouchers for Small and Medium Enterprises", Secretariat for Research and Technology (GSRT) of the Ministry for Education Lifelong Learning and Religion: "*Research and development of innovative tools for palm pest management, *Rhynehophorous ferrugineus* (Coleoptera: Curculionidae)*" (Coordinator Dr. M. Konstantopoulou).

Duration: 6/2011-10/2011

Total funding (lab): 7.000 €

Funding of the lab for 2012: 7.000 €

Research Program "Thalis", Strengthening of inter-scientific and inter-institutional research and innovation with possibility of attraction of high quality scientists from abroad through conduction of basic and applied research of excellence: "*Genomic and functional approach to understand the resistance of insects and mites against insecticides and development of applications for its management*" Scientific Coordinator: J. Vontas, University of Crete. Coordinating Scientist for research team at NCSR "Demokritos" [K. Iatrou (team coordinator), L. Swevers, V. Labropoulou, M. Konstantopoulou].

Duration: 1/9/2012-31/8/2015

Total funding: 90.000 €

Funds (2012) for the laboratory derive through the total Coordinating Scientist for research team at NCSR "Demokritos funds.

Research Group: **Chronobiology**

Research Staff

Anastassia Prombona, Senior Researcher
Anastasia Repouskou, Postdoctoral Fellow
Aggeliki Galeou, Graduate Student

Research Interests

Investigation of the biological clock function in plants

Study of rhythmically expressed genes in *Phaseolus vulgaris*. Regulation of genes involved in the synchronization of the circadian clock by light signals and photoperiodism. Oscillator function in *Phaseolus vulgaris*.

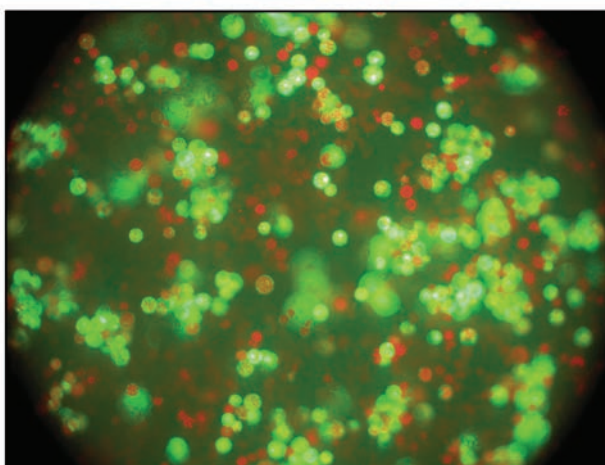
Involvement of the biological clock function in metabolism and carcinogenesis

Regulation by the circadian clock of liver metabolic pathways and the metabolic syndrome.
Regulation by the biological clock of cell cycle/proliferation in mouse fibroblasts and cancer cell lines.
Study of modulated acetylation levels during the application of drugs on the biological clock function and the cell cycle.

2012 Findings

Investigation of the biological clock function in plants

Experiments of A. Galeou aimed at exploring the light resynchronization of the *Phaseolus vulgaris* clock by light signals during the night, under the photoperiod of long night (6 hour light/18 hour dark). The study of the responsiveness of a set of clock genes showed that the evening specific genes (*PvTOC1*, *PvELF4*) are involved in resynchronization at the beginning of the night, when they reach the peak phase of their rhythmic expression. Later at night resynchronization is achieved by the acute response of the morning phased gene (*PvLHY*), when it is at the ascending part of the expression rhythm.



Protoplasts (plant cells after the enzymatic removal of the cell wall) isolated from bean primary leaves that express the green fluorescent protein (GFP). Excitation by UV/blue light (450-490 nm) results in the green to orange color of transformed protoplasts (merging of green fluorescence of GFP and red fluorescence of chlorophyll) and the red color of the untransformed protoplasts (fluorescence of chlorophyll). Variation in the expression levels of GFP in different cells results in colors from intensive green to orange.

In order to explore the molecular mechanism that regulates these responses, the promoter regions of approx. 2000 bases length of the morning specific *PvLHY* and the evening specific *PvTOC1* were isolated. The cloning of these and of truncated fragments in a transient expression plant vector with Luciferase as reporter is in progress. The promoters' activity will be tested in *Phaseolus* protoplasts that by the application of our protocol reach a transformation efficiency of ~80%. Testing of rhythmic expression of *PvLHY* and *PvTOC1/PvELF4* showed that protoplasts possess a functional circadian clock before and after transformation.

Involvement of the biological clock function in metabolism

Dr. A. Repouskou explores as a Postdoctoral Researcher "The role of acetyltransferase Tip60 in the circadian regulation of genes that are involved in metabolism and the metabolic syndrome" in a collaboration program (Support of Postdoctoral Researchers, ESPA 2007-2013) in the laboratory of Prof. G. Eichele, MPI for Biophysical Chemistry, Goettingen, Germany (years 2012-2013). The project involves the production of transgenic mice with liver specific deletion of Tip60 and the identification of rhythmic genes that are deregulated *in vivo* by this tissue specific deletion. In microarrays several genes were identified, that are under study. In addition, the identification of lysine-acetyltransferase substrates of Tip60 that relate to central clock function is in progress.

Publications

Angeliki Galeou, Anastasia Prombona (2012). Light at night resynchronizes the evening-phased rhythms of *TOC1* and *ELF4* in *Phaseolus vulgaris*. *Plant Science* 184, 141–147

M. Xydous, K.E. Sekeri-Pataryas, A. Prombona, T.G. Sourlingas (2012). Nicotinamide treatment reduces the levels of histone H3K4 trimethylation in the promoter of the *mper1* circadian clock gene and blocks the ability of dexamethasone to induce the acute response. *Biochimica et Biophysica Acta* 1819, 877–884.

Impact factor (for 2 publications): 7,796

Citations 2012 (without self-citations): 8

Total Citations 2008- 2012 (without self-citations): 35

h-factor: 5

Common Usage IB-A Equipment in the Laboratory (In charge: A. Prombona)

Thermal Cycler 2 blocks (Biorad)

Thermal Cycler (MJ Research)

Electroporator (BTX, ECM 399)

Hybridization Oven (Stuart Scientific)

Spectrophotometer (Hitachi)

French Press (Aminco)

Incubator 37°C (Gallenkamp)

Current External Funding

Programme entitled *Histone acetyltransferase Tip60 is a potential modulator of clock-controlled genes implicated in liver metabolism and obesity* funded by GSRT (Support of Postdoctoral Researchers, ESPA 2007-2013) with Postdoc Researcher Dr. A. Repouskou (in collaboration with the Laboratory "Genes and Behavior", Head: Prof. G. Eichele at the Max-Planck Institute for Biophysical Chemistry, Karl Friedrich Bonhoeffer Institute, Göttingen-Germany).

Duration: 22/2/2012-21/2/2015

Total funding of the lab: €150.000.

Funding of the lab for 2012: €30.000

Note:

Submitted research proposals:

1. «Aristeia II»

Title: The role of the circadian biological clock and chromatin remodelling in mood disorder

In collaboration with the Medical School of the Kapodistrian University of Athens(E.K.P.A.) and Dr. T.

Sourlingas, Inst. of Biosciences and Applications, Laboratory of Chromatin Function

Scientist in charge: Prof. D. Dikaios, Medical School, EKPA, 1st Psychiatric Clinic, Aiginiteion Hospital

2. «Aristeia II»

Title: Nuclear Imaging Technologies in Tomography

In collaboration with the Faculty of Physics and the Medical School of the Kapodistrian University of Athens (E.K.P.A.)

Scientist in charge: Associate Prof. E. Styliaris, Faculty of Physics, Department of Nuclear and High Energy Physics

Research Group: **Microbial Molecular Genetics**

Research Staff

Vassiliki Sophianopoulou, Research Director

Eleftherios Sideris, Emeritus Scientist

Christos Gournas, Postdoctoral Fellow

Ioannis Vaggelatos, Graduate Student

Alexandros Athanassopoulos, Graduate Student

Pavlos Geranios, Collaborating Graduate Student (*MSc*)

Iliana Zoi, Undergraduate Student

Interests

Our group is primarily interested in several aspects concerning i) the expression, trafficking, function and evolution of transport proteins and ii) the lateral compartmentation of plasma membrane. Our model organism of choice is the non-pathogenic ascomycetes *Aspergillus nidulans*, a classic model genetic system since the 1950's. The two last decades several *A. nidulans* transporters specific for amino acid transport have been cloned and studied in respect to their transcriptional, post-translational and cellular control of expression. The last 4 years punctate structures of the plasma membrane called eisosomes have been extensively studied in respect to their subcellular localization and function in *A. nidulans*.

A. Transporters of medical, pharmacological and agricultural importance

Recognition and transport of amino acid-neurotransmitters (proline, glutamate), through cellular membranes via specific transmembrane transporters

Activities:

a) identification and regulation of the expression of genes encoding amino acid transporters b) isolation and characterization of *trans* factors that regulate directly or indirectly the activity of amino acid transporters i.e. *trans*-acting molecular determinants involved in topogenesis/recycling/endocytosis/trafficking of amino acid transport systems (ER proteins, CKI kinases, FbA aldolases) c) studies on structure-function relationships of amino acid transporters and d) studies concerning the evolution of amino acid transporters in the Aspergilli.

Intermediate and long-term objectives: better understanding of the molecular basis of neurodegenerative diseases, possible identification of new pharmaceutical targets and future development of highly-targeted drugs.

B. Fungal membrane organization

Activities:

a) identification molecular characterization and cellular localization of eisosomal and eisosome-associated proteins involved in punctate lateral compartmentation of fungal plasma membrane b) functional characterization and c) structure-function analysis of eisosome and eisosome-associated proteins.

Intermediate and long-term objectives: knowing the role of specific fungal proteins, including those in contact with the cells of the host, is one of the strategies to develop very specific anti-mycotic drugs with little or no side effects on human patients, which is not the case with most of the first-line anti-mycotic drugs used today.

The principal current interest of the lab is to use *A. nidulans* as a model system for:

- a) Genetically and biochemically dissecting the **structure-function relationships** underlying **amino acid transporter** trafficking, function and specificity
- b) Identifying metabolic pathways and elucidate the molecular mechanisms involved in **trafficking and internalization/endocytosis** of specific transporters in response to various physiological, developmental and genetic signals
- c) Studying the role of **eisosomal proteins** in fungal membrane lateral organization in response to trafficking and endocytosis of specific transporters, cell cycle progression and animal/human infections

2012 Findings

A. Transporters of medical, pharmacological and agricultural importance

- 1) We identified *agtA*, a gene that encodes the specific dicarboxylic amino acid transporter of *Aspergillus nidulans*. The deletion of the gene resulted in loss of utilization of aspartate as a nitrogen source and of aspartate uptake, while not completely abolishing glutamate utilization. Kinetic constants showed that AgtA is a high-affinity dicarboxylic amino acid transporter and are in agreement with those determined for a cognate transporter activity identified previously. The gene is extremely sensitive to nitrogen metabolite repression, depends on AreA (a general nitrogen activator) for its expression, and is seemingly independent from specific induction. We showed that the localization of AgtA in the plasma membrane necessitates the ShrA protein and that an active process elicited by ammonium results in internalization and targeting of AgtA to the vacuole, followed by degradation (*see A. Apostolaki et al., 2012; <http://www.ncbi.nlm.nih.gov/pubmed/22489878>*).
- 2) In the context of structure-function relationships of APC transporters we constructed a new 3D imaging model of the major proline transporter PrnB of *A. nidulans*, based on the structural alignment of PrnB with the crystal structure of AdiC, the agmatine-arginine antiporter of *Escherichia coli* (**Gao et al., 2010. *Nature***).

The experimental confirmation of the model's predictions is currently underway. Site-directed substitutions of amino acid residues that are predicted to be important for specificity are performed. The mutant alleles, which are normally targeted at the plasma membrane, will be functionally, kinetically and physiologically analyzed.

In addition, cystein scanning mutagenesis will be performed in 23 residues of the 6th transmembrane segment (TMS6) of PrnB, which is predicted to be important for substrate binding. More specifically, in a fully functional cystein-less PrnB allele, single cystein substitutions of TMS6 have been constructed. NEM accessibility and substrate protection assays, as well as the effect of alkylation of these single cysteins on the transporter's kinetic profile, are expected to unravel the structure of TMS6 and its role in the translocation mechanism of YATs. (**C. Gournas and V. Sophianopoulou, unpublished results**)

B. Fungal membrane organization

We have studied *A. nidulans* core eisosomal proteins, stated to be involved in lateral compartmentation of fungal plasma membranes (*see I. Vangelatos et al., 2010 <http://www.ncbi.nlm.nih.gov/pubmed/20693301>*), which reveal striking differences in the assembly and developmental fate in two model organisms *A. nidulans* and *Saccharomyces cerevisiae* (*see C. Scazzocchio et al., 2011 <http://www.ncbi.nlm.nih.gov/pubmed/21509182>*) during the sexual cycle of *A. nidulans*. We have showed that the core eisosome proteins PilA, PilB and SurG are not expressed in hülle cells or early ascospores but are expressed in mature ascospores. All eisosomal proteins form punctate structures at the membrane

of late ascospores. In mature but quiescent ascospores, PilA and PilB form punctate structures at the plasma membrane. PilA structures are static and PilB are highly concentration at the areas where the two halves of ascospores are joined together. Finally, SurG was localized both at the membrane of ascospores and perinuclearly. In germlings originating from ascospores the punctate structures were shown to be composed only of PilA. PilB is diffused in the cytoplasm and SurG was located in vacuoles and endosomes. In germinated ascospores PilA foci did not colocalise with the highly mobile and transient peripheral punctate structures of AbpA, a marker for sites of clathrin-mediated endocytosis. Deletions of each one or all the three core eisosomal genes do not affect viability or germination of ascospores. In the presence of myriocin – a specific inhibitor of sphingolipid biosynthesis – PilA-GFP foci of ascospore germlings were less numerous and their distribution was significantly altered, suggesting a correlation between PilA foci and sphingolipid biosynthesis [see, A. Athanasopoulos et al., 2013 <http://www.ncbi.nlm.nih.gov/pubmed/23395641>]. Moreover, we showed, using *in vitro* site-directed mutagenesis, that Leucine 87 of PilA BAR-domain affects the membrane punctate localization of PilA. More precisely, substitutions of L87 with D and R resulted in the cytoplasmic distribution of PilA (P. Geranios and V. Sophianopoulou, unpublished results).

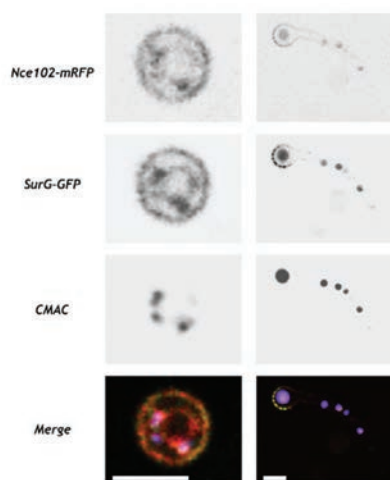


Figure 1: Cellular distribution of proteins involved in furrow-like nanoscale invaginations of *Aspergillus nidulans* plasma membrane. Images of confocal laser microscopy processed with the 3-D blind deconvolution method. Subcellular localization of Nce102 – a transmembrane protein- and SurG- a core eisosomal protein- tagged with mRFP and GFP, respectively. Bar 5 μ m.

Publications

Apostolaki, A., Harispe, L., Calcagno-Pizzarelli, AM., Vangelatos, I., Sophianopoulou, V., Arst Jr, A., Peñalva, MA., Sotiris, A., and Scazzocchio, C. (2012). *Aspergillus nidulans* CkiA is an essential casein kinase I required for delivery of amino acid transporters to the plasma membrane. **Mol. Microbiol.** 84(3):530-49. (IF 5) <http://www.ncbi.nlm.nih.gov/pubmed/22489878>.

Articles in Press

Athanasopoulos, A., Boleti, H., Scazzocchio, C., Sophianopoulou, V. (2013). Eisosome distribution and localization in the meiotic progeny of *Aspergillus nidulans*. **Fungal Genet. Biol.** (IF 3.8) (<http://www.ncbi.nlm.nih.gov/pubmed/23395641>).

Articles in Books and Conference Proceedings

Athanasopoulos A., Geranios P., Gournas C., Boleti H. and Sophianopolou V. (2012). Eisosomes in the sexual cycle of *Aspergillus nidulans*. International Conference on Membrane Domains, Dijon, France. Abstract book (on line).

Gournas C., Athanasopoulos A., Geranios P. and Sophianopolou V. (2012). Summer School NCSR «Demokritos» July 2012. Life Sciences: Plasma membrane function and organisation: the contribution of

model microbial organisms. Abstract book pages 119-120

Athanasopoulos A., Boleti H., Scazzocchio, C. and Sophianopolou V. (2013). Eisosome distribution and localization in the meiotic progeny of *Aspergillus nidulans*. 27th Fungal Genetics Conference, Asilomar Conference Center in Pacific Grove, California, USA. Abstract book pages 291-292.

Educational Activities

Four-hour lecture on "Microbial Biotechnology-Model Systems of Molecular Microbiology" a Post-Graduate Course at the Department of Biology, National University of Athens (V. Sophianopolou).

