

**NATIONAL CENTRE FOR SCIENTIFIC RESEARCH
"DEMOKRITOS"**

INSTITUTE OF BIOLOGY

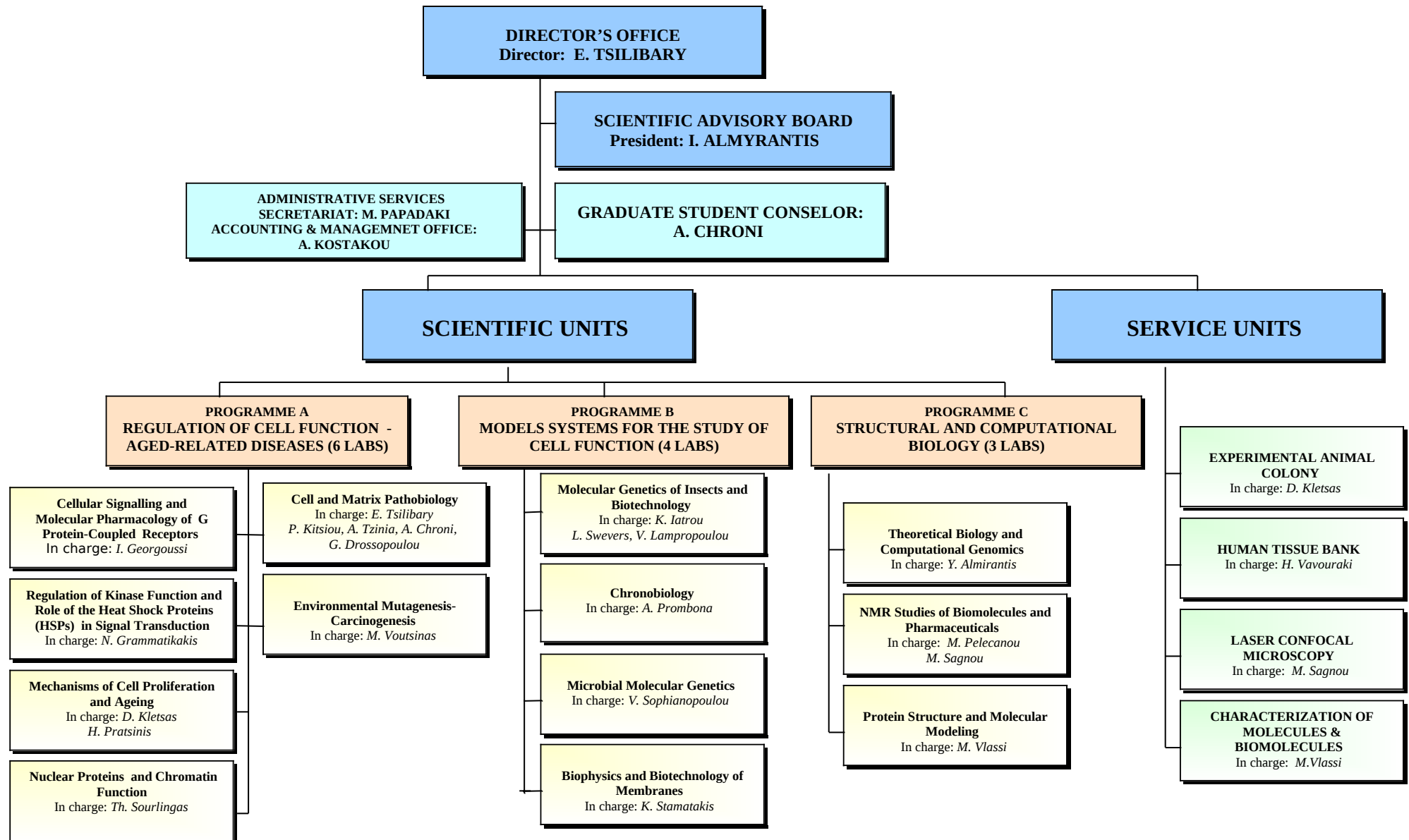
**2010
ANNUAL REPORT**

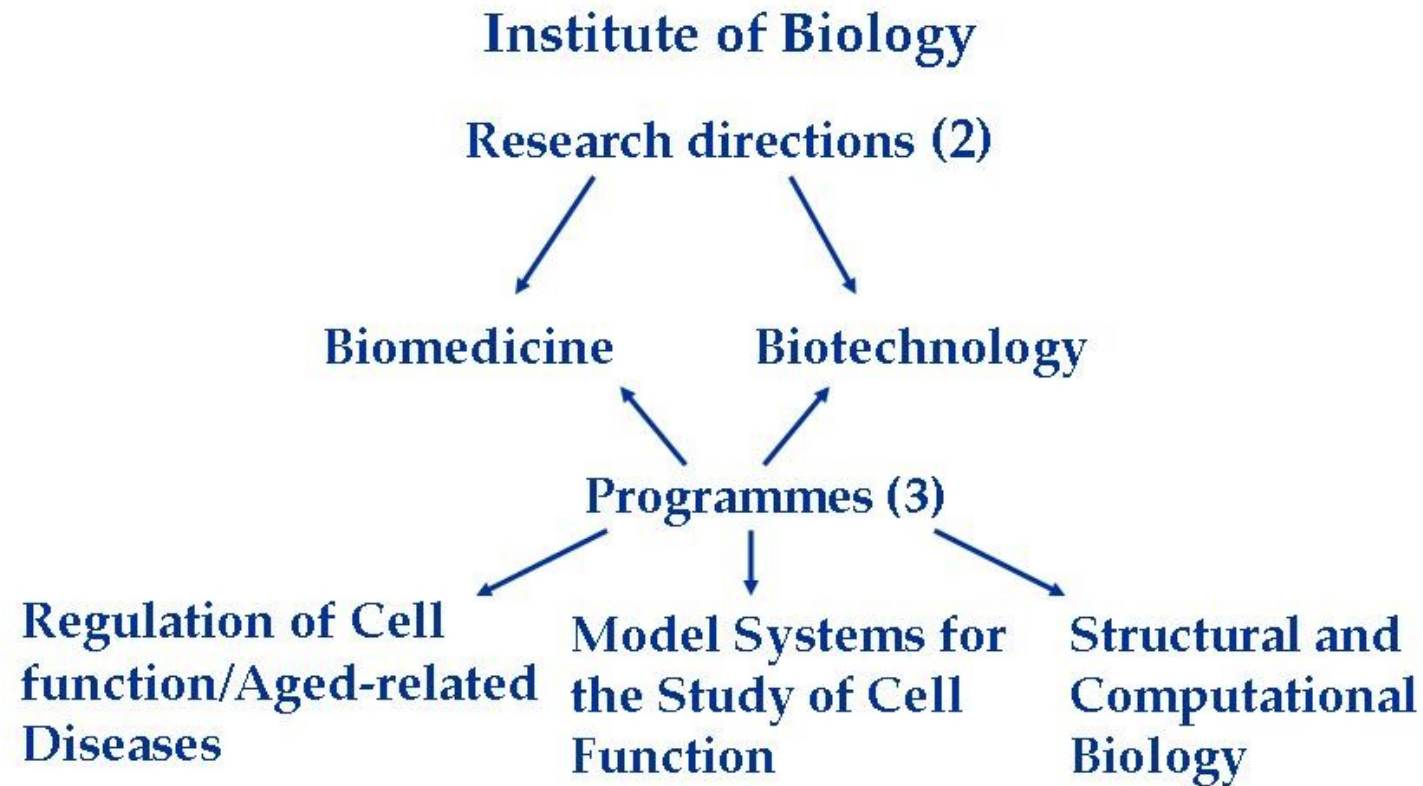
TABLE OF CONTENTS

| | |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| ORGANISATION CHART..... | 5 |
| RESEARCH DIRECTIONS AND PROGRAMMES OF THE INSTITUTE OF BIOLOGY..... | 6 |
| PERSONNEL | 7 |
| DIRECTOR | |
| PERSONNEL | 7 |
| DIRECTOR | 7 |
| ACTING DIRECTOR | 7 |
| SCIENTIFIC STAFF | 7 |
| RESEARCH TECHNICIANS | 8 |
| ADMINISTRATIVE STAFF | 8 |
| EMERITUS & COLLABORATING SCIENTISTS | 8 |
| POSTDOCTORAL FELLOWS | 8 |
| GRADUATE RESEARCH ASSOCIATES | 9 |
| COLLABORATING GRADUATE STUDENTS | 9 |
| UNDERGRADUATE STUDENTS AND OTHER IN TRAINING | 9 |
| INTRODUCTION | 11 |
| PROGRAMME A: | 12 |
| REGULATION OF CELL FUNCTION | 12 |
| AGED-RELATED DISEASES | 12 |
| Research Group: Environmental Mutagenesis -Carcinogenesis | 36 |
| 2. Allelic Imbalance of Expression and Epigenetic Regulation within the Alpha-Synuclein Wild-Type and p.Ala53Thr Alleles in Parkinson Disease | 36 |
| PROGRAMME B: | 40 |
| MODEL SYSTEMS FOR THE STUDY OF CELL FUNCTION | 40 |
| Research Group: Molecular Genetics of Insects and Biotechnology | 41 |
| Research Group: Pending (Collaboration with the laboratory of Molecular Genetics of Insects and Biotechnology –In charge: Dr. K. Iatrou) | 48 |
| PROGRAMME C: | 60 |

| | |
|------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| <u>STRUCTURAL AND COMPUTATIONAL BIOLOGY.....</u> | <u>60</u> |
| <u>Research Group: Theoretical Biology and Computational Genomics</u> | <u>61</u> |
| <u>Research Group: NMR Studies of Biomolecules and Pharmaceuticals</u> | <u>64</u> |
| <u>Research Group: Protein Structure and Molecular Modeling</u> | <u>70</u> |
| <u>SERVICE UNITS</u> | <u>73</u> |
| <u>HUMAN TISSUE BANK</u> | <u>74</u> |
| <u>EXPERIMENTAL ANIMAL COLONY.....</u> | <u>76</u> |
| <u>CHARACTERIZATION OF PROTEINS & BIOACTIVE MOLECULES</u> | <u>78</u> |
| <u>EDUCATIONAL ACTIVITIES.....</u> | <u>79</u> |
| <u>EDUCATION</u> | <u>80</u> |
| <u>COMPLETION/AWARD</u> | <u>82</u> |
| <u>OF DOCTORAL THESES IN 2010.....</u> | <u>82</u> |
| <u>Dimitra Bouzarelou</u> | <u>82</u> |
| <u>LECTURE CONTRIBUTIONS TO.....</u> | <u>83</u> |