Four-hour lecture on Molecular Biology: Systemic and *in silico* Approaches", a Graduate Mandatory Course of the Interdisciplinary (Faculty of Biology and Medical School, National and Kapodistrian University of Athens {NKUA}, Athens, Greece) Two-Years Graduate Program "Applications of Biology in Medicine" (V. Sophianopolou).

Scientific seminar at IB-A: Structure-function relationships: Life after crystals: The example of the APC family (C. Gournas)

Summer School NCSR «Demokritos» July 2012. Life Sciences: Plasma membrane function and organization: the contribution of model microbial organisms (C. Gournas)

Bibliographic seminar: Reassessment of the role of plasma membrane domains in the regulation of vesicular traffic in yeast (A. Athanasopoulos).

Research seminar: Organization of protein microdomains in the fungal plasma membrane: study of the subcellular distribution of eisosomal proteins of *Aspergillus nidulans* (A. Athanasopoulos).

Discrimination- awards

1. Invitation to co-convene a concurrent session entitled «Membrane trafficking and molecular organization» of the 27th Fungal Genetics Conference in 2013, at the Asilomar Conference Center in Pacific Grove, California, USA (V. Sophianopolou)
2. Invitation to join the *Aspergillus* genome project (website EUFGEN). Project: species comparison of *Aspergillus* amino acid transporters (V. Sophianopolou)

Other Scientific activities

V. Sophianopolou

- Editorial Advisor Board Member of "The Open Mycology Journal" (Bentham Science Publishers)
- Referee for Molecular Membrane Biology and the Journal of Molecular Biochemistry
- Member of the Advisory Committee for the PhD thesis of I. Vangelatos, University of Ioannina
- Member of the Advisory Committee for the PhD thesis of A. Athanasopoulos, National and Kapodistrian University of Athens
- Member of the Advisory Committee for the MA thesis of P. Geranios, National and Kapodistrian University of Athens

Other Activities for the Institute of Biosciences & Applications

V. Sophianopolou:

- Member of the Scientific Consulting Board at IB-A (resignation 06/2010)
- President of the Education Committee at IB-A (until 07/2010)
- Member of the Education Committee at NCRS Demokritos (until 07/2010)
- Supervision of the PhD. thesis of the graduate student I. Vangelatos at the IB-A (University of Ioannina)

- Supervision of the PhD thesis of the graduate student A. Athanasopoulos at the IB-A (University of Athens)
- Supervision of the Master thesis of the graduate student P. Geranios at the IB-a (University of Athens)
- Supervision of Diploma Thesis of the undergraduate student V. Pantazopoulou at the IB-A (University of Athens). Completed 19/03/12
- Supervision of Diploma Thesis of the undergraduate student Ilianna Zoe at the IB (University of Athens).
- Deputy member of the Examination Committee for the recruitment or promotion of two Researchers C at the IB-A
- Deputy member of the committee responsible for receiving the IB materials from the regular budget of 2012

Impact Factor (for 1 publication): 5

Citations 2012 (without self-citations): 42

Total Citations 2008-2012 (without self-citations): 185

h-factor: 14

Laboratory Equipment

Power supply Consort E432 και E861, Gel Drier Heto GD1, Hybridization oven Stuart S1 20H, apparatus of Pulsed-Field Gel electrophoresis Rotaphor Type V computerized (Biometra), horizontal gel electrophoresis instrumentation (Consort και Pharmacia), vertical gel electrophoresis instrumentation (Boehringer-Ingelheim, Bioproducts BV 101), protein transfer instrumentation (Boehringer-Ingelheim, Bioproducts BB 100), Heat block Heto Chill Master Comfort CB 8-30E and miniplus 3 Gilson, UV Transilluminator UVP, INC TM-20, Analytical balance ADA71/L, BioDoc-It™ Imaging System UVP, Gradient PCR Takara TP600, Microflow Advanced Bio safety Cabinet ClassII, Refrigerated Centrifuge Kubota 7780 (V. Sophianopoulou).

Current External Funding

Project entitled *Structure-function relations of bacteria transporters and their eukaryotic homologues* funded by GSRT ("Thalis" as "Central Research Group"). Coordinator: S. Frillingos, University of Ioannina (Coordinator of Central Research Group: V. Sophianopoulou).

Duration: 2011- 2013

Total funding ~520.000 €

Total funding of the lab: ~78.000 €.

Funding of the lab for 2012: 25.000 €.

Project entitled *Development of genetic and genomic tools with Minos transposon and their applications in model organisms*, funded by GSRT ("Thalis" as as "Member of a Central Research Group"). Coordinator: Ch. Savakis, Biomedical Sciences Research Center Al. Fleming (Coordinating IB scientist: V. Sophianopoulou).

Duration: 2011- 2013

Total funding ~535.000 €

Total funding of the lab: ~20.000 €.

Funding of the lab for 2012: 6.500€.

Note: The following proposals have been submitted and are under evaluation

«Aristeia II» (GSRT-ESPA): Title: Molecular organization of plasma membrane in fungi and how is implicated in fundamental cellular processes and pathogenicity (Coordinator: V. Sophianopoulou).

Research Group: **Biophysics and Biotechnology of Membranes**

Research Staff

Kostas Stamatakis, Senior Researcher

George Papageorgiou, Emeritus Scientist

Meropi Tsimilli – Michael, Collaborating Scientist

Dimitris Vayenos, Collaborating Graduate Student (*MSc*)

Research Interests

The photosynthetic cyanobacteria are advisable candidates for the sequestration of large quantities CO₂ because they can grow in extreme or/and specified environmental conditions (temperature, pressure, salinity, pH, chemical composition) and are capable of binding CO₂ producing chemical compounds of high energy upon their exposure to sunlight. The usage of rapidly growing cyanobacteria constitutes a much promising source for biofuels production which can substitute natural sources of energy. The production and accumulation of sucrose in cyanobacteria is connected to their adaptation to extreme environmental conditions.

We study the time dependent changes (fluorescence induction) of chlorophyll a (Chl a) in cyanobacteria, giving emphasis to the role of the carotenoids as light harvesters and as distributor of the electronic excitation in the reaction centers of the photosystems (PS) I and II (PSI, PSII). On the contrary to the chlorophylls and the phycobilisomes (PBS), the light harvesting mechanism of the carotenoids and the provision electronic excitation by them in the reaction centers of the PSI, PSII has not been described with clarity until now. Our research focuses on the role of carotenoids in the balanced excitation of the reaction centers of PSI and PSII, so that they turn over at the same rate and the quantum yield of photosynthesis becomes maximized.

Technological applications by studying the fluorescence time dependent changes of the cyanobacterial chlorophyll Chl a. Usage of the induction constants of fluorescence (OJIP) as indicators antimicrobial activity

Study of the photosynthetic apparatus resulting Hydrogen (H₂) production.

2012 Findings

The oxidoreduction state of intersystem electron carriers (plastoquinones, Cyt b₆f) is the chemical signal that initiates the state transitions mechanism in cyanobacteria, which regulates the amount of electronic excitation (EE) delivered from peripheral antenna complexes to the core complexes of Photosystems (PS) I and II (PSICC, PSIIIC). We studied, in *Synechococcus* sp PCC 7942 cells, the impact of the hyperosmotic stress in the lighted excitation reallocation (State transitions). The Chl a fluorescence emission spectra at low temperature (77K) is a very useful tool for the exploration of the "State transitions", which answers to questions, if transitions occur and to which direction. The cells which have been adjusted to blue light (immediate excitation of Chl a, 436 nm) are directed to State 1, while the cells which have been adjusted to orange light (immediate excitation PBS, 620 nm) are directed to State 2. When the *Synechococcus* sp PCC 7942 cells float in a hyperosmotic medium, then the cells are "locked" in the State 2 independently from their adjustment light (blue or orange). Such cells that are "locked" in the State 2 are characterized by a reduced transportation of excitation from the PBS to the PS II and a strengthened transportation of excitation to the PS I, in comparison to the cells in hypo osmotic float.

Control of the antimicrobial activity of new textiles using Gram negative cyanobacteria as bacterial

control models. The constant "O" ("O") of the Chl a fluorescence changes against time constitutes an indicator of the cyanobacterial population variation.

Study of the time dependent photosynthetic production of H₂ by photosynthetic algae

Study of the thermodynamic stability of the ApoE3 protein. Its chemical denaturation using hydrochloric guanidine. The 3 steps model is used to calculate the changes of free energy according to Gibbs during the protein denaturation (Georgiadou, D., et al *Journal of Lipid Research* 54, 164-176).

Publications

Benaki, D., Stamatakis, K., Leondiadis, L., Mikros, E., Pelecanou, M. (2012). Spectroscopic investigation of the interaction of curcumin with the beta-amyloid peptide of Alzheimer's disease. *Journal of Peptide Science* 18, S197-S198

Kana, R., Komarek, O., Kotabova, E., Papageorgiou, G.C., Govindjee and Prasil O. (2012) Slow S to M rise in cyanobacteria is due to a state 2 to state 1 transition. *Biochim. Biophys. Acta-Bioenergetics* 1817, 1237-1247

Articles in Press

Georgiadou, D., Stamatakis K., Efthimiadou E.K., Kordas G., Gantz D., Chroni, A., Stratikos, E. (2013). Thermodynamic and structural destabilization of apoE3 by hereditary mutations associated with the development of lipoprotein glomerulopathy. *Journal of Lipid Research* 54, 164-176 (IF 4,917)

Articles in Books and Conferences Proceedings

Papageorgiou, G. C. (2012). Fluorescence emission from the photosynthetic apparatus. In *Photosynthesis: Plastid Biology, Energy Conversion and Carbon Assimilation* (J.J. Eaton-Rye, B.C. Tripathy and T.D. Sharkey, eds.), *Advances in Photosynthesis and Respiration* vol. 34, pp. 415-443. Springer Science+Business Media B.V. 2012

Papageorgiou G. C. (2012). Foreword Invited contribution to the volume "Photosynthesis: Overview of Recent Progress and Future Perspectives" (S. Itoh, P. Mohanty, K.N. Guruprasad, eds.). I. K. International Publishing House, Pvt., Ltd., New Delhi, India (2012). ISBN 9789 381 14 1007

Papageorgiou, G. C. (2012) *Photosynthetica* XX-YY-ZZ Invited Book Review of the following book: Nickelsen, K., Govindjee: *The Maximum Quantum Yield Controversy. Otto Warburg and the Midwestern Gang. – Bern Studies in the History and Philosophy of Science*, Bern 2011. ISBN: 978-3-9523421-9-0, paperback, 138 pp.

Presentations at Scientific Conferences

K. Stamatakis, M. Tsimilli-Michael, GC Papageorgiou (2011). Hyper-osmotic effects on the distribution of electronic excitation between the photosystem II and photosystem I core complexes. *Molecular Bioenergetics of Cyanobacteria: From Cell to Community*, April 10-15, 2011, Sant Feliu de Guixols, Spain.

D. Benaki, K. Stamatakis, E. Mikros, M. Pelecanou (2011). Curcumin and β - Amyloid Peptide: A promising interaction. *Biologically Active Peptides XII*, April 27 – 29, 2011, Praha, Czech Republic.

R Kaňa, O Komárek, E Kotabová, GC Papageorgiou, Govindjee, O Prášil (2011) Is the slow S to M fluorescence rise in cyanobacteria due to a state transition? *Gordon Research Conference on Photosynthesis*, Davidson, NC, USA, June 11-12, 2011

Stamatakis K., Tsimilli-Michael M., Papageorgiou G. C. (2011) On the question of the light-harvesting role of β -carotene in cyanobacteria 12th Congress Hellenic Botanical Society 29/9-2/10 Rethimno Crete.

Other Scientific Activities

Publication editing of scientific books:

Papageorgiou, G. C. "Of Light and Darkness: Modeling Photosynthesis 1840–1960" by historian Kärrin Nickelsen for publication by Springer. The recommendation was "publish."

G. C. Papageorgiou: Associate Editor & Editorial Board Member of *Photosynthetica*. To *Photosynthetica* (Springer)

Participation in the process of judging scientific proposals:

K. Stamatakis: Participation as an expert to certify the realization of the natural objective of the approved towards financing proposals titled: 'Support of Postdoctoral Researchers'

Referee in scientific journals:

K. Stamatakis: *Photosynthetica*, *FEBS letters*

G. C. Papageorgiou: *Biochim Biophys. Acta (Bioenergetics)*, *Photosynth. Research*, *Photosynthetica*, *Journal of Fluorescence*, κ.α.

Other Activities for the Institute of Biosciences & Applications

Member of Scientific Advisory Board of IB

Impact Factor (for 1 publication): 1,807

Citations 2012 (without self-citations): 22

Total citations 2008-2012 (without self-citations): 84

h-factor: 7

Laboratory Equipment and Common Usage Equipment (IB) (Allocated to K. Stamatakis)

- Cultures of photosynthetic microorganisms: Orbital incubator of controlled temperature and light. Orbital incubator Gallenkamp Model INR-400 (London, UK)
- Refrigerated Microcentrifuge. Sorvall RMC-14 Refrigerated Microcentrifuge (Sorvall Dupont USA)
- Dual wavelength absorption spectrophotometers
 - (i) Hitachi-557 dual wavelength absorption spectrophotometer (Hitachi, Tokyo, Japan)
 - (ii) Jasco double beam spectrophotometer UVIDEK 610 (JASCO Japan spectroscopic Co. LTD).
- Fluorometers:
 - I. Constant excitation in 650 nm with time analysis from 1 μ s, PEA-fluorometer (PEA, Hansatech, King's Lynn, Norfolk, UK)
 - II. Pulse excitation and fluorescence measurements (PAM; Heinz Walz, Effeltrich, Germany), and
 - III. Fluorescence spectra measurements Hitachi F-2500 spectrofluorometer (Hitachi High Technologies Corporation, Japan) – Equipment IB, supervisor: K. Stamatakis
- Oxygen measurement devise: Clark-type oxygen electrode (DW1; Oxygraph, Hansatech, King's Lynn, U.K.)

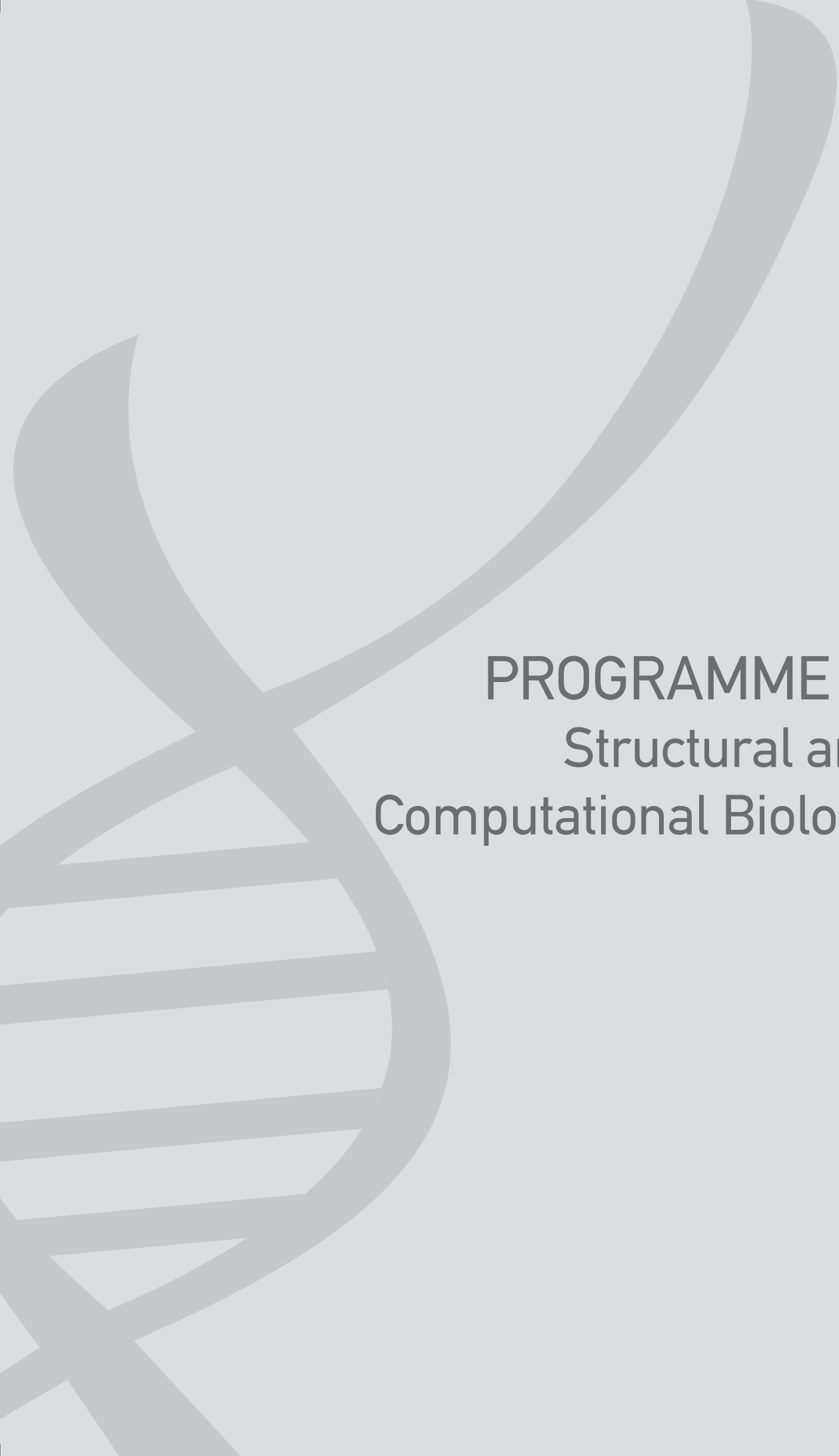
Current External Funding

Participation to the programme IRAKLEITOS II entitled *Green fruit's photosynthesis: correlation with the peculiarities of the internal microenvironment and their photoprotective and metabolic demands*, funded by the Greek Ministry of Education, Lifelong Learning and Religious Affairs (Coordinator: Ass. Professor Y. Petropoulou, Univ. of Patras)

Duration: 2009-2012

Total programme funding: 45.000€

Funding for the lab for 2012: 0€



PROGRAMME C:
Structural and
Computational Biology

Research Group: Theoretical Biology and Computational Genomics

Research Staff

Yannis Almirantis, Research Director

Spyros Papageorgiou, Emeritus Scientist

Konstantinos Apostolou – Karabelis, Graduate Student

Dimitris Polichronopoulos, Collaborating Graduate Student

Research Interests

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

- Deviations from randomness at the level of nucleotide n-tuplets. Patterns related to the functionality of genomic regions and to the global genome structure.
- Deviations from randomness at the “middle” length scale, expressed as clustering of similar nucleotides. Use of such approaches for the distinction of coding and non-coding segments.
- Long range correlations and Zipf laws in the genome structure. Power laws in the distribution of exons and of other genomic functional localizations.
- DNA sequences seen as genomic text – Linguistic features in the genome: redundancy – multiple coding – asymmetry etc.
- “Conservation laws” at the genome structure. The case of “Chargaff’s 2nd parity rule”. The use of deviations from this law in the study of genomic dynamics and evolution.
- Evolution at the genomic level. Formulation of minimal evolutionary scenarios compatible with the observed probabilistic features of genomes. Interpretation of the above mentioned probabilistic features either by selectionist or mutationist causality.

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

- Early development – Left-right asymmetries – Mechanisms of activation of Hox genes during limb development.
- Reaction-diffusion systems – Spontaneous symmetry breaking and pattern-formation in systems with feedbacks.
- Prebiotic and early evolution as a complex self-organization procedure.

2012 Findings

Conserved, ultra-conserved and other classes of constrained elements (collectively referred as CNEs here), identified by comparative genomics in a wide variety of genomes, are non-randomly distributed across chromosomes. These elements are defined by various degrees of conservation between organisms. We here investigate the chromosomal distribution of CNEs by means of the size distribution of inter-CNE distances. We find the widespread occurrence of power-law-like distributions, a feature which is associated with fractality and self-similarity. Given that CNEs are often found to be spatially associated with genes, especially with those that regulate developmental processes, we have verified, after appropriate gene masking, that a power-law-like pattern emerges irrespectively of whether elements found close or inside genes are excluded or not. An evolutionary model is put forward for the understanding of these findings that includes *segmental or whole genome duplication* events and *eliminations (loss)* of most of the duplicated CNEs. Simulations reproduce the main features of the observed size distributions. Power-law-like patterns in the genomic distributions of CNEs are in accordance with current knowledge about their evolutionary history in several genomes. Moreover, these distributional patterns in CNEs, as well as in other classes of genomic elements, are compatible with experimental results showing that chromatin is organized according to a “fractal globule” pattern.

Publications

Klimopoulos A., Sellis D., Almirantis Y. (2012). Widespread occurrence of power-law distributions in inter-repeat distances shaped by genome dynamics. *Gene* DOI: 10.1016/j.gene.2012.02.005
Papageorgiou S. (2012). Comparison of models for the collinearity of Hox genes in the developmental axes of vertebrates. *Current Genomics*, 13, 245-251.

Articles in Press

Almirantis Y. (2013). Homeopathy – at the edge between tradition and modern science: remedies as carriers of *significance*. *Homeopathy (in press)* (Impact Factor: 1,14)

Presentations at Scientific Conferences

G.Tsatsaronis, M.Schroeder, G.Paliouras, Y.Almirantis, I.Androutsopoulos, E.Gaussier, P.Gallinari, T.Artieres, M.R.Alvers, M.Zschunke. (2012). BioASQ: A Challenge on Large-Scale

Biomedical Semantic Indexing and Question Answering. *Information Retrieval and Knowledge Discovery in Biomedical Text: Papers from the AAAI Symposium*; Lana Yeganova, Rezarta Islamaj Dogan, Vahan Grigoryan, Mark Dredze, Cochairs November 2–4, 2012, Arlington, Virginia.

Other Scientific Activities

Participation in the organizing committee of the satellite meeting “Genomic Complexity” <http://geco2012.chem.demokritos.gr> held in the framework of ECCS' 12 Brussels, 3-7 Sept. 2012 <http://geco2012.chem.demokritos.gr> (Y. Almirantis).

Reviewer of scientific papers for: Entropy, Journal of Theoretical Biology, Computational Biology Journal (Y. Almirantis).

Talk given in the framework of the international series ‘Embryo Physics Course’, with subject: *A biophysical model can explain the multiscale phenomenon of collinearity of clustered HOX genes*. <http://embryogenesisexplained.com/2012/01/biophysical-model.html> (S.Papageorgiou).

Educational Activities

- Teaching (16H) of the course “An Introduction to Computational Genomics” in the framework of the “Post Graduate Specialisation Diploma” in Bioinformatics, Biology Department, U.O.A.
- Teaching (12H) of the course “An Introduction to Computational Genomics” in the framework of the Post Graduate Specialisation Diploma in Clinical Biochemistry and Molecular Diagnostics, Biology Department, U.O.A.

Other Activities for the Institute of Biosciences & Applications

President of the Scientific Advisory Board of the Institute (Yannis Almirantis)

Impact Factor (for 2 publications) : 2.55

Citations 2012 (without self- citations): 15

Total Citations 2008-2012 (without self- citations): 92

h-factor: 12

Current External Funding

Project entitled: *BioASQ: A challenge on large-scale biomedical semantic indexing and question answering*, funded by EE with Coordinator Dr G. Paliouras (II&T, NCSR “Demokritos”) participation from IB-A Y.

Almirantis, D. Polychronopoulos.

Duration: 2012-2014

Total programme funding: 1.270.000 €

Funding of the lab for 2012: 3.923 €

Research Group: **Structural Studies of Biomolecules and Pharmaceuticals with NMR**

Research Staff

Maria Pelekanou, Research Director

Marina Sagnou, Researcher

Demetra Benaki, Postdoctoral Fellow

Angeliki Panagiotopoulou, Technical Specialist

Myrta Kostomoiri, Graduate Student (MSc)

Mariliza Vassiliadou, Training Student

Research Interests

Studies of the **structure**, **interactions**, and **structure/activity** relationship of compounds of pharmacological interest for the diagnosis and/or therapy of various diseases.

Our efforts are focused on two major groups of molecules:

1. Bioactive organic compounds designed for targeted pharmacological action either on their own or after suitable labeling/complexation

The activity includes synthesis and/or chemical modification of pharmacophoric molecules, study of their structural characteristics, dynamic properties and interactions with biomolecules, the investigation of their coordination properties to metals, as well as the evaluation of their biological properties in appropriate model systems. In recent years, our interest is directed towards bioactive natural products with special focus on the investigation of new derivatives of curcumin as multi-modal agents for multi-targeted treatment of common ailments.

In an established and ongoing collaboration with the Institute of Nuclear and Radiological Sciences, Energy, Technology and Safety (INRASTES), our group is engaged in the development of novel complexes of rhenium and technetium for the diagnosis and/or therapy of prevailing diseases like cancer and Alzheimer's disease. Furthermore, in collaboration with the University of Athens dual-targeted anticancer complexes of platinum and palladium are developed.

2. Peptides

The activity includes studies with NMR and CD on the conformation and interactions of bioactive peptides with special focus on the β -amyloid peptide (β -AP) of Alzheimer's disease. A thorough examination of the structural transition of β -AP(1-40) to amyloid fibrils - the principal component of amyloid plaques that characterize the disease - and its interactions with compounds, like thioflavin T, oleuropein, curcumin, that may intervene in the toxic aggregation process, has been conducted. In addition, in collaboration with INRASTES, the structure-activity relationship of peptides of the neuroprotective family of Humanin is studied along with the development of specially designed labeled derivatives to be used in the investigation of the mechanism of action of this peptide family.

2012 Findings

In 2012 our on-going collaboration with INRASTES in the field of design, synthesis and biological evaluation of Re and ^{99m}Tc complexes for diagnostic applications in Alzheimer's disease and cancer have led to four scientific publications. In the area of Alzheimer's disease, a Re complex of 2-(4'-aminophenyl) benzothiazole was synthesized which exhibits selective binding to amyloid plaques and may therefore

be applied towards the development of a radiodiagnostic. In the area of cancer, a series of complexes of suitably modified dextrans was synthesized towards radioimaging of the sentinel node, the first site of tumor metastasis. These large-sized complexes which do not crystallize and cannot be characterized by MS spectrometry due to their polymeric nature, were successfully characterized with NMR. Moreover, NMR was used in the characterization of the neuroprotective peptide bombesin with the cyclam chelate designed to incorporate copper for PET diagnostic applications.

Our involvement with the natural product curcumin continued in 2012 with the study of its interaction with the β -amyloid peptide of Alzheimer's disease, the design and synthesis of new multimodal derivatives to improve its diverse pharmacological properties, and the development of potential Pgp inhibitors to reverse or diagnose multidrug resistance in cancer chemotherapy. In the area of natural products, and in collaboration with the Cell and Matrix Pathobiology laboratory, it was shown that oleuropein, the main antioxidant of olive oil, add the commas reduces the production and aggregation of β -AP in neuronal cell lines.

In parallel, in the area of peptides, labelled derivatives of the neuroprotective colivelin (CL) were synthesised as tools for the investigation of the CL mode of action with biochemical/immunochemical techniques and biodistribution experiments. Within this framework, the interaction of β -amyloid with CL was shown with immunochemistry and circular dichroism experiments offering a new perspective in the investigation of the mechanism of the neuroprotective action of CL.

Publications

Sagnou, M., Tzanopoulou, S., Raptopoulou, C. P., Psycharis, V., Braband, H., Alberto, R., Pirmettis, I. C., Papadopoulos, M., Pelecanou, M. * (2012). A phenylbenzothiazole conjugate with the tricarbonyl fac-M(I)(CO)³⁺ (M = Re, ⁹⁹Tc, ^{99m}Tc) core for imaging of β -amyloid plaques. *Eur. J. Inorg. Chem.*, **27**, 4279–4286.

Sagnou, M.* Mitsopoulou, K. P., Koliopoulos, G., Couladouros, E. A., Michaelakis, A.* (2012). Evaluation of naturally occurring curcuminoids and related compounds against larvae of *Culex pipiens*. *Acta Tropica*, **123**, 190-195.

Benaki, D., Zikos, C., Karachaliou, C.-E., Tsitsilonis, O., Leondiadis, L., Kalbacher, H., Voelter, W., Papadopoulos, M., Pirmettis, I., Pelecanou, M.*, Livaniou, E.* (2012). Complexes of an Alpha-thymosin derivative with 185/187Re and ^{99m}Tc: Structural analysis and initial biological evaluation. *Chem. Biol. Drug Des.* **80**, 545-553.

Pirmettis, I., Arano, Y, Tsoதாக, T., Okada, K. Yamaguchi, A. Uehara, T., Morais, M, Correia, J. D. G, Santos, I., Martins, M., Pereira, S., Kyprianidou, P., Triantis, C., Pelecanou, M., Papadopoulos, M. (2012). New ^{99m}Tc(CO)₃ mannosylated dextrans bearing S-derivatized cysteine chelator for sentinel lymph node detection. *Mol. Pharm.*, 1681-1692.

Makris, G., Karagiorgou, O., Papagiannopoulou, D., Panagiotopoulou, A., Raptopoulou, C. P., Terzis, A., Psycharis, V., Pelecanou, M., Pirmettis, I., Papadopoulos, M. S. (2012). Rhenium(I) and technetium(I) tricarbonyl complexes with [NⁿSO]-type chelators: Synthesis, structural characterization, and radiochemistry. *Eur. J. Inorg. Chem.*, 3132-3139.

Liolios, C. C., Zikos, C., Fragogeorgi, E., Benaki, D., Pelecanou, M., Pirmettis, I., Ioannidis, N., Sanakis, Y., Raptopoulou, C., Terzis, A., Boschetti, F., Papadopoulos, M., Sivolapenko, G., Varvarigou, A. (2012). A new bombesin-copper complex based on a bifunctional cyclam derivative. *Eur. J. Inorg. Chem.*, 2877-2888.

Chiotellis, A., Tsoukalas, C., Pelecanou M., Pirmettis I, Papadopoulos, M. (2012) Synthesis, characterization and biological evaluation of novel neutral fac-M(CO)₃(SNO) complexes (M=Re, ^{99m}Tc) bearing the o-methoxyphenylpiperazine moiety. *Appl. Radiat. Isotopes*, 70, 957-964.

Kostomoiri, M., Fragkouli, A., Sagnou, M., Skaltsounis, L. A., Pelecanou, M., Tsilibary, E. C., Tzinia, A. K., (2013). Oleuropein, an antioxidant polyphenol constituent of olive favors α -secretase cleavage of the amyloid precursor protein (APP). *Cell. Mol Neurobiol.*, 33 ,147-154.

Articles in Press

Giglio, J., Fernández, S., Jentschel, C., Pietzsch, H.-J., Papadopoulos, M., Pelecanou, M., Pirmettis, I., Paolino, A., Rey, A. (2013). Design and development of ^{99m}Tc "4+1" labelled dextrane-mannose derivatives as potential radiopharmaceuticals for sentinel lymph node detection. *Cancer Biother. Radio.* (IF 1.787)

Articles in Books and Conference Proceedings

Kostomoiri, M., Zikos, C. Benaki, D., Paravatou-Petsotas, M., Tsotakos, T., Triantis C., Pirmettis, I., Papadopoulos, M., Pelecanou, M., Livaniou, E. Neuroprotective peptide colivelin and labeled derivatives (2012) Structural, in vitro and in vivo evaluation. In *J. Pept. Sci.*, vol.18, issue S1, p. S87. *Proceedings in Peptides 2012*, pp 242-3. (G. Kokotos, V. Constantinou-Kokotou, J. Matsoukas, Eds).

Benaki, D., Stamatakis, K., Leondiadis, L. Mikros, E., Pelecanou, M. Spectroscopic investigation of the interaction of curcumin with the β -amyloid peptide of Alzheimer's disease (2012). In *J. Pept. Sci.*, vol.18, issue S1, p.S197. *Proceedings in Peptides 2012*, pp 664-665. (G. Kokotos, V. Constantinou-Kokotou, J. Matsoukas, Eds).

Presentations at Scientific Conferences

M. Kostomoiri, C. Zikos, D. Benaki, M. Paravatou-Petsotas., T. Tsotakos., C. Triantis., I. Pirmettis, M. Papadopoulos, M. Pelecanou, E. Livaniou (2012). Neuroprotective peptide Colivelin and labeled derivatives: Structural, in vitro and in vivo evaluation. 32nd European Peptide Symposium, September 2-7, Athens, Greece

D. Benaki, K. Stamatakis, L. Leondiadis, E. Mikros, M. Pelecanou (2012) Spectroscopic investigation of the interaction of curcumin with the β -amyloid peptide of Alzheimer's disease. 32nd European Peptide Symposium, September 2-7, 2012, Athens, Greece

D. Benaki, Ch. Zikos, Ch. Tsoukalas, Ch. Liolios, C.- E. Karachaliou, P. Bouziotis, E. Livaniou, M. Papadopoulos, I. C. Pirmettis, M. Pelecanou (2012). Structural characterization with NMR of derivatized dextrans as cancer diagnostic agents Euromar, July 1-5, Dublin, Ireland.

A. Panagiotopoulou, Th.Tsotakos, Ch. Triantis, I. Pirmettis, M. Papadopoulos, M. Pelecanou (2012). Characterization of new fac-[Re(L)(P)(CO)₃] and Re(L)(P)₂(CO)₂ complexes by NMR. 3rd ARCADE Workshop Advanced Mass Spectrometric and NMR Methods. May 28-30, National Hellenic Research Foundation, Athens, Greece.

M. Kostomoiri, C. Zikos, D. Benaki, M. Paravatou-Petsotas, T. Tsotakos, C. Triantis, I. Pirmettis, M. Papadopoulos, M. Pelecanou, E. Livaniou (2012). Structural and Biological Evaluation Studies of Colivelin through the use of labeled derivatives. 15th Hellenic Symposium on Medicinal Chemistry May 25-27, National Hellenic Research Foundation, Athens, Greece.