| <u>THE 2010 SUMMER SCHOOL.....</u> | <u>83</u> |
| <u>OF THE NCSR “DEMOKRITOS”</u> | <u>83</u> |
| <u>(July 2010)</u> | <u>83</u> |
| <u>SEMINAR PROGRAMME 2010.....</u> | <u>84</u> |
| <u>INSTITUTE OF BIOLOGY</u> | <u>84</u> |
| <u>COLLECTIVE DATA</u> | <u>86</u> |
| <u>FINANCIAL REPORT 2010</u> | <u>87</u> |
| <u>3.EXTERNAL FUNDING FROM THE PROGRAMMES OF THE INSTITUTE (Programmes that are coordinated by the Head of IB are included).....</u> | <u>88</u> |
| <u>COLLECTIVE DATA ON PRODUCTIVITY OF SCIENTIFIC PROGRAMMES</u> | <u>89</u> |
| <u>2.....</u> | <u>89</u> |
| <u>CHANGES OF STAFF 2007 -2010.....</u> | <u>91</u> |
| <u>EXTERNAL FUNDING, PUBLICATIONS AND CUMULATIVE IMPACT FACTORS 2007 - 2010.....</u> | <u>93</u> |
| <u>CITATIONS 2007-2010</u> | <u>94</u> |

ORGANISATION CHART





PERSONNEL



DIRECTOR

Tsilibary Effie MD, Cell Biologist

ACTING DIRECTOR

Almirantis Yannis Chemist

SCIENTIFIC STAFF

Research Directors

| | |
|--------------------------|-------------------------------------------------|
| Almirantis Yannis | Chemist |
| Iatrou Kostas | Professor of Biochemistry and Molecular Biology |
| Kletsas Dimitris | Biologist |
| Pelecanou Maria | Pharmacist |
| Sophianopoulou Vassiliki | Biologist |
| Tsilibary Effie | MD, Cell Biologist |
| Vlassi Metaxia | Physicist-Chrystallographer |

Senior Researchers

| | |
|--------------------------|----------------|
| Chroni Aggelika | Biologist |
| Georgoussi Zafiroula-Iro | Biochemist |
| Grammatikakis Nikolaos | Cell Biologist |
| Labropoulou Vassiliki | Biochemist |
| Prombona Anastasia | Biologist |
| Stamatakis Konstantinos | Biologist |
| Swevers Luc | Biologist |
| Tzinia Athina | Biochemist |
| Voutsinas Gerassimos | Biologist |

Researchers

| | |
|-----------------------|-----------------|
| Kitsiou Paraskevi | Biologist |
| Konstantopoulou Maria | Biologist |
| Sourlingas Thomae | Biologist |
| Vavouraki Helen | Radiopharmacist |

Lecturers

| | |
|-------------------------|--------------------|
| Drossopoulou Garifallia | Biologist |
| Pratsinis Haris | Chemist |
| Sagnou Marina | Biologist/ Chemist |

Technical Specialists

| | |
|--------------------------|------------|
| Panagiotopoulou Angeliki | Biochemist |
| Stefanou Dimitra | Agronomist |

RESEARCH TECHNICIANS

Argyri Letta
 Avgeris Socrates
 Doulgeridis George
 Kakkos Stilianos
 Kopanelis Dimitris (Retired)
 Kotsopoulou Eleni
 Pantazi-Mazomenou Anastassia
 Stefou Vassiliki
 Zafiropoulos Ioannis

ADMINISTRATIVE STAFF

| | |
|---------------------|------------|
| Kostakou Athanassia | Accountant |
| Papadaki Margarita | Secretary |

EMERITUS & COLLABORATING SCIENTISTS

Emeritus Scientists

Ignatiadou Lydia (Dr. Hydrobiologist) - *Emeritus*
 Mamalis Anastasios (DDS)
 Martinou Kelly (PhD in Environment) - *Collaborating Scientist*

LLaboratory

Iatrou K.
 Vavouraki H.
 Konstantopoulou M

| | |
|----------------------------------------------------------------------------|-------------------|
| Papageorgiou George (Dr. Biochemist) - <i>Emeritus</i> | Stamatakis K. |
| Papageorgiou Spyros (Dr. Physicist) - <i>Emeritus</i> | Almirantis I. |
| Sekeri Kalliope (Dr. Biochemist) - <i>Emeritus</i> | Sourlingas Th. |
| Sideris Eleftherios (Dr. Geneticist) - <i>Emeritus</i> | Sophianopoulou V. |
| Stathakos Dimitrios (Dr. Biochemist) - <i>Emeritus</i> | Kletsas D. |
| Tsimilli - Michael Meropi (Dr. Biologist) - <i>Collaborating Scientist</i> | Stamatakis K. |

POSTDOCTORAL FELLOWS

Fellow

Agalou Adamantia (NCSR "Demokritos")
 Benaki Demetra (NCSR "Demokritos")
 Efrose Rodica (Programme)
 Furla Danai (Programme)
 Fragouli Apostolia (NCSR "Demokritos")
 Koussis Konstantinos (Programme)
 Mavrogonatou Eleni (Programme)
 Tsitoura Panagiota (Programme)
 Vamvakas Sotirios - Spiridon (NCSR "Demokritos")
 Vlachakis Dimitris (NCSR "Demokritos")

Supervisor

Georgoussi Z.
 Pelekanou M.
 Iatrou K.
 Georgoussi Z.
 Tsilibary E.
 Iatrou K.
 Kletsas D.
 Iatrou K.
 Kletsas K.
 Vlassi M.

GRADUATE STUDENTS

| Student | Supervisor |
|--------------------------------------------------------|-----------------------------------------|
| Aliberti Sofia (NCSR "Demokritos") | Grammatikakis N. |
| Armatas Andreas (NCSR "Demokritos") | Kletsas D. |
| Apostolou - karabelis Konstantinos (NCSR "Demokritos") | - <i>Rotation</i> |
| Athanassopoulos Alexandros (NCSR "Demokritos") | Sophianopoulou V. |
| Bouzarelou Dimitra (NCSR "Demokritos") | Sophianopoulou V. - <i>PhD obtained</i> |
| Daniil George (NCSR "Demokritos") | Chroni A. |
| Dafnis Ioannis (Programme) | Chroni A. |
| Dimozi Anastasia (NCSR "Demokritos") | Kletsas D. |
| Galeou Aggeliki (NCSR "Demokritos") | Prombona A. |
| Georganta Irene (Programme) | Georgoussi Z. |
| Handris Panagiotis (NCSR "Demokritos") | Kletsas D. - <i>PhD obtained</i> |
| Ioannidis Konstantinos (NCSR "Demokritos") | Iatrou K. |
| Kapodistria Katerina (NCSR "Demokritos") | Kitsiou P. |
| Karkoulis Panagiotis (NCSR "Demokritos") | Voutsinas G. |
| Kolliopoulou Anna (NCSR "Demokritos") | - <i>Rotation</i> |
| Konstantinou Vassos (Programme) | Kletsas D. |
| Koutmos Theodore (NCSR "Demokritos") | Tsilibary E. |
| Kostomiri Mirto (NCSR "Demokritos") | Pelecanou M. |
| Leontiadis Leonidas (NCSR "Demokritos") | Georgoussi Z. |
| Magkrioti Christiana (Programme) | Iatrou K. |
| Papadopoulou Adamadia (Programme) | Kletsas D. |
| Papakonstantinou Maria (NCSR "Demokritos") | Georgoussi Z. |
| Repouskou Anastasia (NCSR "Demokritos") | Prombona A. |
| Salpea Paraskevi (NCSR "Demokritos") | Sourlingas Th. |
| Tsotakos Nikos (NCSR "Demokritos") | Tsilibary E. |
| Vaggelatos Ioannis (NCSR "Demokritos") | Sophianopoulou V. |
| Xidous Marios (NCSR "Demokritos") | Sourlingas Th. |

GRADUATE RESEARCH ASSOCIATES

| Fellow | Supervisor |
|--------------------------------------|-------------------|
| Rapropoulos Dimitris (Dr. Biologist) | Iatrou K. |
| Sellis Diamadis (<i>MSc</i>) | Vlassi M. |

COLLABORATING GRADUATE STUDENTS

| Student (University) | Supervisor |
|----------------------------------------------------------|-------------------------------------|
| Anastassiou Dimitra (Univ. of Athens) | Voutsinas G. |
| Athanassopoulou Labrini (Athens Polytechnic School) | Almyrantis I. - <i>MSc obtained</i> |
| Fotopoulos Vassilis (Univ. of Ioannina) | Tzinia A. |
| Kachrilas Stefanos (Univ. of Athens) | Voutsinas G. |
| Klimopoulos Alexandros (Univ. of Athens, <i>MSc</i>) | Almyrantis I. - <i>MSc obtained</i> |
| Konstantatou Evmorphia (Univ. of Athens) | Voutsinas G. |
| Lagopati Nefeli (Athens Polytechnic School) | Tsilibary E./Tzinia A. |
| Leventis Minas (Univ. of Athens) | Vavouraki H. |
| Polichronopoulos Dimitris (Univ. of Athens, <i>MSc</i>) | Almyrantis I. |
| Peristeri Eleutheria (Univ. of Athens, <i>MSc</i>) | Voutsinas G. |
| Rapti Maria - Aikaterini (Univ. of Athens, <i>MSc</i>) | Kletsas D. |
| Tsiagas Ioannis (Univ. of Athens, <i>MSc</i>) | Almyrantis I. |
| Verouti Sofia (Univ. of Athens) | Tsilibary E. . |

UNDERGRADUATE STUDENTS AND OTHER IN TRAINING

| Student (University) | Supervisor |
|-----------------------------|-------------------|
|-----------------------------|-------------------|

Arvaniti Maria (Univ. of Athens)
Bassoyianni Angeliki - Stephania (Univ. of Athens)
De Wilde Ruben (Univ. of Gent, Belgium)
Georgakopoulos Soares Elias (European School
Brussels III)
Ioannidi Konstantia (Univ. of Patras)
Krezias George (Univ. of Athens)
Mender Ilgen (Hacettepe University, Turkey)
Pantazopoulou Vassiliki (Univ. of Athens)
Panteleri Rafaela (Univ. of Thessaloniki)
Papadopoulou Natalia (Univ. of Athens)
dissertation completed

Tsilibary E./Tzinia A.
Voutsinas G.
Swevers L.
Stamatakis K.
Sophianopoulou V.
Sophianopoulou V.
Kletsas D.
Sophianopoulou V.
Konstantopoulou M.
Kletsas D. - *undergraduate*

INTRODUCTION



The Institute of Biology (IB) is one of eight institutes of National Center for Scientific Research (NCSR) Demokritos. The Center has the unique feature of combining scientists with different expertise who conduct basic research and collaborate. The thrust is optimal research and technology progress in the thematic areas covered by research interests of researchers from the different institutes.