Educational Activities

M. Pelecanou "Applications of NMR in Medicine" in the framework of the graduate course "Introduction to Research Methodology", School of Medicine, Univ. of Athens, December 2012 (3 hours, 30 students), M. Pelecanou

M. Sagnou CityUnity College - Postlyceum Education Centre, Dept. of Psychology - University of Seattle - Course modules and Seminars in Neuropsychology (30 h, 12 students), Psychopharmacology (30 h, 14 students), and Psychology of Addiction (30 h, 30 students)

Other Scientific Activities

Member of the Local Organizing Committee of the International Conference Euromar 2013 that will be held in Crete in July 2013 (M. Pelecanou)

Reviewer for the scientific journals: Inorganic Chemistry, Journal of Medicinal Chemistry (M. Pelecanou)

Other Activities for the Institute of Bioesciences & Applications

M. Pelecanou:

- Member of the Scientific Advisory Board of the Institute of IB-A
- Co-responsible (with M. Vlassi) for the operation of the Circular Spectropolarimeter (CD) with the support of the specialized technical scientist Dr. A. Panagiotopoulou
- Co-responsible for the NMR Lab of the NCSR "D", (Internal Project 949, Protocol Number. A.C.305/Subj. 18).
- Safety Responsible for IB-A
- Member of the Committee of Administrative and Personnel Affairs of NCSR "D"
- Deputy Director of IB-A
- Member of the committee for the appointment of a Reseacher C in the field of "Regulation of Cell Multiplication and Ageing" in IB-A, November 2012
- Member of the committee for the appointment of a Researcher C in the field of "Regulation of Gene Expression" in IB-A, December 2012

M. Sagnou:

- Responsible for the operation of the Confocal microscope of IB-A
- Deputy for educational affairs
- Responsible for graduate student seminars
- Member of the team for the preparation of the proposal of IB-A within the framework of KRIPIS on research infrastructures (General Secretariat for Research and Technology, EPAN-II, ESPA)
- Responsible for the promotion and marketing of the products of the Human Tissue Bank of IB-A

D. Benaki:

- Responsible for the NCSR "D" Blood Bank

Impact Factors (for 8 publications): 24,684

Citations 2012 (without self-citations):

M. Pelecanou: 78

M. Sagnou: 22

Total Citations 2008-2012 (without self-citations):

M. Pelecanou: 258

M. Sagnou: 86

h-factor:

M. Pelecanou: 16

M. Sagnou: 6

Laboratory Equipment

Circular Spectropolarimeter (CD). The instrument, JASCO J-720 equipped with a Peltier temperature controller, is placed in IB and belongs to the Crystallography Center. Responsible for IB: Dr. M. Vlassi and M. Pelecanou. Dr. A. Panagiotopoulou (Technical Specialist) is responsible for the maintenance and service rendering for NCSR "D" users as well as of other research and academic institutions.

NMR Spectrometers 250 and 500 MHz, Bruker. The spectrometers are placed in the building "Sholi" and they belong to three institutes, i.e. Biology, Physical Chemistry, and RRP. Responsible for IB: M. Pelecanou. The Technical Specialist Dr. A. Panagiotopoulou is responsible for the maintenance and service rendering for NCSR "D" users as well as of other research and academic institutions.

HPLC (old, from Dr. S. Loucas Lab); recent service by the electronic's engineer D. Gkirklemis

High precision balance of six decimals (from Dr. S. Loucas Lab)

UV for TLC, rotary evaporator, glassware oven

Current External Funding

Program entitled *Development of Novel Natural Product-based Imaging Probes for Early Diagnosis and Therapeutic Application in Multi-Drug Resistant Tumors (PITHEAS)*, funded by EU with Dr M. Sagnou as scientific coordinator.

Duration: 2012-2014

Total Funding : 400.800 €

Funding of the lab for 2012: 20.000 €

Program entitled *European Research initiative to develop Imaging Probes for early In-vivo Diagnosis and Evaluation of response to therapeutic Substances*, funded by EU with Dr Varvarigou (IRRP, NCSR "Demokritos") as scientific coordinator of the Hellenic team; IB-A collaborator: Dr. M. Sagnou.

Duration: 2012-2016

Total Funding: 6.995.000 €

Funding of the lab for 2012: 9.000 €

Program entitled *Development and screening of novel beta amyloid peptide inhibitors for Alzheimer's disease*, funded by GSRT (Within the framework of "Collaboration" NSRF 2007-2013) and Coordinating Institute The Goulandris Natural History Museum, GAIA Research Center, Bioanalytical Department (IB-A Subcontractor, Dr. M. Pelecanou).

Duration: 2012-2015

Total Funding: 2.431.800 €

Total Lab Funding: 100.000 €

Funding of the lab for 2012: 35.000 €

Program entitled *Biosynthesis and genetic selection of cyclic peptides with therapeutic potential against Alzheimer's disease; Inhibitors of the aggregation of the A β protein*, funded by GSRT (Within the framework of "Thalis", NSRF 2007-2013) and Coordinator: Dr. Efstathios Gonos, Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation (IB-A Subcontractor, Dr. M. Pelecanou).

Duration: 2012-2016

Total Funding: 510.000 €
Total Lab Funding: 29.000 €
Funding of the lab for 2012: 0 €

Program entitled *Biosynthesis and genetic selection Directed Evolution of Small-Molecule Therapeutics Against Neurodegenerative Diseases*, funded by GSRT (Within the framework of "Aristeia 201" NSRF 2007-2013) and Coordinator: Coordinator Dr. George Skretas, Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation (IB-A Subcontractor, Dr. M. Pelecanou).

Duration: 2012-2014

Total Lab Funding: 15.300 €
Funding of the lab for 2012: 0 €

Program entitled *Helium Recovery, towards the creation of a National Helium Reserve*, funded by GSRT (Within the framework of "Cooperation" NSRF 2007-2013) and Coordinator: Dr. B. Petrouleas, Institute for Advanced Materials, Physicochemical Processes, Nanotechnology & Microsystems, NCSR "D" (Participation of the NMR Laboratory (Drs K. Yannakopoulou, L. Leondiadis, M. Pelecanou, A. Panagiotopoulou) on helium recycling).

Duration: 2013 – 2015

Funding for the NMR Lab: 15000 €

Program entitled *Study of the potential anti-addiction activity of the natural product curcumin and its derivatives in experimental models of rats*, private sponsoring by Professor G. Paxinos

Scientific responsible: M. Sagnou

Duration: 2012

Total funding: 2000 €
Funding for 2012: 1.000€

Note 1 : the following proposals have been approved in 2012, funding however has not yet started
Program with title: "*Development of new targeted tools of rhenium and technetium with dual tumor imaging potential*"

Empeirikion Foundation (2010 Research Grant call)

Scientific responsible: M. Pelecanou

Total budget: 15.000 €

Program with title: "*Synthesis, characterization and biological evaluation of novel radioiodinated tracers for imaging of multidrug resistance in pathological cancer models*"

Empeirikion Foundation (2010 Research Grant call)

Scientific responsible: A. Varvarigou, Institute of Radio and Safety INRASTES

Total budget: 11500 €

Note 2 : Submitted in 2012, under review

Within the framework of "Aristeia 2012" NSRF 2007-2013

"*Development of multimodal imaging agents for sentinel node detection and cancer diagnosis*"

Scientific responsible: I. C. Pirmettis, INRASTES, NCSR "D".

"*Novel technetium-99m tricarbonyl complexes bearing the 4-[3-bromophenyl] quinazoline moiety as a biomarker for EGFR-TK imaging*"

Scientific responsible: M. Papadopoulos, INRASTES, NCSR "D".

"*TeraMed – THz imaging for biomedical applications: from molecule to tissue properties*"

Scientific responsible: K. Ouzounoglou, National Technical University of Athens

Research Group: **Protein Structure and Molecular Modeling**

Research Staff

Metaxia Vlasi, Research Director

Diamadis Sellis, Graduate Associate (MSc)

Research Interests

Our current research interests focus on

- Structural studies of proteins with emphasis on aminoacid-sequence-repeat containing proteins aiming at elucidating their sequence/structure relationships and at identifying the structural determinants of sequence-repeat-mediated protein interactions. The approach we follow includes mainly *in silico* techniques such as, homology/comparative modeling and threading, molecular dynamics simulations, molecular docking, etc.
- Studying the dynamics of proteins by means of molecular dynamics (MD) simulations aiming at understanding their structure/function relationships, and development of related computing tools.
- 3D-modeling of enzymes of mainly medical importance and of their interactions aiming at unraveling, at atomic detail, their activation/inhibition mechanisms towards a full understanding of their function, as a tool for rational development of new pharmaceuticals.

2012 Findings

1. 3D-modeling of Arginine-Serine (RS) repeats:

Our work on the conformation of Arginine-Serine (RS) repeats, which are found in several proteins and are involved in protein-interactions regulated by SRPK1-mediated phosphorylation, was published in 2012 (see *Sellis et al, 2012*).

In summary, using mainly **molecular dynamics (MD) simulations** on the **RS-domain of Lamin-B Receptor (LBR)**, in combination with biochemical data, we showed that even short RS-domains may constitute part of recognition platforms for SRPK1, which in turn uses the same, distal to the active site, docking groove (see Figure) to recognize its RS-substrates, irrespective of their length. Furthermore, we showed that binding to SRPK1 may promote unfolding of the RS-repeats destined to be phosphorylated, whereas their phosphorylation induces compact structures, irrespective of the RS-repeat length, probably serving in recognition of positively charged partners (e.g. histone H3 for LBR).

In total, our studies on the RS repeats, shed light on the conformational preferences of an important class of aminoacid-sequence repeats and contribute to the elucidation of their phosphorylation mechanism and of their role in protein interactions.

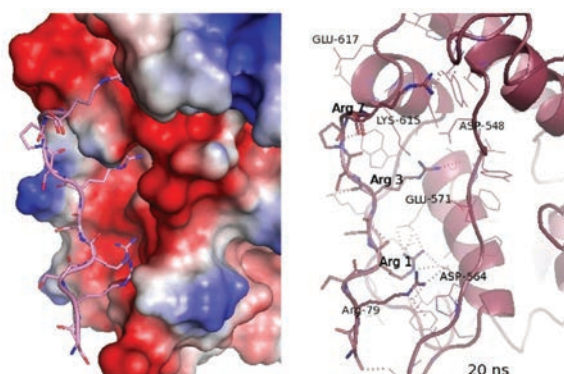


Figure: 3D-model of the RS-containing docking peptide of human LBR (in stick-model) in complex with the kinase, SRPK1 (shown as surface colored by electrostatic potential, left panel and as ribbon; right panel) after a 20 ns molecular dynamics simulation in explicit water. Important residues predicted to be involved in molecular recognition are labeled. The model, in combination with literature and biochemical data showed, for the first time, that SRPK1 uses the same, distal to the active site, acidic docking groove (in red in left panel) to recognize its RS-containing substrates irrespective of their length.

2. 3D- modeling of novel 10-aminoacid repeats

In 2012, using *in silico* techniques, we modeled the structure of a **new class of evolutionary conserved 10-aminoacid repeats**, identified by our collaborator M. Adrande-Navarro (Berlin-Germany) (*Manuscript in preparation*).

3. The Gromita.v3 software, a tool for performing molecular dynamics simulations of proteins in solution

Recently, we developed a **new version (v.3)** of the **Gromita GUI** developed previously in our lab (Sellis et al., 2009, *Bioinformatics & Biology Insights* 2009:3,99-102), which is compatible with the latest versions of the molecular simulation package, Gromacs-4.

In 2012, we created an updated web-site (<http://gromita.bio.demokritos.gr>) including a new download engine, as well as installation instructions and manual for the new Gromita.v3. More than 20 (academic) users have registered to the new site and signed a license agreement to download and use the updated version of our software.

4. 3D-Modeling of PARN/inhibitor interactions

Our work including **molecular docking (in silico)** of new **synthetic nucleoside analogues**, drastic inhibitors of human poly(A)-specific ribonuclease (**PARN**), into the active site of the enzyme and comparison with our previous docking data on similar compounds with **antitumor activity** (Balatsos et al, 2009, *Biochemistry* 48(26):6044-6051), is published in 2012 (*see Balatsos et al, 2012*).

In total, these two *in silico* studies, together with kinetic data, reinforce the notion that PARN can be established as a novel molecular target for the development of novel anticancer agents and show that the most effective inhibitor (uracil-based compound, U1), acting through slow-binding and slow-release from the active site of the enzyme, can be used as leading compound for further rational design of new more potent PARN inhibitors.

Publications

Sellis D, Drosou V, Vlachakis D, Voukkalis N, Giannakouros T, Vlassi M. (2012) Phosphorylation of the arginine/serine repeats of lamin B receptor by SRPK1-insights from molecular dynamics simulations. *BiochimBiophysActa (General Subjects)* 1820(1):44-55.

Balatsos N, Vlachakis D, Chatzigeorgiou V, Manta S, Komiotis D, Vlassi M, Stathopoulos C (2012) Kinetic and *in silico* analysis of the slow-binding inhibition of human poly(A)-specific ribonuclease (PARN) by novel nucleoside analogues. *Biochimie.* 94(1):214-21.

Presentations at Scientific Conferences

D. Sellis, T. Giannakouros, M. Vlassi (2012) Recognition of arginine/serine repeats by SR protein kinase 1: Insights from molecular dynamics simulations. 22nd IUBMB & 37th FEBS Congress, Sept 4-9, 2012, Seville, Spain (*FEBS Journal* 279 (Suppl. 1) (2012) pp: 553-554).

D. Sellis, T. Giannakouros, M. Vlassi* (2012) Molecular dynamics simulations of arginine/serine repeats: Implications for their role in molecular recognition. 6th International Conference of the Hellenic Crystallographic Association, Sept 28-29, 2012, Univ. of Athens, Athens, Greece (**oral presentation*).

Other Scientific Activities

- Member of the evaluation committee of the University of Thessaly (School of Health Sciences, Dept. of

Biochemistry & Biotechnology) for the appointment of an Associate Professor in the field of Biophysics with emphasis on structure/function studies of proteins

- Member of the evaluation committee of IB-A/NCSR "D" for the appointment of an Associate Research Scientist in the field of the "Design, Synthesis and Evaluation of Chemicals with Biological and Pharmaceutical Activity"
- Member of the national network BE/OPT-XFEL (Network to Optimize use of the European X-FEL by the Greek Research Community)
- Member of the national network INSTRUCT-EL ("INSTRUCT/ESFRI: An Integrated Structural Biology Infrastructure for Europe)
- Member of the "Center for Crystallographic Studies of Macromolecules" network

Educational Activities

Lectures on "Principles of X-Ray Crystallography: Applications in Structural Biology" in the framework of the post-graduate program (towards a Masters degree) entitled "Clinical Biochemistry – Molecular Diagnosis" (Dr. M. Vlassi, Dept. of Biology, National & Kapodistrian University of Athens)

Lecture entitled "Prediction of protein structure using molecular dynamics simulations" to students of the University of Texas at Arlington (UTA) in the framework of the "Study abroad program" (<http://studyabroad.iit.demokritos.gr/>) co-organized by UTA and NCSR "D" (Dr. M. Vlassi, NCSR "Demokritos", May 28-30, 2012).

Member of the PhD Thesis Committee of the graduate student, A. Karabellis (IB-A and Democritus Univ. of Thrace)

Other Activities for the Institute of Biology

Member of the Scientific Advisory Board of IB-A

Surrogate Member of the Scientific Advisory Committee of NCSR "D"

Co-responsible (with Dr. M. Pelecanou) for the operation of the Circular Dichroism (CD) laboratory with the help of the technical specialist, Dr. A. Panagiotopoulou

Co-responsible (scientific responsibility) for the computing equipment of IB-A

Impact Factors (for 2 publications): 8,022

Citations 2012 (without self- citations): 37

Total Citations 2008-2012 (without self- citations): 155

h-factor: 12

Laboratory Equipment

The current equipment includes various desktops and servers

Current External Funding

Program entitled *The new Biology of intrinsically disordered proteins: A targeted, multidisciplinary analysis of IDP structure, function and properties in real time and true cellular conditions*, funded by GSRT (THALIS) with Prof. S. Georgatos (Univ. of Ioannina) as scientific coordinator.