The IB with 23 faculty members, has recently obtained significant new and upgraded equipment through competitive funding for infrastructure for the total amount of over 500.000 €; Two major service laboratories, the animal colony and the human tissue bank have been certified by international standards (ISO 9000/2001); these laboratories are gradually upgraded by adding new equipment, offices and/or office space, and space for experimentation.

The IB has significant independent and collaborating activities aiming at internationally competitive research and the achievement of excellence. One of the features of the IB is multidisciplinary biomedical and biotechnological research, evident from the nature of the three programs and the published research papers as well. Interdisciplinary research related to life sciences and the environment is a unique and characteristic advantage (an “earmark”) of NCSR Demokritos and the IB as well. Presently, the conducted interdisciplinary research focuses on the environment, the development of innovative molecules and biomolecules for diagnostic and therapeutic use, nanomaterials for medical and imaging purposes, targeted drug delivery, etc. These activities form a dynamic research cluster which includes many IB researchers. Moreover, the main independent IB activities focus on the one hand on biomedical research with different biochemical, cellular, molecular, pharmaceutical, proteomic and other approaches; on the other hand, biotechnological research is performed related to the environment, using similar approaches. Finally, structural and theoretical approaches make up one more research direction of the IB.

Retired, emeritus researchers proved to be very active once again contributing publications, seminars, participation to IB research projects etc, thus being a significant part of IB productivity.

I wish to thank the members of the IB scientific advisory committee (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. Vlassi, who substantially contributed to a smooth operation of the IB and helped with my numerous administrative tasks.

Despite the various obstacles and difficulties (particularly intense this year due to the financial crisis), 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 towards excellence. It is my belief that based on objective indexes such as: funding, number of publications, citations, etc, the IB progressively becomes recognized as an internationally competitive institute. I have trust in the research potential of IB researchers who on a daily basis prove their contribution and keep trying harder and getting better, and I extend to all IB members my best wishes for continuous success and recognition.

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

Effie C. Tsilibary, MD, PhD

Director of IB
March 2011

**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, Senior Researcher

Adamantia Agalou, Postdoctoral Fellow

Danai Fourla, Postdoctoral Fellow

Leonidas Leontiadis, Graduate Student

Irene Georganta, Graduate Student

Maria Papakonstantinou, Graduate Student

Research Interests

The research interests of our group are focused on the **elucidation of the molecular mechanisms governing the heptahelical G protein-coupled receptors (GPCRs) signalling**. We use as a model system the opioid receptors because of their involvement in pain perception and in mechanisms related to tolerance and dependence upon chronic drug administration.

More specifically our objectives aim to identify:

- ◆ novel opioid-or other GPCR interacting partners and signaling pathways in order to define novel pharmacological factors
- ◆ transcription factors and genes whose action is altered upon opioid receptor activation,
- ◆ and characterize pharmacologically novel ligands that bind to the μ , δ and κ -opioid receptors as potential new compounds- analgesics- to alleviate chronic pain.

2010 Findings

Novel interacting partners of the μ -, δ - and κ - opioid receptors

In an attempt to investigate the signaling pathways mediated upon activation of the **μ , δ and κ -opioid receptors** (μ -OR, δ -OR, κ -OR) we found a novel interacting protein such as the **Regulator of G protein Signalling, RGS4** protein. To further understand the mode of action of RGS proteins in opioid signalling we investigated whether selectivity of coupling between the μ -OR, δ -OR and κ -OR with other members of RGS proteins exist. We identified that RGS2 interacts differentially with the three opioid receptor subtypes and demonstrated the ability of this protein to modulate opioid receptor signalling. At the same time we demonstrated that spinophilin, a neural scaffolding protein, interacting with RGS2 and RGS4, interacts also with μ -OR and δ -OR. Binding of spinophilin modifies cAMP levels and MAPK phosphorylation mediated by both opioid receptors, thus establishing spinophilin as a new interacting partner of opioid receptor function.

Activation of transcription factors upon δ -opioid receptor stimulation and their role in neurite outgrowth

In an attempt to elucidate the molecular mechanisms involved in the phosphorylation of transcription factors after opioid administration, we demonstrated that the δ -opioid receptor (δ -OR) serves as a platform for the formation of a multi-component signalling complex, consisting of p-STAT5B- c-Src kinase and specific subunits of G proteins. This "signalosome" formed is implicated in cell survival and plays a critical role in neurite length as we detected in various neuronal cells upon δ -OR activation with specific agonists (Fig. 1).

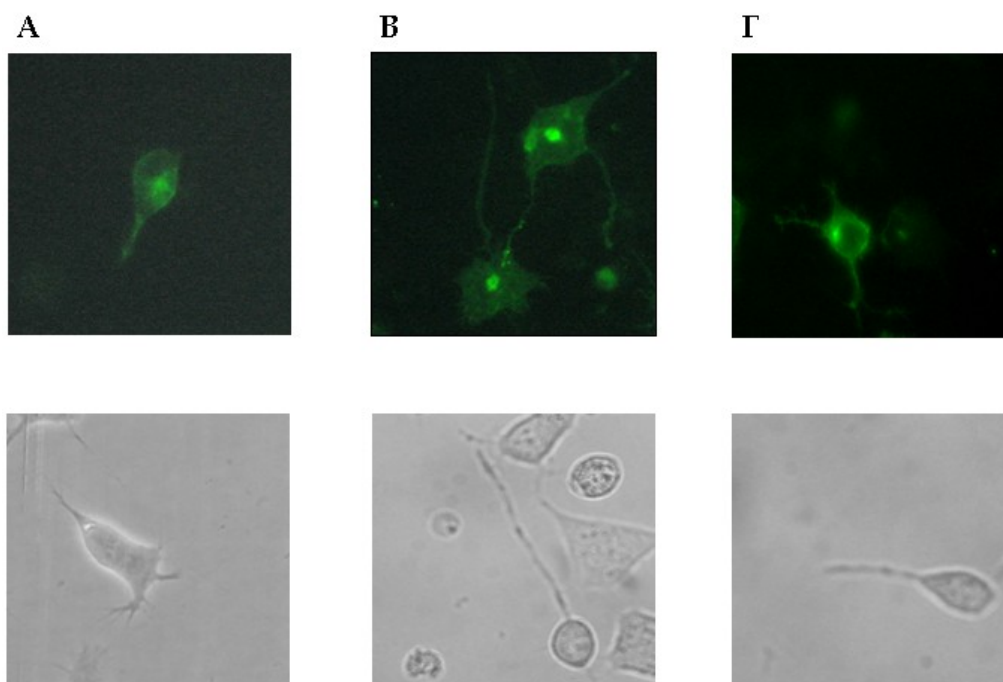


Fig. 1. Neurite outgrowth of Neuro2A cells (A) without addition of opioid agonist, (B) upon δ -OR stimulation with DSLET. (Γ) Reduction of neurite length upon expression of a negative mutant of STAT5B confirming the involvement of STAT5 transcription factor in neurite growth