Duration: 2012-2015

Total Lab Funding: 10.000€ (mainly for the purchase of an advanced computer system: 64-core server)

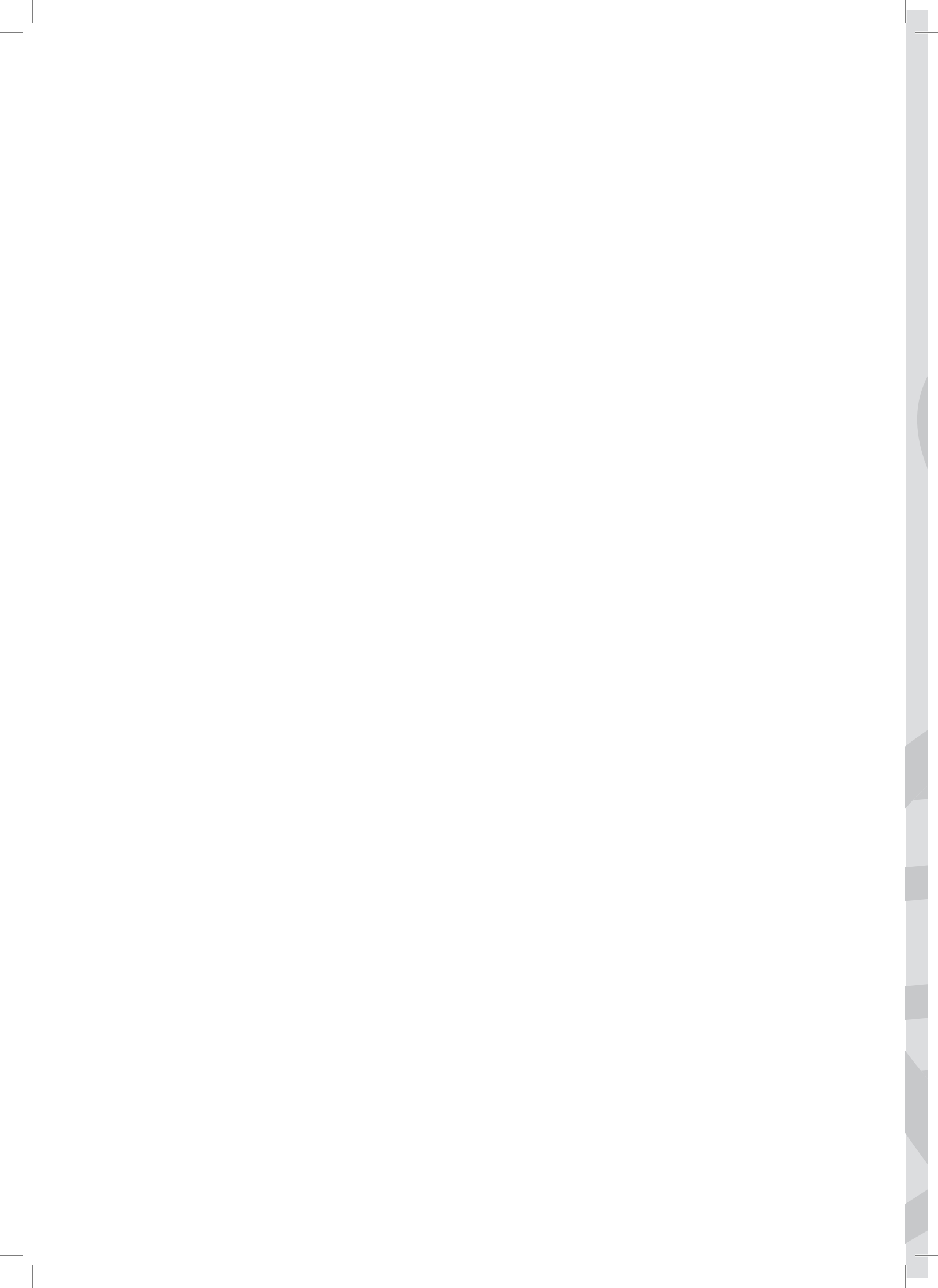
Funding of the lab for 2012: 0 €

Program entitled *Mitochondrial dysfunction in neurodegenerative disorders*, funded by GSRT (THALIS) with Prof. A. Plaitakis (Univ. of Crete) as scientific coordinator.

Duration: 2012-2015

Total Lab Funding: 20.000€ (mainly salary for a scientific collaborator to be hired)

Funding of the lab for 2012: 0 €





SERVICE UNITS

Human Tissue Laboratory/ Bank

Experimental Animal Colony

Laser Confocal Microscopy

Circular Dichroism (CD) Laboratory

Laboratory of Cell & Matrix Pathobiology

Laboratory of Molecular Diagnosis of Genetic Diseases

Laboratory of Cell-Based Assay Systems
and Bioactive Compounds

Provision of Biotechnology Products & Services

HUMAN TISSUE LABORATORY / BANK

Research Staff

Helen Vavouraki, Researcher

Photini – Effie Tsilibary, Director of IB-A (Administrative Responsible)

Marina Sagnou, Researcher (Marketing & Promotion)

Kostas Anagnostakos, Graduate Research Associate - MD

Avgi Kyriazi, Graduate Research Associate- MD

Stilianos Kakkos, Research Technician

Margarita Papadaki, Secretarial Support



Lab Description – Research Interests

Human Tissue Bank is one of the first legislated laboratories of NCSR “DEMOKRITOS”, and always mentioned in the Ministry of Health laws regarding transplantations. Bank operational aim is the collection of various human tissues, their processing and the production of grafts to be used in reconstructive surgery. It is conforming to the European Directives 23/2004, 17/2006 and 86/2006 concerning human tissues and cells as well as to the recommendations of IAEA.

A Member of the European Association of Tissue Banks, the laboratory is unique for Greece applying its “know how” in the processing of a great variety of human tissues and the expertise that has gradually developed in each procedure (procurement, tissue processing, irradiation sterilization and disposal of tissue grafts) is constantly improved by the bank’s Scientific Supervisor whereas all the members of the team keep working hard in order to extend provided services and different types of grafts. During the 40 years of continuous functioning it has delivered more than 46000 tissue preparations of flawless quality. All procedures taking place in the Bank are fully computerized and accredited according to ISO 9001/2008. Constant care is practiced regarding quality control issues, and compliance with the Greek and European standards.

The grafts preparations are delivered to hospitals, health clinics, medical and dental laboratories, all over the country, but mainly in the area of Attica.

The tissue banking research interests are focused mainly in the study of the activity of the produced grafts, in the optimization of the production methods, the introduction of new techniques, the process of new tissues and the development of new grafts. Presently, the laboratory is in the process of developing bovine as well as synthetic bone fragments (chemical synthesis processes) as well as other related products (collagen membranes, etc.) for use in implantology (M. Sagnou, E. Tsilibary, in charge).

The laboratory’s research activities are based on collaborations with university medical and other departments and hospitals, in order to promote Public Health by launching improved products, publishing original papers and participating in doctorate degrees (Ph.D. theses).

The highly specialized services of this unique facility in Greece, relies on collecting human tissues from national hospitals according to European directives of tissue donation and banking, to ensure donor traceability and tissue safety. The tissues are stored under appropriate conditions until they are processed accordingly. More than 90% of the products regard human bone allografts of various sizes. The production and supply of human bone allografts follows the increasing demands of processed bone tissue products in bone implants and regeneration processes in orthopedic, general and plastic surgery, neurosurgery, upper/lower jaw surgery, implantology, periodontology etc. which has been expected to Reach \$3.18 Billion in the United States by 2015, According to New Report by Global Industry Analysts.

Services, Products and Activities

Bone Processing, includes neutralization of HIV and Hepatitis B virus, tissue dehydration/ lyophilization (final water content $\leq 3\%$), packaging in vials resistant to radioactivity and sterilization with γ -radiation (^{60}Co) until the tissue receives 2.5 Mrad as recorded by Perspex-type dosimeters (EN 552/94). Final microbiological/sterility tests and pyrogen tracing is compulsory before the product is available to the clinic.

The Tissue Bank process is fully computerized: donor-patient medical history, batch processing, sterility test results, labeling and financial-stocking documentation.

Our product list regarding Human Bone Allografts includes:

Cancellous or Cortical or Mixed (cortico/cancellous) bone

| | | |
|--------------------------------|--------------------------------|--------------------|
| ≤ 1 mm, 0.5 cc (granular) | ≤ 2 mm, 1.0 cc (granular) | 4-10 mm, 10.0 cc |
| ≤ 1 mm, 1.0 cc (granular) | ≤ 2 mm, 2.0 cc (granular) | 10x10x10 mm (cube) |
| ≤ 1 mm, 2.0 cc (granular) | ≤ 2 mm, 5.0 cc (granular) | 15x15x15 mm (cube) |
| ≤ 2 mm, 0.5 cc (granular) | 4-10 mm, 5.0 cc | 23x15x3 mm (cube) |

2012 Graft production - Findings

For our collaboration with the private sector which promoted our grafts, we delivered 55 bone grafts to be used in the Orthopedic Surgery and prepared 291 grafts for Dental Surgery. Tissues were derived from living donors.

We have delivered also various other tissue preparations for scientific collaboration purposes, to hospitals, University labs, e.t.c. in order to establish tissue procurement regulations as well as the preparation and study of new products.

The new Technological Research Project – Research , Development and Delivery of Tissue grafts-products of Tissue Repair- which is recently approved, is based on the above collaborations

Publications

Minas D. Leventis, Efstathios Eleftheriadis, Panagiota Oikonomopoulou, Helen Vavouraki, Lubna Khaldi, Konstantinos I. Tosios, Emmanouil Vardas, Konstantinos D. Valavanis, and Ismene Dontas. "Experimental Study of the Effect of Autologous Platelet-Rich Plasma on the Early Phases of Osteoinduction by Allogenic Demineralized Bone Matrix". *Implant Dentistry*, 21 (5), pp 399-405, 2012.

Pantou A.L, Markopoulou C.E., Dereka X.E., Vavouraki H., Mamalis A., Vrotsos I.A. "The effect of Platelet-Rich Plasma (PRP) combined with a bone allograft on human Periodontal Ligament (PDL) cells". *Cell and Tissue Banking*, 13 (1) , pp: 81-88, 2012

Articles in Press

Pantou A.L, Markopoulou C.E., Dereka X.E., Vavouraki H., Mamalis A., Vrotsos I.A. "The effect of Platelet-Rich Plasma (PRP) combined with a bone allograft on human Periodontal Ligament (PDL) cells". *Cell and Tissue Banking*, (in press) Epub 2010 Dec 1.(I.F 2010:1,157)

Presentations at Scientific Conferences

Pantelis Bochlogyros, Helen Vavouraki, Rassa Jebrin. "Immediate implantation in fresh extraction sockets". 13o Peloponese Dental Congress, University of Sparti, June2012

Helen Vavouraki, Pantelis Bochlogyros, Rassa Jebrin. «New design implant for immediate implantation in fresh extraction sockets». 13o Peloponese Dental Congress, University of Sparti, June 2012

Other Activities for the Institute of Biosciences & Applications

Scientific Responsible of Human Tissue Bank.

Quality Manager of Human Tissue Bank (ISO 9001/2008)

Training visits of University students in the Bank.

Other Scientific Activities

Reviewer in the scientific Journals: Platelets, Cell and Tissue Banking

Member of European Committee for the establishment of unique nomenclature of human tissues and cells

Member of European auditors-net of human tissue and cells banks

Expert of National Transplant Organization and Ministry of Health in the field of Human Tissue Banking

Impact Factors (for 2 publications): 4,772

Citations 2012 (without self- citations): 11

Total Citations 2008-2012 (without self- citations): 34

h-factor: 5

Laboratory Equipment

Deep freezers, refrigerators for the preservation of tissues and their derivatives

Operating Room equipment

Customized surgical saw

Cutting mill

Lyophilizer

Laminar Flow

Packaging equipment

Sterility tests equipment

General laboratory equipment

Note:

A Project under the name "Research, development and delivery of tissue grafts-tissue repair products" has been suggested and approved by the Directory Board of NCSR "D"

EXPERIMENTAL ANIMAL COLONY

Research Staff

Dimitris Kletsas, Research Director

Ioannis Zafiropoulos, Research Technician

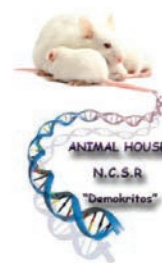
George Doulgeridis, Research Technician

Description

The animal facility maintains and reproduces inbred strains of experimental animals.

The following strains are currently available:

- Mice, strain SWR SWISS ALBINO
- Rats, strains WISTAR ALBINO, ETB & transgenic SPRAGUE DAWLEY
- Rabbits, strain NZW ALBINO
- Mice, strain SCID



During 2012 the Animal Facility provided the following animals:

| <i>Users</i> | <i>Rats WISTAR</i> | <i>Rats ETB</i> | <i>Mice</i> | <i>Rabbits NZW</i> | <i>Mice SCID</i> |
|---|------------------------|---------------------|-------------|------------------------|----------------------|
| Institute of Biology | 0 | 0 | 46 | 3 | 65 |
| Institute of Radioisotopes & Radiodiagnostics | 37 | 0 | 299 | 16 | 459 |
| External Users | 583 | 165 | 0 | 0 | 0 |
| Total | 620 | 165 | 345 | 19 | 524 |

During 2012 the Experimental Animal Colony received the permission from the supervising authorities for the development of an Animal Experimentation Facility (code: EL 25 BIO 039). Accordingly, a special room with all relevant infrastructure has been created.

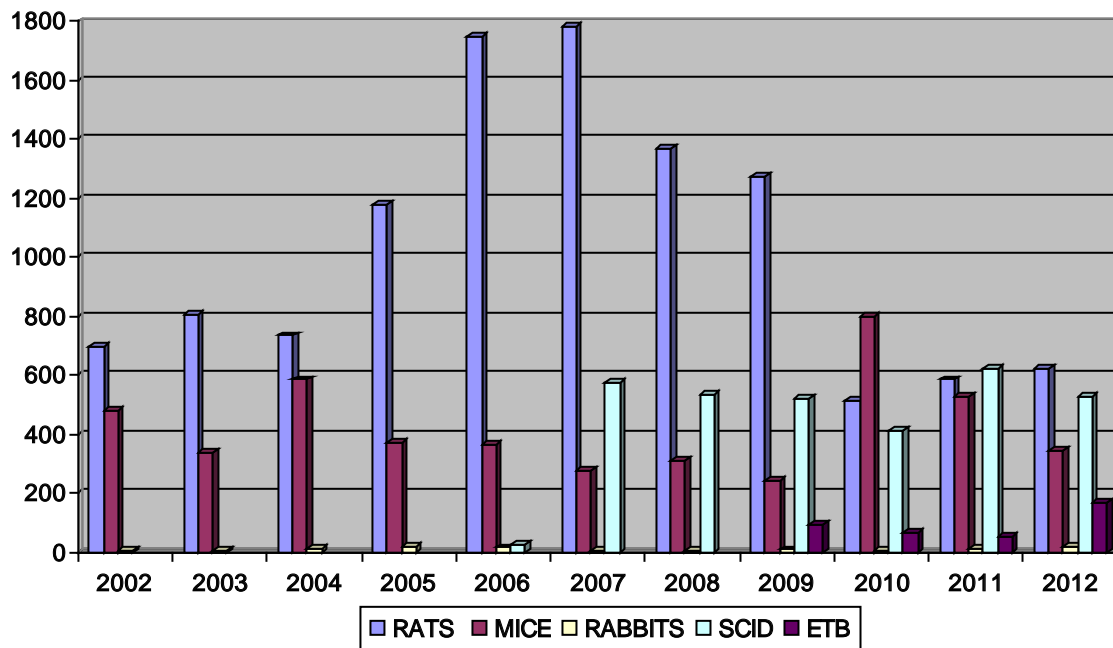
The certification according to ISO 9001:2008 has been renewed.

The number and species of animals produced are dictated by the needs of research programs within the Institutes of NCSR "Demokritos", mainly the Institutes of Biology and Radioisotopes-Radiodiagnostic Products. In addition, animals are provided outside the Centre in research laboratories, Universities, pharmaceutical companies, etc.

The colonies of mice, rats and SCID mice have been renewed. New colonies of transgenic animals have also been developed.

The building has (and is currently being) upgraded, new instruments have been purchased, the quality of animals has been tested locally by a veterinarian and by a certified laboratory abroad, in order to conform with the ISO 9001:2008 certification.

DISPOSAL OF LABORATORY ANIMALS 2002-2012



LASER CONFOCAL MICROSCOPY

Research Staff

Marina Sagnou, Researcher

Description

The current Unit activities include:

- a) The study of cellular, molecular and biochemical phenomena on cells and tissues using confocal microscopy imaging techniques
- b) The use of confocal microscopy as a tool to explore the surface area and penetration potential of novel and known material
- c) The application of immunohistochemistry, phase-contrast, Nomarsky etc techniques on both fixed and living cells.

2012 Findings

During the year 2012, there seemed to be a rather increased demand for the the study of cellular, molecular and biochemical phenomena using confocal microscopy imaging techniques by both the Local Institute of Biology researchers, and those from the University of Athens, The Agricultural University, the Technical University as well as some Hospital Units.

Furthermore, it was this year's achievement, to initiate the exploration of the surface area and penetration potential of novel and known material, as a new ground of application for this technique, by both NCSR "D" researchers and external industry collaborators.



CIRCULAR DICHROISM (CD) LABORATORY

Research Staff

Metaxia Vlassi, Research Director

Maria Pelecanou, Research Director

Aggeliki Panagiotopoulou, Technical Specialist

Users' Committee:

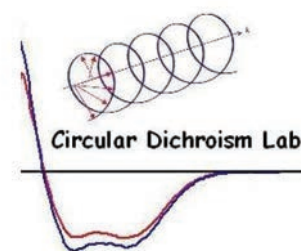
Dr. Metaxia Vlassi

Dr. Maria Pelecanou

Dr. Angeliki Chroni

Dr. Georgios Nounesis (INRASTES)

Dr. Stratos Stratikos (INRASTES)



Description of the Laboratory - Objective

The infrastructure of the Circular Dichroism (CD) laboratory was acquired in 1998 within the framework of the "Center for Crystallographic Studies of Macromolecules (CCM)" which was financed through a grant from the General Secretariat for Research and Technology (EPET) as a network of three Institutes of NCSR "D" (the former: Biology, Physical Chemistry, Radioisotopes & Radiodiagnostic Products) and other Greek academic institutions. The CD equipment includes a JASCO-715 spectropolarimeter equipped with Peltier system for temperature control, it is located at Room Y35 of IB-A and has been operating since 1998 under the supervision of research scientists of IB-A. The application for the establishment of a separate CD Service Unit is in preparation and will be submitted for approval to the NCSR Research Committee.

The objective of the CD lab is to perform circular dichroism experiments in liquid samples. Circular dichroism is a well-established spectroscopic technique based on the differential absorption of circularly polarized light from optically active molecules and its applications include, among others:

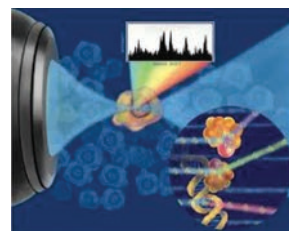
- Conformational analysis of biological macromolecules
- Investigation of interactions between macromolecules as well as of interactions/complexation of macromolecules (e.g. proteins) with various ligands, drugs, inhibitors, activators, stabilizers, etc
- Comparative conformational studies of proteins from various sources.
- Stability studies under various conditions
- The CD method is non-destructive to the sample, highly accurate, sensitive, and reproducible.

2012 Findings

The CD facility is widely used since 1998 by research groups both from NCSR "D" and from other academic/research institutions in Greece, for conformation and interaction studies of biological macromolecules. It should be mentioned that the CD lab is one of few existing in Greece and the only one in the area of Attika offering services to external users. The CD facility has largely contributed to scientific publications, PhD theses, as well as to the establishment of collaborations between research groups, both within and outside NCSR "D" and to the submission of joint grant applications. In addition, the CD lab has contributed to the education of new users (students and research scientists) and has provided support in technical and scientific problems related to the application of CD.

In 2012, the CD lab offered services to research projects of at least 12 research groups from three NCSR Institutes as well as from other Greek academic Institutions, such as: University of Athens (Pharmacy & Chemistry Depts), National Technical University of Athens, University of Patras, and National Hellenic Research Foundation.

LABORATORY OF CELL & MATRIX PATHOBIOLOGY



Research Staff

Fotini-Effie Tsilibary, Research Director

Athina Tzinia, Senior Researcher

Angelika Chroni, Senior Researcher

Paraskevi Kitsiou, Senior Researcher

Garyfallia Drossopoulou, Researcher

Description of the Laboratory - Objective

The specialized services and products provided by the laboratory of Cell & Matrix Pathobiology include:

- Isolation /characterization of stem cells and other cell types from various sources
- Products such as matrix proteins and matrix-derived peptides related to regenerative medicine
- Specialized assays for cell survival and apoptosis /cell toxicity
- Assessment of the quality (antiatherogenic properties) of high-density lipoproteins (HDL)

Services, Products and Activities

The laboratory provides expertise and services related to regenerative medicine and clinical analyses including:

- Isolation and characterization of human umbilical cord and human umbilical cord blood (UCB) stem cells, stem cells from other sources (skin, fat tissue, etc).
- Isolation and subcultures of human and mouse olfactory neuroblasts
- Isolation of other cells types (endothelial cells, neuronal cells, etc).
- Mouse neurospheres (in collaboration with Dr. P. Politis from BRFAA)
- Other stem cells and products related to regenerative medicine (matrix proteins, e.g. collagens, laminin, fibronectin, proteolytic fragments of these glycoproteins, matrix-related peptides)
- Measurement of antioxidant and other antiatherogenic properties of HDL

Infrastructure

The laboratory has equipment to support cell biology, biochemical, and molecular biology experiments. The following list indicates major pieces of equipment:

- Tissue culture room with laminar flow hood, incubators for cell cultures, light microscope, stereoscope, etc.
- Table-top refrigerated centrifuge and microfuge
- Vibratome, horizontal and vertical gel & blotting apparatus with power supplies, FPLC, etc.
- Refrigerators, freezer, deep freezer, balances, PH meters, sonicator, PCR, etc.
- Tissue baths, magnetic stirrers, incubator for general use, digital pipettes
- Liquid nitrogen container for cell cryopreservation

Collaborations and Clients

Biophylaxis, S.A. (a company focusing on isolation of stem cells from various sources, and stem cell cryoprotection, for use in regenerative medicine), orthopedic surgeons, neurosurgeons, implantologists, pharmaceutical companies and companies interested in stem cells and matrix-related products, etc.

LABORATORY OF MOLECULAR DIAGNOSIS OF GENETIC DISEASES

Research Staff

Gerassimos Voutsinas, Senior Researcher

Socratis Avgeris, Research Technician

Description of the Laboratory - Objective

This lab is performing Genetic Testing activities and offering Genetic Counseling services for the hereditary dominant neurocutaneous disorders Tuberous Sclerosis Complex and Neurofibromatosis type 1.

Services, Products and Activities

- Direct sequencing of 23 PCR products for gene *TSC1* and 42 PCR products for gene *TSC2*, using genomic DNA isolated from peripheral blood lymphocytes
- Direct sequencing of 24 cDNA-based PCR products of gene *NF1*, using RNA isolated from peripheral blood lymphocytes
- Genetic Counseling for Tuberous Sclerosis Complex and Neurofibromatosis type 1

Infrastructure

The Laboratory of Molecular Diagnosis of Genetic Diseases is hosted by the Laboratory of Environmental Mutagenesis and Carcinogenesis (Institute of Biosciences and Applications, NCSR Demokritos), using the same equipment, as well as common instruments and facilities of the Institute of Biosciences and Applications, e.g. Laminar flows, CO₂-incubators, Freezers and Deep-Freezers, Centrifuges, Thermal Cyclers, QPCR, Speed-vac, all types of Electrophoresis Apparatuses, Waterbaths, Image analysis systems, ABI Prism 310 Genetic Analyzer, etc.

Collaborations and Clients

Collaborators of the Laboratory of Molecular Diagnosis of Genetic Diseases are

- Laboratory of Molecular Diagnostics, Institute of Nuclear and Radiologic Sciences and Technologies (INRASTES), NCSR Demokritos
- Greek Association for Tuberous Sclerosis
- Greek Alliance for Rare Diseases
- 3 specialized Child Neurologists (MDs S. Youroukos, A. Papavassiliou, A. Dinopoulos)
- 1 specialized Pediatrician (MD A. Xaidara)
- The clients are individual patients and their families

LABORATORY OF CELL-BASED ASSAY SYSTEMS AND BIOACTIVE COMPOUNDS

Personnel

Dimitris Kletsas, Research Director

Harris Pratsinis, Researcher

Eleni Mavrogonatou, Post-doctoral Fellow

Adamantia Papadopoulou, Graduate Research Associate (*MSc*)

Maria Lefaki, Graduate Research Associate (*MSc*)

Eleni Liakou, Graduate Research Associate (*MSc*)

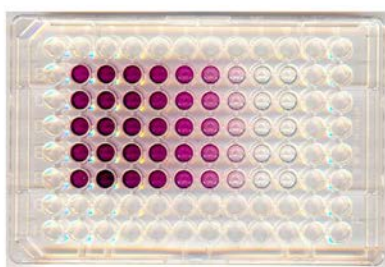
Description

The activities of the Laboratory comprise the isolation of primary somatic or mesenchymal stem cells and the development of the appropriate cell assay systems for the evaluation of synthetic or natural bioactive products regarding their wound healing, anti-ageing, and anti-cancer properties.

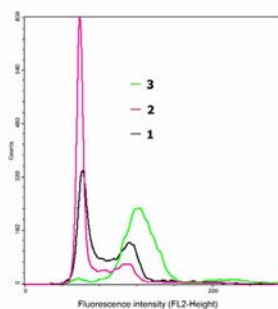
2012 Findings

The following studies were implemented during 2012: cytotoxicity and matrix metalloproteinase expression studies following assigned by Korres S.A. Natural Products, estrogenic activity studies assigned by Danville Materials Inc. (San Ramon CA, USA), and antioxidant activity studies assigned by Lavipharm S.A.

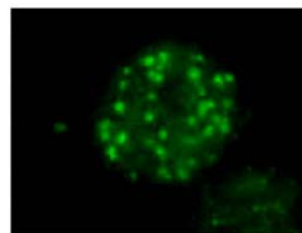
CYTOTOXICITY ASSESSMENT



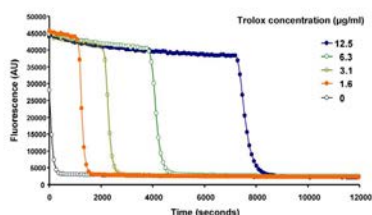
CELL-CYCLE ANALYSIS



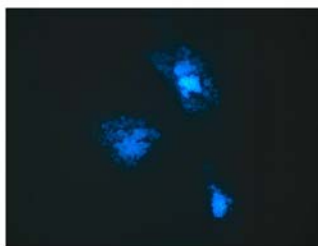
DNA DAMAGE ANALYSIS



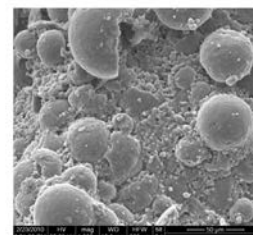
ANTIOXIDANT ACTIVITY ASSAY



STUDY OF APOPTOSIS



ORGANOTYPIC CELL CULTURES



PROVISION OF BIOTECHNOLOGY PRODUCTS & SERVICES

Personnel

Kostas Iatrou, Research Director (Facility Director)

Zafiroula Georgoussi, Research Director

Maria Konstantopoulou, Senior Researcher

Christos Meristoudis, Research Technician

Dimitra Stefanou, Technical Specialist

Description of Products and Services

This service facility was created in November 2011. The spectrum of products and services offered to third parties includes:

1. Recombinant proteins produced by genetically modified insect cells. Such proteins may be used as
 - candidate therapeutic agents (pilot production);
 - cosmetics additives with desired added-value properties;
 - subunit vaccines for protection of livestock from infectious diseases;
 - antigens for antibody development; and
 - reagents for other research uses.
2. Essential oils and other types of plant extracts derived from fresh samples of an in-house collection of more than 300 Greek plants, most of them aromatic and many of them endemic, for use in screening programs for identification of bioactive compounds with defined specificities against specific targets.
3. Development and use of platform technologies based on genetically modified cell lines of mammalian and insect origin. Such cell lines express proteins-targets and allow the undertaking of fast identification of the presence and isolation of bioactive compounds of desired specificities in compound mixtures derived from endemic plants including aromatic ones. Such bioactive compounds may be used by
 - the pharmaceutical industry as lead compounds for development of new pharmacological agents with enhanced beneficial properties;
 - the chemical industry as cosmetics additives with desired added-value properties;
 - the chemical industry and companies developing products for plant protection as active ingredients for environmentally friendly control of insect pests of agricultural and medicinal importance;
 - the chemical industry and companies or organizations, which address issues related to the protection of Public Health and the Environment from environmental pollutants;
 - the research community for research purposes (e.g., activators or inhibitors of protein enzymes).
4. Construction of genetically modified insect viruses (baculoviruses) for environmentally safe control of agricultural insect pests.
5. Development of novel baculovirus-derived gene delivery and expression vectors for mammalian cells for the purpose of producing recombinant proteins with complex (mammalian-type) post-translational modification requirements.
6. Generation and use of vectors for DNA vaccination of livestock for protection from infectious diseases.
7. Licensing of the use of specialized recombinant protein expression vectors to third parties for in-house of production of recombinant proteins.

8. Licensing of the use of specialized recombinant protein expression vectors and/or specific screening platforms to third parties for development of high throughput screening systems for discovery of bioactive leads against protein targets of choice.

Available instrumentation

- Cell culture: laminar flow hood, incubators for cell cultures, cell bioreactor, light and fluorescent microscopes, stereoscope, table-top refrigerated centrifuge and microfuges;
- Experimental insets: incubators for production and maintenance of silkworms and other lepidopteran and dipteran insects;
- Recombinant protein production and purification: HPLC, FPLC, affinity chromatography purification systems;
- Molecular Biology: ηλεκτροφόρηση DNA, RNA and protein electrophoresis, microcentrifuges, ultracentrifuge, electroporation systems, sonicator, microfasmometer, centrifugal vacuum concentrator, scintillation counter;
- Cell Biology: Coulter counter, Fluorescent Activated Cell Sorter, fluorescent microscope, confocal microscope;
- High Throughput Screening Systems for detection of bioactive compounds and FRT-based protein-protein interactions: fluorescent and luminescent microtiter plate readers for reactions employing GFP, rhodamine, fluorescein, beta-galactosidase, luciferase and other reporter photoproteins.

Findings 2012

During 2012 no service contracts were obtained, due to insufficient efforts for raising the awareness of potential customers abroad about the existence of the specific service facility and, importantly, the adverse economic climate in this country in relation to potential Greek customers. Nevertheless, important biological materials were provided during this period to researchers in the public and private sectors abroad following the signing of Uniform Biological Materials Transfer Agreements (UBMTAs). The specific UBMTAs covered primarily the provision of patent-protected expression vectors for generation of recombinant proteins and methods of use and allowed the use of the transferred materials for research purposes only while prohibiting explicitly their use for commercial applications. Notably, the materials were provided to those who requested them free of charge except for shipping costs. Moreover, arrangements were made for the generation and provision of specific recombinant proteins to Greek users but, again due to the adverse financial situation of the Greek research institutions, the requested materials were prepared for distribution on a collaborative basis rather than for sale.



EDUCATIONAL ACTIVITIES

EDUCATION

The Institute of Biosciences & Applications continues its Graduate Course Programme, which has been successfully carried out for the past 40 years. This Programme includes:

- a. Training of young scientists at the postdoctoral level
- b. Pre-graduate and graduate thesis work
- c. Courses at the graduate level
- d. Lecture Contributions to the Summer School of the NCSR "Demokritos"

During the year 2012, 9 scientists were trained at the postdoctoral level at our Institute. Furthermore, 23 graduate students worked toward the completion of their doctoral thesis research work under the supervision of scientists of the Institute and on projects which were given to them by their respective supervisors.

During the year 2012, 5 of our graduate students finished their thesis work and became PhDs and 4 students completed their Master thesis.

Moreover, 9 students from Greek Universities are carrying out their pre-graduate project thesis work at the Institute. Additionally, during 2012, one graduate student from abroad visited IB-A and 3 students did practical job training in laboratories at the Biology Institute.

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

- *Lecture entitled "Cytotoxicity study on conventional and targeted chemotherapeutic drugs" (seminar and practical laboratory exercise)* included in the course for "Cell and tissue cultures", in the framework of the Post-Graduate Specialization Diploma "Biological Applications in Medicine" (Dr. G. Voutsinas, Department of Biology and Medical School, University of Athens)
- *Lectures entitled "Pharmacological targeting of Hsp90" included in the course for "Molecular Biology – Systemic and in silico approaches", in the framework of the Post-Graduate Specialization Diploma "Biological Applications in Medicine"* (Dr. G. Voutsinas, Department of Biology and Medical School, University of Athens)
- *Lectures entitled "Molecular diagnosis of genetic diseases" included in the course for "Molecular Biology – Systemic and in silico approaches", in the framework of the Post-Graduate Specialization Diploma "Biological Applications in Medicine"* (Dr. G. Voutsinas, Department of Biology and Medical School, University of Athens)
- *Lecture entitled "G protein- coupled receptors in health and disease" in the framework of the postgraduate course "Biochemistry"* (Dr. Z. Georgoussi, Department of Biology, University of Athens)
- *Teaching in the framework of the postgraduate course "Molecular Base of Human Diseases"* (Dr. Z. Georgoussi, Department of Biology, University of Athens)
- *Lecture entitled "Cell senescence and tissue homeostasis" in the framework of the postgraduate course "Physiology"* (Dr. D. Kletsas, Medical School, University of Athens)
- *Lecture entitled "Cell senescence and tissue homeostasis" in the framework of the undergraduate course "Oncogenes and growth factors in cancer biology"* (Dr. D. Kletsas, Medical School, University of Athens)

- Lecture entitled “Cell senescence and Carcinogenesis” in the framework of the undergraduate course “Thoracic Oncology” (Dr. D. Kletsas, Medical School, University of Athens)
- Teaching in the framework of the postgraduate programme “Application of Biology in Medicine”, the course “Cell cultures – Tissue cultures” (Dr. D. Kletsas, Dr. H. Pratsinis and Dr. E. Mavrogonatou, Department of Biology, University of Athens)
- Lecture entitled “Cell Cycle: Checkpoints and Consequences for Physiological Cell Function” in the framework of the postgraduate course: “Applications of Biology to Medicine” (Dr. Th. Sourlingas, Department of Biology & Medical School, University of Athens).
- Lecture entitled “Molecular mechanisms and therapeutic approaches of diabetes mellitus” in the framework of the course “Pathobiochemistry” (Dr. E. Tsilibary, Department of Biology, University of Athens)
- Lecture entitled “The pros and cons of the process of cell apoptosis in diseases” in the framework of the course “Molecular and applied physiology”, (Dr. E. Tsilibary, Medical School, University of Athens)
- Lecture entitled “Lipids and apolipoproteins: From cardiovascular disease to Alzheimer’s disease” in the framework of the graduate course “Clinical Chemistry II” (Dr. A. Chroni, Department of Chemistry, University of Athens)
- Lecture entitled “Lipoprotein metabolism pathways and atherosclerosis. The association between atherosclerosis and Alzheimer’s disease” in the framework of the postgraduate course “Human Biochemistry” (Dr. A. Chroni, Department of Chemistry, University of Athens)
- Lectures entitled “Eukaryotic microorganism as model systems for functional expression and characterization of transmembrane transporters of higher organisms” in the framework of the postgraduate course “Microbial Biotechnology – Model Systems of Molecular Microbiology” (Dr. V. Sophianopoulou, Department of Biology, University of Athens)
- Lectures entitled “Molecular Biology: Systemic and in silico Approaches” in the framework of the postgraduate course “Applications of Biology in Medicine” (Dr. V. Sophianopoulou, Department of Biology Medical School & , University of Athens)
- Teaching in the framework of the postgraduate program “Bioinformatics”, the course “Introduction to Computational Biology” (Dr. I. Almyrantis, Department of Biology, University of Athens)
- Teaching in the framework of the postgraduate program “Clinical Biochemistry and Molecular Diagnostics”, the course “Introduction to Computational Biology” (Dr. I. Almyrantis, Department of Biology, University of Athens)
- Lecture on “Principles of X-Ray Crystallography: Applications in Structural Biology” in the framework of the post-graduate program (towards a Masters degree) entitled “Clinical Biochemistry – Molecular Diagnosis” (Dr. M. Vlassi, Dept. of Biology, University of Athens)
- Teaching in the framework of the graduate course “Introduction to Research Methodology”, the course “Applications of NMR in Medicine” (Dr. M. Pelecanou, Medical School, University of Athens)

During July 2012, the Summer School of NCSR “Demokritos” was held and had included talks from the researchers of the Institute of Biosciences & Applications and of invited speakers coming from other Greek Institutions and abroad. The seminars of IB-A related to the Summer School are presented analytically in the following pages.