Pharmacological evaluation of new selective compounds targeting the different opioid receptors

Under the 6th Framework we participate in the EU consortium "NORMOLIFE" (LSHC-CT2006-037733). Our group characterized new pharmacological ligands (synthesized by other members of the consortium) that bind to μ -OR, δ -OR or κ -opioid receptors, with mixed agonistic and antagonistic properties, displaying also potent *in vivo* analgesic effects as assessed using brain-region specific primary network co-cultures and microelectrode neurochip technology prior to animal studies.

Molecular mechanisms mediated by the olfactory receptors of the mosquito *Anopheles gambiae*

In collaboration with the group of Insect Molecular Genetics and Biotechnology of the Institute of Biology headed by Prof. K. Iatrou and under the 7th FP framework we also participate in a second EU consortium termed "ENAROMaTIC". Our efforts focused on the elucidation of the signalling mechanisms regulating the *Anopheles* mosquito olfactory receptors. We found that the mosquito OR1 and OR2, odorant receptors, exhibit a reverse orientation as compared to the classical GPCRs. Studies on the binding parameters of these receptors using tritiated ligands is under investigation.

Publications

Georganta, E-M., Agalou, A., Georgoussi, Z. (2010). Multi-component signaling complexes of the δ -opioid receptor with STAT5B and G proteins. *Neuropharmacology* 59, 139-148.

Tsitoura, P., Andronopoulou, E., Tsikou, D., Agalou, A., Papakonstantinou, M-P, Kotzia, G.A., Labropoulou, V., Swevers, L., Georgoussi, Z., Iatrou K. (2010) Expression and Membrane Topology of *Anopheles gambiae* Odorant Receptors in Lepidopteran Insect Cells. *PLoS ONE*, 5(11): e15428.

Articles in Press

Georgoussi, Z., Georganta, E-M., Milligan, G. (2011). The other side of opioid receptor signalling: Regulation by protein-protein interaction. *Curr. Drug Targets, in press* (i.f. 3.932).

Vandormael, B., Fourla, D., Gramowski, A., Weiss, D., Georgoussi, Z. and Tourwé, D. (2011) Design and Synthesis of Dmt1-[dermorphin] tetrapeptide as μ/δ opioid receptor agonists. *Submitted to the J. Med.Chem.*

Pasquinucci, L., Parenti, C., Turnaturi, R., Aricò, G., Marrazzo A., Prezzavento O., Ronsisvalle S., Georgoussi Z., Fourla D-D., Scoto G-M., and Ronsisvalle G. (2011) The benzomorphan-based LP1 ligand is a suitable MOR/DOR agonist for chronic pain treatment. *Submitted to Life Sciences.*

Articles in Books and Conference Proceedings

Leontiadis, L. J., Papakonstantinou M -P. and Georgoussi Z. (2010) Regulator of G protein signaling 4: a novel regulator of μ - and δ -opioid receptor signaling. *Review of Clinical Pharmacology and Farmakokinetikes International Edition*, 24 (2), 168-170,

Georganta, E-M., Agalou, A., Georgoussi, Z. STAT5B forms dynamic complexes with the δ -opioid receptor and selective G protein subunits. *Review of Clinical Pharmacology and Farmakokinetikes International Edition*, 24 (2), 91-94

Georgoussi, Z (2010) «Seven Transmembrane Receptors and G proteins: Health, pathogenesis and development of new drugs » Summer School N.C.S.R. "DEMOKRITOS" p.117-118

Presentations at Scientific Conferences

M-P. Papakonstantinou, L.J. Leontiadis, F. Nikolos, M. Sarris and Z. Georgoussi (2010) RGS2 and RGS4 proteins differentially modulate delta and kappa opioid receptor signaling. 35th FEBS Congress, June 26 – July 1, 2010 Göteborg, Sweden (Poster and short oral presentation).

E-M. Georganta, A. Agalou, Z. Georgoussi (2010). STAT5B forms dynamic complexes with the δ -opioid receptor and selective G protein subunits. 6th Panhellenic Congress of Pharmacology, June 4-6, 2010, Heraklion, Crete, Greece (oral presentation).

L. J. Leontiadis, M -P. Papakonstantinou and Z. Georgoussi. (2010) Regulator of G protein signaling 4: a novel regulator of μ - and δ -opioid receptor signaling. 6th Annual Meeting of the Greek Society of Pharmacology, June 4-6, Heraklion, Crete (Poster)

L. Pasquinucci, R. Turnaturi, C. Parenti, G. Aricò, G. M. Scoto, Z. Georgoussi, D.-D. Fourla and G. Ronsisvalle. New benzomorphan-based lp1 ligand as suitable mixed mop/dop receptors agonist for chronic pain treatment in: *Molecular Targets for novel pain therapeutics, From Basic Research to Clinical Translation*, 22-24 September 2010, Calabria, Italy

M-P Papakonstantinou, L. Leontiadis, F. Nikolos, M. Sarris and Z. Georgoussi. (2010) Regulators of G protein Signaling RGS4 and RGS2: novel modulators of delta and kappa opioid receptor signaling. Neuroscience Days of the Hellenic Society of Neurosciences, October 1-2, Athens (poster)

D.D. Fourla, A. Agalou, E.-M. Georganta, M.-P. Papakonstantinou, Z. Georgoussi. Spinophilin is a novel interacting partner of the δ -opioid receptor. Neuroscience Days of the Hellenic Society for Neuroscience, 1-2 October 2010, Athens, Greece (poster).

P. Tsitoura, A. Lioupis, L. Swevers, Z. Georgoussi, K. Iatrou. Functional expression and characterization of the human 5-hydroxytryptamine 4a receptor in insect cells. Neuroscience Days of the Hellenic Society for Neuroscience, 1-2 October 2010, Athens, Greece (poster).

E-M. Georganta, A. Agalou, Z. Georgoussi (2010). A signalosome formed between the δ -opioid receptor, STAT5B and G protein subunits: possible mediator of neurite outgrowth. Neuroscience Days of the Hellenic Society for Neuroscience, October 1-2, 2010, Athens, Greece (poster).