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 (2012) are presented analytically in the following pages.

Finally, the educational endeavours of IB-A also include those accomplished by Dr. H. Pratsinis and Dr. A. Prombona (from 7/2011), who give informative seminars to High School, University and Military School students.

COMPLETION/AWARD OF DOCTORAL THESES IN 2012

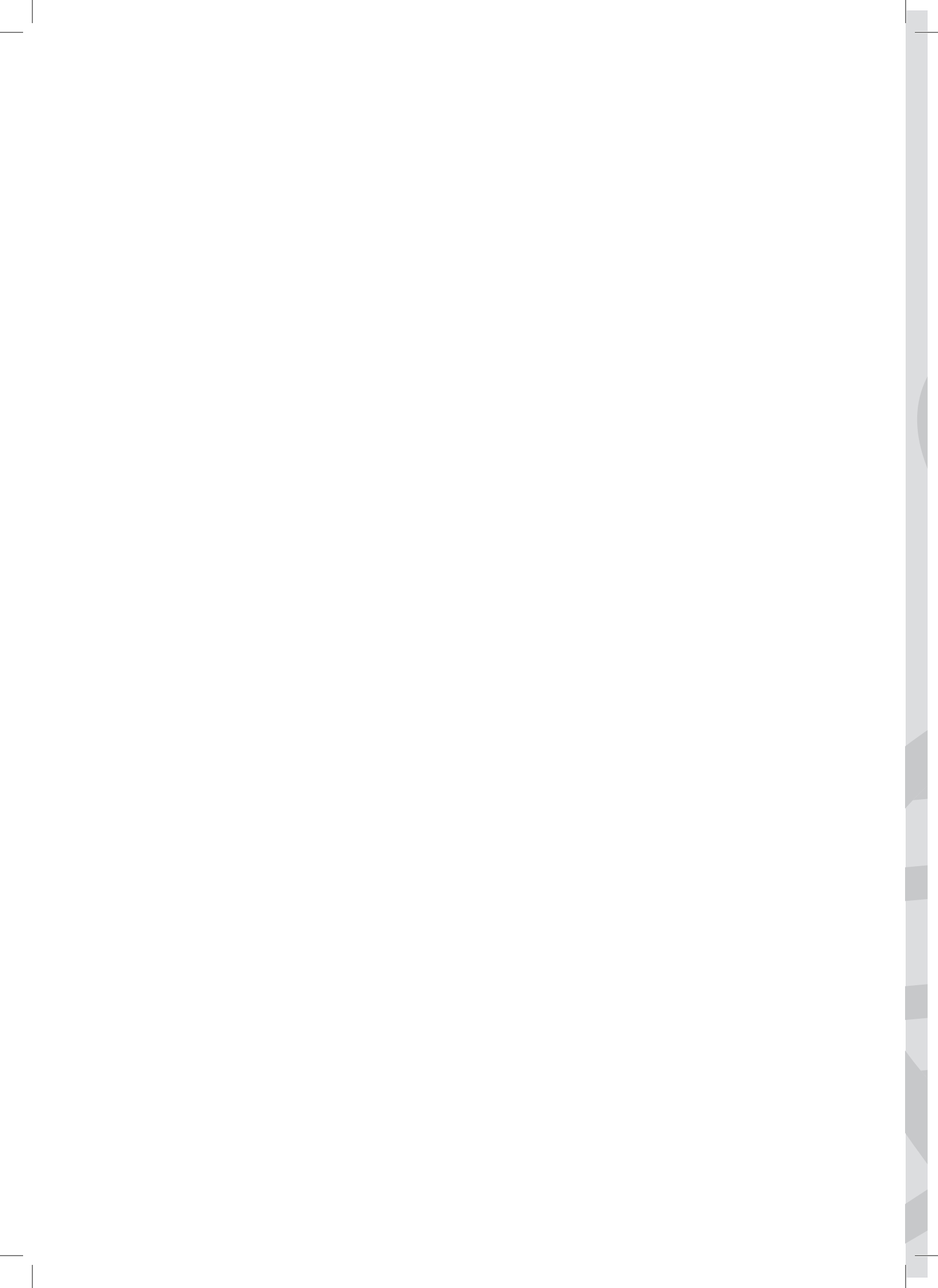
| GRADUATE STUDENT | TITLE OF DOCTORAL THESIS | ADVISOR (in IB-A) | UNIVERSITY |
|--------------------------------|--|------------------------|---|
| Leonidas Leontiadis | Opioid receptor signaling beyond the G protein paradigm | Z. Georgoussi | Department of Biology University of Athens |
| Irene – Maria Georganta | Novel Signalling pathways leading to alteration in gene expression and synaptosomal plasticity | Z. Georgoussi | Department of Biology University of Athens |
| Dimitrios Konstantonis | Effect of donor age on human periodontal fibroblasts' response to mechanical stimulation | D. Kletsas | School of Dentistry University of Athens |
| Marios Xedous | The effect of histone acetylation and methylation levels in the regulation of the mammalian biological clock | Th. Sourlingas | Medical School University of Athens |
| Theodore Georgomanolis | Characterization of protein interactions and functional role of the SH3 protein of <i>Bombyx mori</i> | K. Iatrou & L. Swevers | Department of Biology University of Athens |

**LECTURE CONTRIBUTIONS
TO THE 2012 SUMMER SCHOOL
OF THE NCSR "DEMOKRITOS"
(July 2012)**

| DATE | SPEAKER | TITLE |
|---------|--|--|
| 11/7/12 | Dr. K. Stamatakis IB-A, NCSR "Demokritos" | Study of photosynthetic apparatus resulting hydrogen production |
| 16/7/12 | Dr. E. Tsilibary – Dr. G. Drossopoulou IB-A, NCSR "Demokritos" | Basic Research: From bench to patient- Nephropathies |
| 16/7/12 | Dr. A. Prombona IB-A, NCSR "Demokritos" | Biological clock: role in health and disease |
| 16/7/12 | Dr. V. Sophianopoulou- Dr. C. Gournas IB-A, NCSR "Demokritos" | Plasma membrane function and organisation: the contribution of model microbial organisms |
| 16/7/12 | Dr. K. Iatrou IB-A, NCSR "Demokritos" | The olfactory process of mosquitoes as target for the control of the spread of malaria and other infectious diseases |
| 16/7/12 | Dr. L. Swevers IB-A, NCSR "Demokritos" | The RNAi mechanism in lepidopteran insects: can it be exploited for insect pest control |
| 18/7/12 | Dr. A. Chroni IB-A, NCSR "Demokritos" | Alzheimer's Disease: Is it in our genes? |
| 18/7/12 | Dr. D. Kletsas IB-A, NCSR "Demokritos" | Cell senescence and tissue homeostasis |

SEMINAR PROGRAMME 2012
INSTITUTE OF BIOSCIENCES & APPLICATIONS

| DATE | SPEAKER | TITLE |
|---------|---|--|
| 26/1/12 | A. Koliopoulou IB-A, NCSR "Demokritos" | Study of small RNA pathways in the silkworm <i>Bombyx Mori</i> : in vivo and in vitro approach |
| 2/2/12 | K. Kapodistria IB-A, NCSR "Demokritos" | Survival mechanisms of pancreatic beta cells: A possible role of nephrin signalling pathway on PI3K/ Akt pathway of pancreatic beta cells |
| 23/2/12 | A. Galeou IB-A, NCSR "Demokritos" | Study of function and synchronisation of the circadian biological clock in <i>Phaseolus vulgaris</i> |
| 23/2/12 | M. Kostomoiri IB-A, NCSR "Demokritos" | Development of labeled derivatives of the neuroprotective peptide Colivelin for biological studies |
| 1/3/12 | G. Daniil IB-A, NCSR "Demokritos" | Elucidation of the interactions among various proteins (apoA-I, cholesterol transporters, enzymes, lipid transport proteins) of the HDL pathway. |
| 1/3/12 | L. Argyri IB-A, NCSR "Demokritos" | A simple experimental procedure for the expression and purification of the recombinant apoE4 |
| 15/3/12 | K. Apostolou – Karambelis IB-A, NCSR "Demokritos" | A study of the nature of deviations from Chargaff's second parity rule- the significance of dinucleotides odds ratios |
| 23/3/12 | D. Polychronopoulos IB-A, NCSR "Demokritos" | Computational analysis of conserved not expressed sequences (CNEs) in several genomes |
| 24/4/12 | Dr. C. Gournas IB-A, NCSR "Demokritos" | Structure-function relationships: Life after crystals: The example of the APC family |
| 26/4/12 | E. Georganta IB-A, NCSR "Demokritos" | Novel signal transduction pathways leading to alterations of transcription factors implicated in synaptosomal plasticity |
| 3/5/12 | A. Papadopoulou IB-A, NCSR "Demokritos" | Anticancer therapies and aging |
| 3/5/12 | A. Athanassopoulos IB-A, NCSR "Demokritos" | Organization of protein microdomains in the fungal plasma membrane: study of the subcellular distribution of eisosomal proteins of <i>Aspergillus nidulans</i> |
| 25/6/12 | Dr. D. Chan Dpt. of Biochemistry, Univ. of Hong Kong | Genetics of Intervertebral Disc Degeneration |
| 25/6/12 | Dr. D. Samartzis Univ. of Hong Kong | Disc Degeneration: a Real "Pain" in the Back |





COLLECTIVE DATA

FINANCIAL REPORT 2012



INTERNAL FUNDING FROM THE SPECIAL ACCOUNT DEPARTMENT

| <u>INCOME</u> | PROGRAMME (COORDINATOR: E. TSILIBARY, Head of IB-A) |
|-------------------------------|---|
| | 464 |
| CARRIED OVER FROM 2011 | 31.010,64 |
| FUNDING FROM NCSR "D" | 0,00 |
| MATCHING FUNDS | 0,00 |
| INCOME FROM SERVICES | 0,00 |
| DONATIONS FROM COMPANIES | 0,00 |
| TRANSFER FROM OTHER RESOURCES | 11.038,25 |
| <u>TOTAL INCOME</u> | 42.048,89 |
| | |
| <u>EXPENSES</u> | |
| EQUIPEMENT | 4.104,19 |
| SUPPLIES | 10.628,53 |
| SALARIES | 500,00 |
| TRAVELS | 767,29 |
| OTHER EXPENSES | 7.186,11 |
| PAYMENT IN ADVANCE | -427,56 |
| COMMITTED | 202,86 |
| TRANSFER FROM OTHER RESOURCES | 6.442,89 |
| <u>TOTAL EXPENSES</u> | 29.404,31 |

| PROGRAMMES FROM SERVICES | | | | |
|--------------------------|-----------------|-------------------|------------------|-------------------|
| 1334 | 1475 | 1164 | 1618 | TOTAL |
| -20.000,00 | 2.154,45 | 90.529,96 | 9.466,63 | 82.151,04 |
| 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 0,00 | 0,00 | 0,00 | 0,00 | |
| 12.411,20 | 0,00 | 68.709,71 | 3.000,00 | 84.120,91 |
| 0,00 | 0,00 | 0,00 | 0,00 | |
| 2.000,00 | 0,00 | 25.141,26 | 100,00 | 27.241,26 |
| -5.588,80 | 2.154,45 | 184.380,93 | 12.566,63 | 193.513,21 |
| | | | | |
| 949,80 | 0,00 | 7.786,82 | 0,00 | 8.736,62 |
| 2.537,42 | 369,00 | 37.217,41 | 7.307,08 | 47.430,91 |
| 5.800,00 | 0,00 | 5.000,00 | 2.400,00 | 13.200,00 |
| 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| 5.511,56 | 0,00 | 20.071,91 | 450,00 | 26.033,47 |
| 600,00 | 0,00 | -1.625,00 | 0,00 | -1.025,00 |
| 46,00 | 0,00 | 350,08 | 0,00 | 396,08 |
| 1.214,63 | 0,00 | 15.296,61 | | 16.511,24 |
| 16.659,41 | 369,00 | 84.097,83 | 10.157,08 | 111.283,32 |

EXTERNAL FUNDING FROM THE PROGRAMMES OF THE INSTITUTE
(Programmes that are coordinated by the Head of IB-A are included)

| SOURCE OF FUNDING (number of programmes) | FUNDING (in EUROS) | | | |
|--|--------------------|----------------|---------------|----------------|
| | Programme A | Programme B | Programme C | INSTITUTE |
| European Union (5) | 26.350 | 219.217 | 32.923 | 278.490 |
| General Secretariat for Research & Technology (8) | 72.000 | 79.000 | 50.000 | 201.000 |
| Scholarship by Prof. G. Paxinos (1) | - | - | 1.000 | 1.000 |
| Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease w (2) | 5.000 | - | - | 5.000 |
| Abbot Hellas/Abbvie (1) | 4.000 | - | - | 4.000 |
| TOTAL | 107.350 | 298.217 | 83.923 | 489.490 |

COLLECTIVE DATA ON PRODUCTIVITY OF SCIENTIFIC PROGRAMMES

| | P R O G R A M M E | | | INSTITUTE |
|--|------------------------|------------------------|------------------------|-------------------------------------|
| | A | B | C | |
| Researchers | 11 | 7 | 4 | 23* |
| Technical Specialist | 1 | 1 | 1 | 3 |
| Emeritus & Collaborating Scientists | 1 | 4 | 1 | 6 |
| Postdoctoral Fellows | 4 | 4 | 1 | 9 |
| Graduate Students | 11 | 6 | 2 | 19 |
| Collaborating Graduate Students | 10 | 2 | 1 | 13 |
| Graduate Research Associates | 1 | 1 | 1 | 3 ^{!!!} |
| Undergraduate Students | 16 | 4 | 1 | 21 |
| Research Technicians | 2 | 2 | - | 7 [@] |
| Administrative Staff | - | - | - | 4 |
| Total Personnel | 57 | 31 | 12 | 110 |
| Publications in Peer-Reviewed Journals | 23 | 14 | 12 | 48[#] |
| Publications (Average) in Peer-Reviewed Journals per Scientist | 2.090 | 2 | 3 | 2.086 |
| Cumulative Impact Factor in Peer-Reviewed Journals (number of publications) | 75.472 (23) | 33.297 (14) | 37.386 (12) | 148.403[#] (48) |
| Average Impact Factor in Peer-Reviewed Journals | 3.281 | 2.738 | 3.155 | 3.090 |
| Cumulative Impact factor per Scientist | 6.861 | 4.756 | 9.346 | 6.450 |
| Proceedings to Conferences | 3 | 4 | 2 | 9 |
| Proceedings (Average) per Scientist | 0.272 | 0.571 | 0.5 | 0.391 |
| Total Publications | 26 | 18 | 14 | 57[#] |
| Publications (Average) per Scientist | 2.363 | 2.571 | 3.5 | 2.478 |
| Citations | 1072 | 457 | 152 | 1692* |
| International Patents | - | - | - | - |
| Greek Patents | - | - | - | - |
| Presentations to International Conferences | 17 | 10 | 7 | 36[£] |
| Presentations (Average) per Scientist to International Conferences | 1.545 | 1.428 | 1.75 | 1.565 |
| Presentations to Greek Conferences | 17 | 8 | 3 | 28 |
| Presentations (Average) per Scientist to Greek Conferences | 1.545 | 1.142 | 0.75 | 1.217 |
| Total Presentations to Conferences | 34 | 18 | 10 | 64[£] |
| Presentations (Average) per Scientist to Conferences | 3.090 | 2.571 | 2.5 | 2.782 |