M-P. Papakonstantinou, L.J. Leontiadis, F. Nikolos, M. Sarris and Z. Georgoussi Z. (2010) Delta and kappa opioid receptor signaling is differentially modulated by RGS4 and RGS2 proteins, 61st Conference of the Hellenic Society for Biochemistry and Molecular Biology, October 15-17, Alexandroupolis (Oral Presentation)

E-M. Georganta, A. Agalou, Z. Georgoussi (2010). A novel dynamic complex between the delta-opioid receptor, STAT5B and selective G protein subunits: possible mediator of neuronal survival and neurite outgrowth. 61st Greek Conference of Biochemistry and Molecular Biology, October 15-17, 2010, Alexandroupoli, Greece (poster).

P. Tsitoura, Lioupis, A., Swevers, L., Georgoussi, Z. and Iatrou, K. (2010). Expression of transmembrane receptors in insect cells: biochemical properties and functional assays. 61st Conference of the Hellenic Society for Biochemistry and Molecular Biology, October 15-17, Alexandroupolis, Greece (poster).

L. Schultz, O. H.-U. Schroeder, K. Jügelt, A. W. Lipkowski, A. Misicka-Kesik, Z. Georgoussi, D. Tourwé, D. G. Weiss, A. Gramowski. New multi-target opioid peptides in drug development of cancer pain. 40th Annual Meeting, Society of Neuroscience 13-17 November 2010, San Diego, CA, USA

Educational Activities

Coordinator of the postgraduate lecture studies of the Institute of Biology in 'Signal Transduction Mechanisms'

Supervision of the Ph.D theses of the graduate students E. Georganta L. Leontiadis, and M. Papakonstantinou (Universities of Athens and Patras)

Member of the Recruitment Committee of postdoctoral fellows in the I.B.

Member of the examination Committee for the recruitment of new postgraduate students at the IB (Biochemistry)

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.

Lecturing on the Postgraduate course in the Sector of Biochemistry and Molecular Biology in Department of Biology of the University of Athens, with the title "7TM Receptors and G proteins in health and disease".

Teaching in the Postgraduate course on "Molecular Base of Human Diseases" of the University of Athens Sector of Biochemistry and Molecular Biology in the Department of Biology, December 2010, 2 hours, 25 students.

Graduate Program, "Molecular basis of Human Disease" of the University of Athens Department of Biochemistry and Molecular Biology, University of Athens, December 2010, 2 hour lecture with title: G proteins in health and disease".

Two-hour presentation with the title «Seven Transmembrane Receptors and G proteins: Health, pathogenesis and development of new drugs » Summer School N.C.S.R. "Demokritos"

Orientation and briefing on the latest developments in research and technology, Summer School 2010 of N.C.S.R. "DEMOKRITOS, «Seven Transmembrane Receptors and G proteins: Health, pathogenesis and development of new drugs »

Participation in the Educational Exchange Program of the University of Catania, Sicily, Italy

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

Research Seminars held by I. Georganda and M. Papakonstantinou under the postgraduate program of the IB.

Other Scientific Activities

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

Reviewer in: Molecular Pharmacology, Journal of Neurochemistry, Journal of Pharmacology and Experimental Therapeutics, Cellular Signaling, Neuropharmacology, Neuropharmacology Journal of Biotechnology, Physiological Genomics and Journal of Neuroscience.

Reviewer of grant proposals of the General Secretary for Research of Cyprus (RPF), Cyprus Chair of the Organizing Committee for "Neuroscience Days 2010" of the Hellenic Society for Neurosciences (1-2 October 2010)

Member of the Scientific Organizing Committee of the International Neuroscience conference (August 2011)

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

Member of the WISE (Working group of Women in Science) of FEBS

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

Advisor of women scientists under the auspices of the Hellenic Association of Women Scientists AMALIA FLEMING

Member of the Organizing Committee of the 62nd Panhellenic Conference of Biochemistry and Molecular Biology, December 2011, Athens

Other Distinctions and Awards

Financial Award of the Greek Society for Neurosciences in Neuroscience Days Conference of 2010 for the work by M. Georganta, A. Agalou, Z. Georgoussi (2010), entitled «*A signalosome formed between the δ -opioid receptor, STAT5B and G protein subunits implicated in neuronal survival and neurite outgrowth*».

Other Activities for the Institute of Biology

Member of the committee for postdoctoral researchers in the Institute of Biology

Member of the examination committee for recruitment of graduate students (scholarship) in the Institute of Biology

Member of the Committee for organization of seminars "*Road ideas*" of the IB

Scientific responsible for functioning of special equipment of the Institute of Biology

Impact factor (for 2 publications):8,26

Citations for 2010 (without self citations): 28

Citations for 2006-2010 (without self citations): 111

h-factor: 13

Current external funding

Research Program entitled *NORMOLIFE -Development of new therapeutic substances and strategies for treatment of pain patients with advanced stages of cancer*, funded by the European Union coordinated by: Prof. A. Lipkowski, with responsible Scientist from Greece: Z. Georgoussi

Duration: 12/2006-12/2009

Total funding of the program (*consortium*): 2.039.925 €

Total funding (of the lab): 541.331 €

Funding of the lab for the year 2010: 61.951,71 €.

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 2010: 12.000 €

Pending proposals:

- Proposal EU 7FP Marie Curie Mobility Actions-Industry Academia Partnerships and Pathways (IAPP) entitled "*Neurotensin and opioid receptor heterodimers and multivalent ligands: A strategy for developing new analgesics devoid of undesired side effects*" with the Polish Academy of Sciences (Prof. A. Lipkowski, Polish Academy of Sciences) and the German company NeuroProof GmbH (Dr Olaf Schroeder), Brussels July 27, 2009 (Coordinator of consortium: Dr Z. Georgoussi)
- Submission of proposal DISCA **D**istinguished **I**nternational **S**cientist **C**ollaboration **A**ward **N**ational Institute on **D**rug **A**buse in Collaboration with Dr T. Shippenberg, Integrative Neuroscience Branch, Baltimore, USA on: "*Dysregulation of glutamate transmission in addiction pathogenesis*" , December 31st, 2010 (Coordinating scientist: Z. Georgoussi)
- Bilateral joint proposal cooperation between Hungary-Greece (ESPA 2007-13) entitled "*Selective opioid-neurotensin mosaic peptide analogs targeting heterodimerization between respective receptors*" Prof. G. Toth, Hungarian Academy of Science, GSRT, September 25, 2009 (Coordinating scientist: Z. Georgoussi)

Note: Dr Georgoussi's group has submitted the following proposals under evaluation for the program «THALIS» of the General Secretary of Research and Technology as Central Research Group:

- Title: "*Structure and function of CRF₁: design and synthesis of novel CRF₁-selective anxiolytic and anti-depressant drugs*" Coordinator: Prof. G. Liapakis, University of Crete (Coordinating I.B. scientist: Z. Georgoussi)
- Title: "*Prostanoid receptors in the development of insects - study of the signal transduction mechanisms and detection of agonists and antagonists in natural products*". Coordinator: Dr D. Skarlatos, University of Athens (participating scientist: Z. Georgoussi)
- Title: "*Interdisciplinary approach and study of the activation mechanisms, intracellular signaling and biological-pharmacological actions of transmembrane receptors*" Coordinator: Dr M. Mangoura, BRFAA (Coordinating I.B. scientist: Z. Georgoussi)

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).

Citations 2010 (without self- citations): 57

Total Citations 2006-2010 (without self- citations): 405

h-factor: 17

Research Group: Mechanisms of Cell Proliferation and Ageing

Research Staff

Dimitris Kletsas, Research Director

Haris Pratsinis, Lecturer

Dimitrios Stathakos, Emeritus Scientist

Sotirios-Spyridon Vamvakas, Postdoctoral Fellow

Eleni Mavrogonatou, Postdoctoral Fellow

Panagiotis Handris, Graduate Student- *Phd obtained in 2010*

Adamantia Papadopoulou, Graduate Student

Anastasia Dimozi, Graduate Student

Andreas Armatas, Graduate Student

Vassos Constantinou, Graduate Student

Maria - Aikaterini Rapti, Collaborating Graduate Student (*MSc*)

Ilgen Mender, Visiting Graduate Student (*ERASMUS Programme*)

Natalia Papadopoulou, Undergraduate Student - *Undergraduate dissertation completed in 2010*

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 the senescent stromal fibroblasts and adjacent cancer cells. Emphasis is given in 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.

2010 Findings

In our laboratory the role of growth factors in tissue repair is being studied; having in mind the different repair strategies between fetuses and adults, we studied the effects of amniotic fluid, i.e. the physiological milieu of fetal cells. We have shown that amniotic fluid stimulates the proliferation of both fetal and adult human skin fibroblasts. This stimulation is due to the presence of bFGF and PDGF, and is mediated through the MEK/ERK and PI3K/Akt signaling pathways. In addition, in this context, TGF- β was also studied. Although we have previously shown in human skin fibroblasts that this growth factor inhibits the proliferation of fetal cells while in stimulates adult ones, regarding human lung fibroblasts it inhibits cells from both developmental stages through the Smad signaling pathway, suggesting differential roles of TGF- β in the homeostasis of these two tissues.

The proliferative behaviour of intervertebral disc cells was also investigated: it was discovered that exogenous and autocrine growth factors stimulate the proliferation of these cells through the activation of the MEK/ERK and PI3K/Akt pathways. Through immunohistochemical studies in human disc samples, we have identified activation of these two pathways in vivo, especially in cell clusters characterized by intense proliferation (Figure 1). Furthermore, we have shown that the response of disc cells to the above-mentioned growth factors is diminished under conditions of hyperosmotic

stress, while it is enhanced under the hypo-osmotic conditions prevailing in the degenerated discs, possibly leading to increased proliferation and tissue repair.

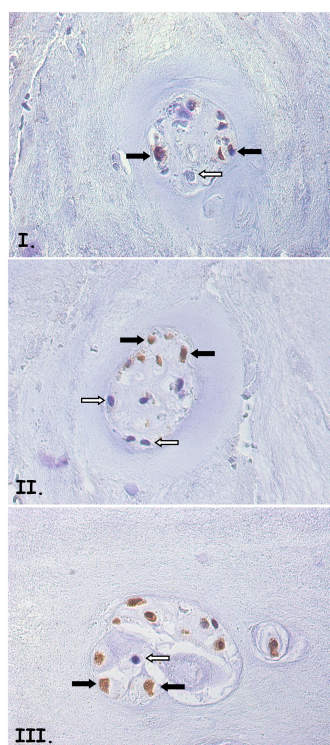


Figure 1. Immunohistochemical localization of intervertebral disc cells expressing Ki-67 (I.), as well as activated ERK (II.) and Akt (III.). Black arrows indicate positive stained cells, while white ones negative.

Main goal of the laboratory is the study of the morphological and functional features of senescent cells, as well as their role in the development of age-related diseases including cancer. We have shown that in senescent fibroblasts the mRNA processing machinery is compromised, a fact implicating also the ATR kinase. Furthermore, the ageing of stromal fibroblasts after induction by ionizing radiation or anticancer compounds, and especially their role in the growth of adjacent cancer cells was also studied. In addition, we continued our research on the mechanisms leading to premature ageing of intervertebral disc cells after various stresses (osmotic, oxidative, and mechanical) related to the physiology of these cells.

Finally, we keep on investigating the cytostatic/cytotoxic, anti-ageing and wound healing activities of natural products and novel synthetic compounds, as well as the effects of materials used in various therapeutical procedures on the homeostasis of the adjoining cells. In this context, we have shown that TEGDMA, a resin used in dental praxis, inhibits the proliferation of gingival fibroblasts through the p53—p21^{WAF1}—pRb pathway, most probably as a cell defence against the genotoxic effects of this compound.

Publications

Chandris, P., Giannouli, C.C., Panayotou, G., Kletsas, D. (2010). Compromise in mRNA processing machinery in senescent human fibroblasts: implications for a novel potential role of Phospho-ATR (ser428). *Biogerontology* 11, 421-436

Chrissouli, S., Pratsinis, H., Velissariou, V., Anastasiou, A., Kletsas, D. (2010). Human amniotic fluid stimulates the proliferation of human fetal and adult skin fibroblasts: the roles of bFGF and PDGF and of the ERK and Akt signalling pathways. *Wound Repair Regen.* 18, 643-654.

Mavrogonatou, E., Eliades, T., Eliades, G., Kletsas, D. (2010). The effect of triethylene glycol dimethacrylate on p53-dependent G2 arrest in human gingival fibroblasts. *Biomaterials* 31, 8530-8538.

Mavrogonatou, E., Kletsas, D. (2010). Effect of varying osmotic conditions on the response of bovine nucleus pulposus cells to growth factors and the activation of the ERK and Akt pathways. *J. Orthop. Res.* 28, 1276-1282.

Pratsinis, H., Kletsas, D., Melliou, E., Chinou, I. (2010). Antiproliferative activity of Greek propolis. *J Med Food* 13, 286-290.

Anastasiadi, M., Pratsinis, H., Kletsas, D., Skaltsounis, A.L., Haroutounian, S.A. (2010). Bioactive non-coloured polyphenols content of grapes, wines and vinification by-products: Evaluation of the antioxidant activities of their extracts. *Food Res. Int.* 43, 805-813.

Levidou, G., Saetta, A.A., Karlou, M., Thymara, I., Pratsinis, H., Pavlopoulos, P., Isaiadis, D., Diamantopoulou, K., Patsouris, E., Korkolopoulou, P. (2010). D-type cyclins in superficial and muscle-invasive bladder urothelial carcinoma: correlation with clinicopathological data and prognostic significance. *J. Cancer Res. Clin. Oncol.* 136, 1563-1571.