* 1 Scientist of Human Tissue Bank is included

!!! 2 Collaborating Graduate Associates of Human Tissue Bank are included

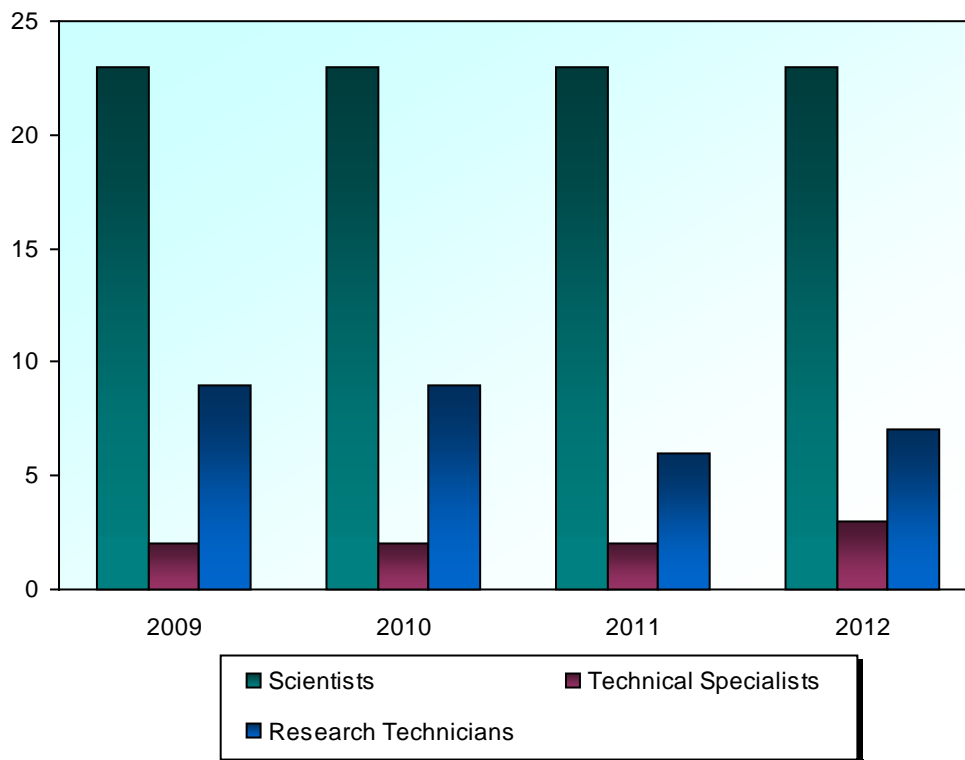
@ 2 Research Technicians who are occupied in Experimental Animal Colony and 1 Research Technician who is occupied in Human Tissue Bank are included

2 publications of Human Tissue Bank are included, 1 common in A and B Programme, 1 common in B and C Programme kai 1 common in A and C Programme

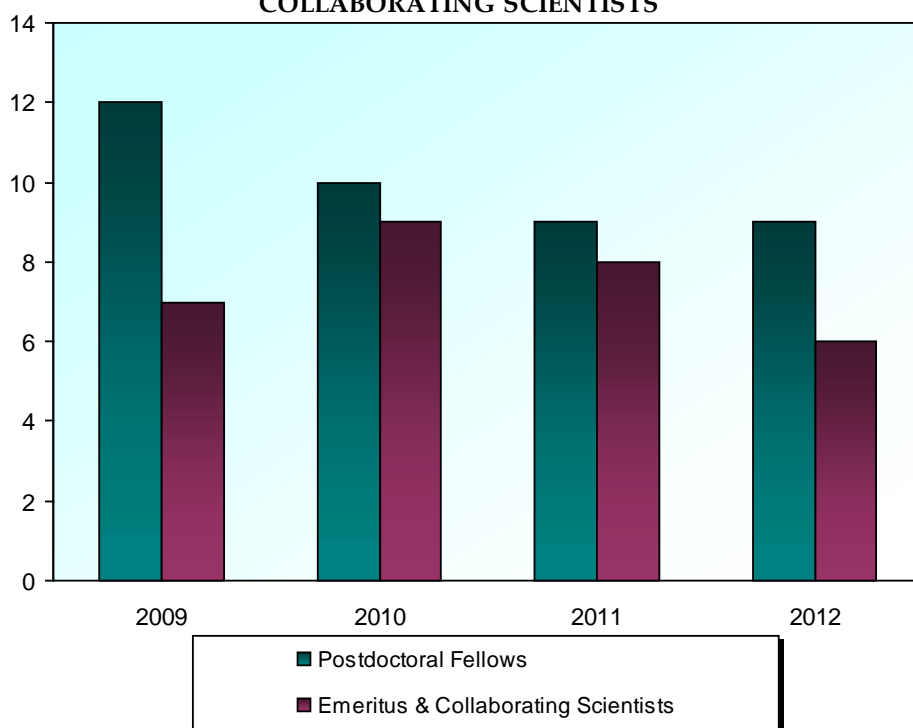
£ 2 presentations of the Human Tissue Bank in International Congresses are included

CHANGES OF IB-A STAFF DURING 2009-2012

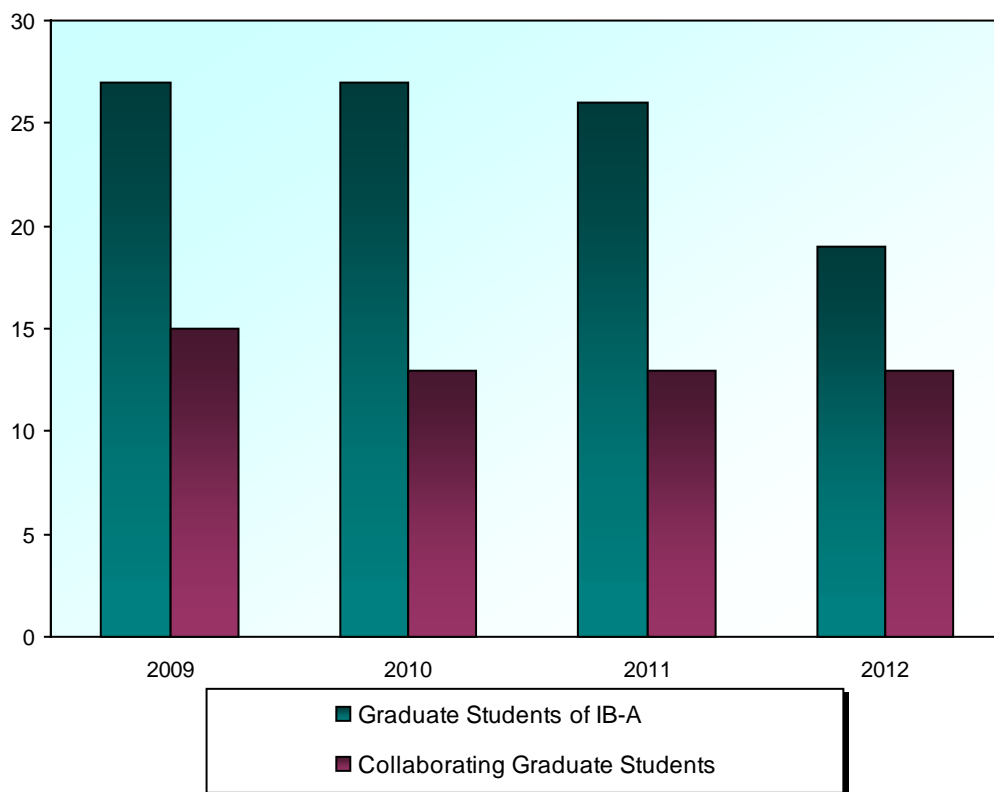
TENURED EMPLOYEES



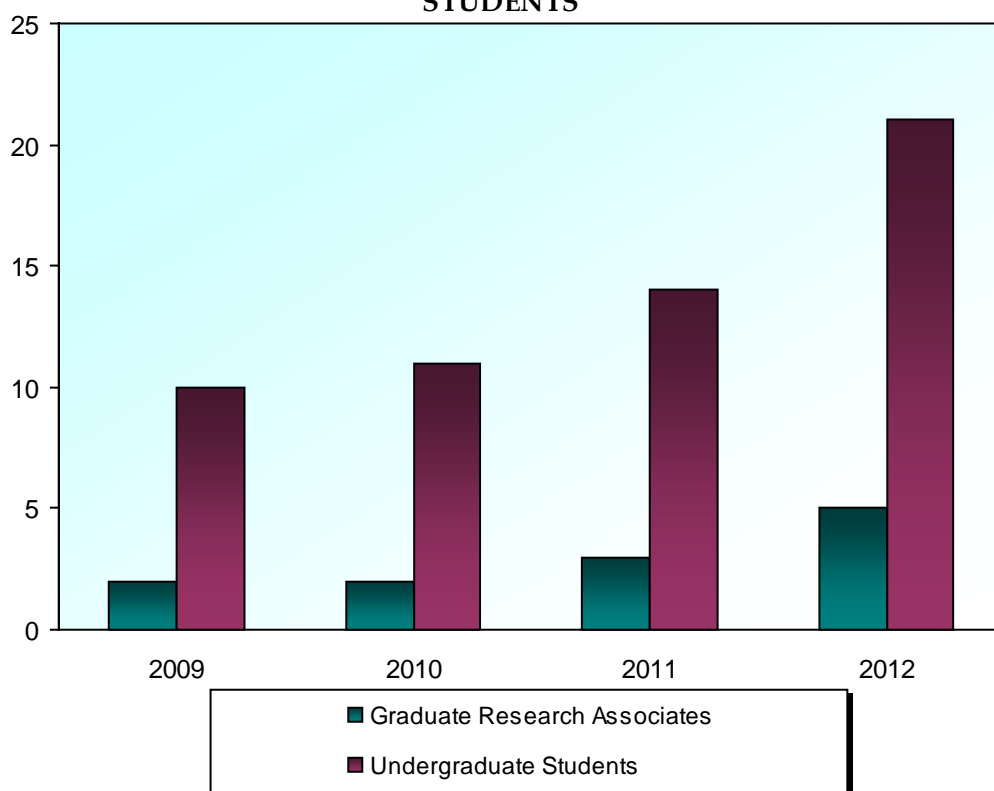
POSTDOCTORAL FELLOWS and EMERITUS & COLLABORATING SCIENTISTS



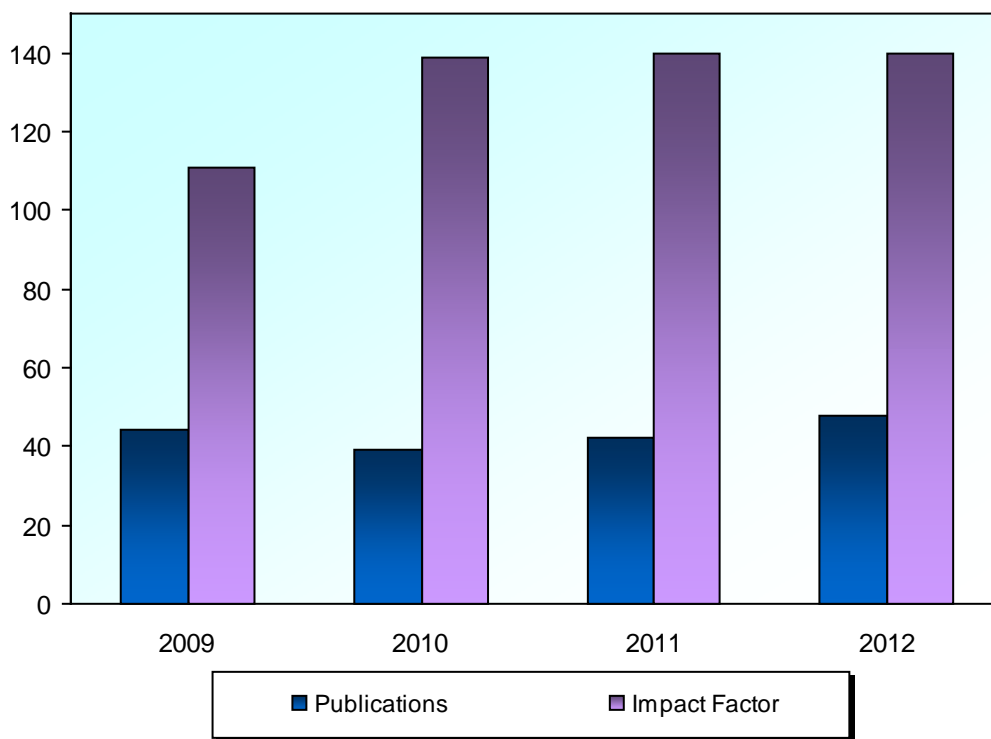
GRADUATE STUDENTS



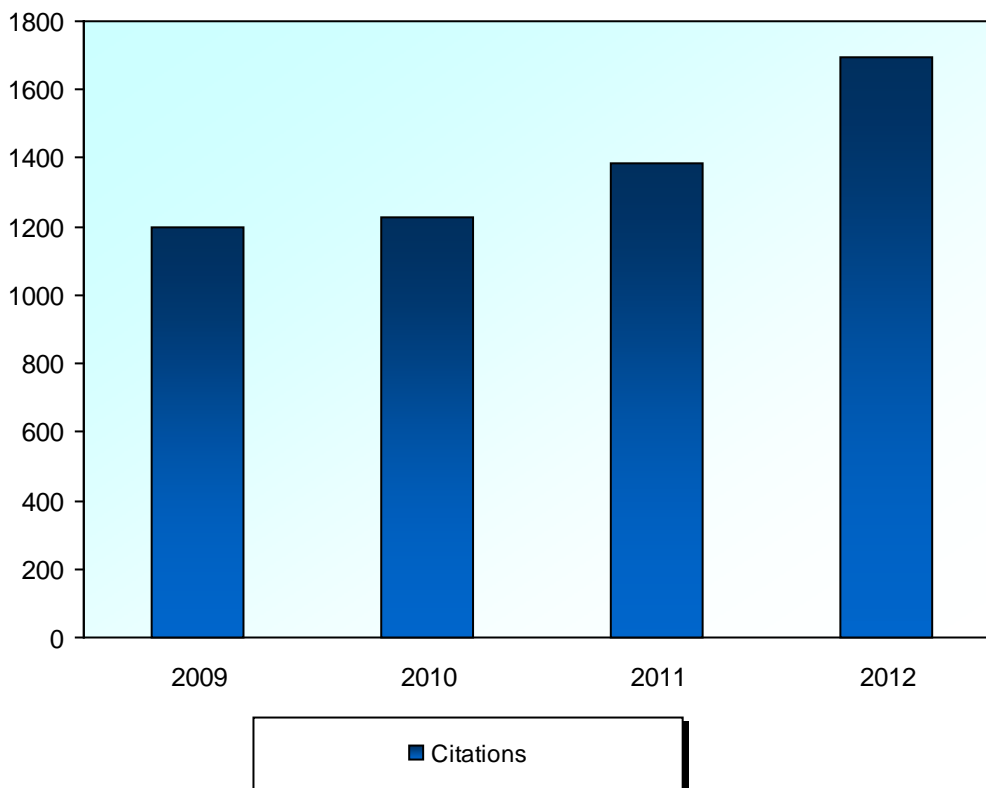
GRADUATE RESEARCH ASSOCIATES AND UNDERGRADUATE STUDENTS



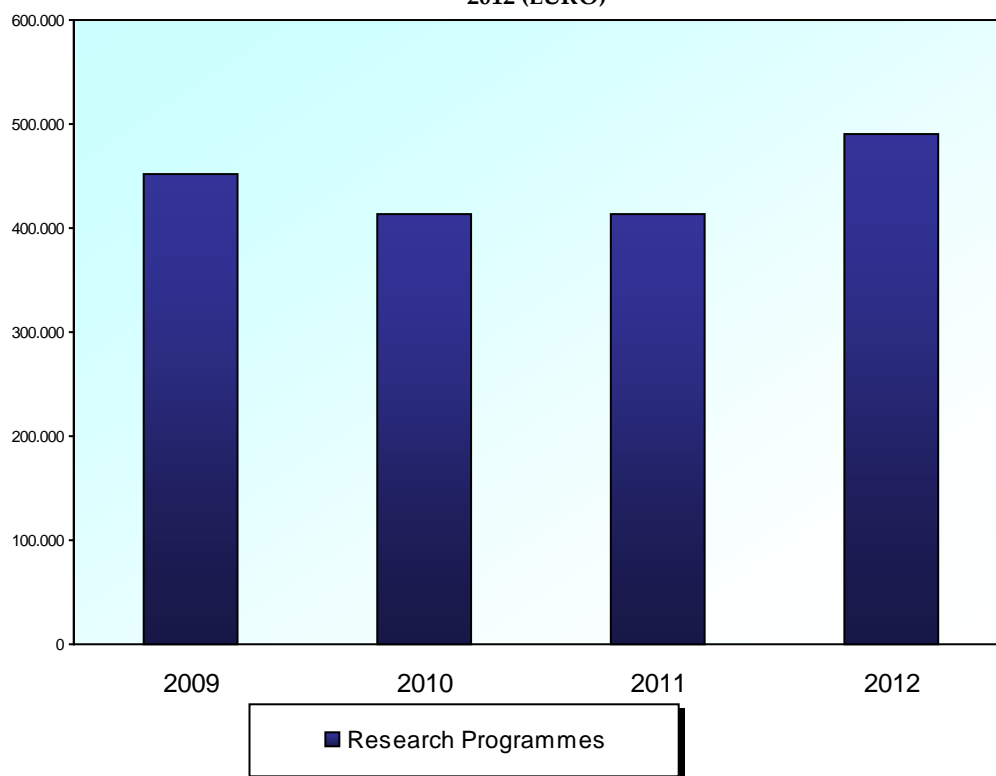
**PUBLICATIONS IN PEER-REVIEWED JOURNALS AND CUMULATIVE
IMPACT FACTOR DURING 2009-2012**



CITATIONS OF THE INSTITUTE 2009-2012



CUMULATIVE EXTERNAL FUNDING OF THE INSTITUTE DURING 2009-
2012 (EURO)





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