Maffeo, D., Lampropoulou, M., Fardis, M., Lazarou, Y.G., Mavridis, I.M., Mavridou, D.A., Urso, E., Pratsinis, H., Kletsas, D., Yannakopoulou, K. (2010). Novel polycarboxylated EDTA-type cyclodextrins as ligands for lanthanide binding: study of their luminescence, relaxivity properties of Gd(III) complexes, and PM3 theoretical calculations. *Org. Biomol. Chem.* 8, 1910-1921.

Metwally, K., Khalil, A., Pratsinis, H., Kletsas, D. (2010). Synthesis, in-vitro cytotoxicity, and a preliminary structure-activity relationship investigation of pyrimido[4,5-c]quinoline-1(2H)-ones. *Arch. Pharm. (Weinheim)* 343, 465-472.

Articles in Press

Pratsinis, H., Dimozi, A., Pilichos, K., Tsagarakis, S., Yiacooumettis, A., Kletsas, D. (2011). Previous chronic exogenous glucocorticoid administration in vivo does not affect functional characteristics and cellular lifespan of human skin fibroblasts in vitro. *Exp. Dermatol.* (in press) IF: 3,239

Magoulas, G.E., Bariamis, S.E., Athanassopoulos, C.M., Haskopoulos, A., Dedes, P.G., Krokidis, M.G., Karamanos, N.K., Kletsas, D., Papaioannou, D., Maroulis, G. (2010). Syntheses, antiproliferative activity and theoretical characterization of acitretin-type retinoids with changes in the lipophilic part. *Eur. J. Med. Chem.* (in press). IF: 3,269

Papaioannou, K.A., Markopoulou, C.E., Gioni, V., Mamalis, A., Vavouraki, H.N., Kletsas, D., Vrotsos, I.A. (2011). Attachment and Proliferation of Human Osteoblast-Like Cells on Guided Bone Regeneration (GBR) Membranes in the Absence or Presence of Nicotine: An In Vitro Study. *Int. J. Oral Maxillofac. Implants.* (in press) IF: 1,978

Zampeli, D., Pratsinis, H., Eliades, T., Eliades, G., Kletsas, D., Papagiannoulis, L. (2011). In vitro estrogenicity of dental resin sealants. *Pediatr. Dent.* (in press) IF: 1,620

Papadopoulou, Ad., Kletsas, D. (2011). Human lung fibroblasts prematurely senescent after exposure to ionizing radiation enhance the growth of malignant lung epithelial cells in vitro and in vivo. *Int. J. Oncol.* (in press) IF: 2,447

Mavrogonatou, E., Kletsas, D. (2011). Differential response of nucleus pulposus intervertebral disc cells to high salt, sorbitol and urea. *J. Cell. Physiol.* (in press) IF: 3,986.

Presentations at Scientific Conferences

D. Kletsas (2010). Cellular senescence: Friend or foe for tissue homeostasis? IWWT / ETRS Congress, 17-19 January, Paris 2010. (invited speaker)

D. Kletsas (2010). Cellular Senescence: Mechanisms and Implications in Tissue Homeostasis. 20th European Tissue Repair Society Congress. 15-17 September 2010, Gent, Belgium. (invited speaker)

E. Mavrogonatou and D. Kletsas "High osmolality reduces the proliferation rate of nucleus pulposus intervertebral disc cells, inhibits their response to exogenous growth factors and activates the mechanism for an enhanced DNA repair" GENODISC Meeting 6-8 September 2010, Ljubljana, Slovenia

A. Dimozi and D. Kletsas "Oxidative stress-induced premature senescence in human intervertebral disc cells" GENODISC Meeting 6-8 September 2010, Ljubljana, Slovenia

H. Pratsinis, V. Constantinou, K. Pavlakis, G. Sapkas and D. Kletsas "Exogenous and autocrine growth factors stimulate human intervertebral disc cell-proliferation via the ERK and Akt pathways" GENODISC Meeting 6-8 September 2010, Ljubljana, Slovenia

H. Pratsinis, S. Chrissouli, V. Velissariou, A. Anastasiou, D. Kletsas. (2010). Amniotic fluid-induced proliferation of human fetal and adult skin fibroblasts is mainly attributed to bFGF and PDGF and is mediated through the ERK and Akt signaling pathways. 61st National Conference of Biochemistry and Molecular Biology, 15-17 October 2010, Alexandroupolis, Greece.

E. Papachristou, Ch. Matragkou, F. Tsitouroudi, D. Kletsas, T. Choli-Papadopoulou. (2010). The importance of the NH₂-terminal region of eukaryotic (cancer/normal cells) ribosomal protein S5 for its cellular trafficking. 61st National Conference of Biochemistry and Molecular Biology, 15-17 October 2010, Alexandroupolis, Greece.

E. Mavrogonatou, D. Kletsas. (2010). High osmolality decreases the proliferative potential of bovine nucleus pulposus intervertebral disc cells in response to growth factors via the regulation of the ERK and Akt pathways. 61st National Conference of Biochemistry and Molecular Biology, 15-17 October 2010, Alexandroupolis, Greece.

E. Mavrogonatou, T. Eliades, G. Eliades, D. Kletsas. (2010). Triethylene glycol dimethacrylate causes a p53-dependent G2-arrest in human gingival fibroblasts that protects the cells against the genotoxic effects of the compound. 61st National Conference of Biochemistry and Molecular Biology, 15-17 October 2010, Alexandroupolis, Greece.

A Dimozi, D. Kletsas (2010) Premature senescence of intervertebral disc cells subjected to oxidative stress. 7th National Conference for Free Radicals and Oxidative Stress. 10-13 June 2010, Spetses, Greece.

D. Kletsas (2010) Ageing of intervertebral disc cells. 16th Seminar of Biomechanics and Biotechnology of the Spine. 3-5 December 2010, Athens, Greece. (invited speaker)

D. Kletsas (2010) Cell senescence: its role in tissue homeostasis and impact on cell replacement therapies. Scientific Meeting "Cell Interactions with extracellular networks and biomaterials. Applications in regenerative medicine and histo technology". 16 December 2010, University of Patras, Patras, Greece (invited speaker)

Educational Activities

"Cell senescence and tissue homeostasis", Summer School of NCSR "Demokritos", 1 hour, 30 students. (D. Kletsas)

Supervisor of the doctorate theses of Adamantia Papadopoulou, Anastasia Dimozi, and Andreas Armatas (D. Kletsas)

"Cellular senescence and tissue homeostasis" Post-graduate Master's Degree in Biochemistry, Chemistry Department of the University of Athens, 2 hours, 15 students. (D. Kletsas)

"Cellular 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 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. and M.Sc. theses in the University of Athens (Department of Chemistry and Dental School) and in the University of Patras. (D. Kletsas)

P. Chandris completed his Ph.D. thesis entitled "Alterations in the structure and function of cell nucleus during ageing" in the Medical School of the University of Crete, which was accepted and awarded the degree "Excellent". (Scientific Supervisor D. Kletsas).

N. Papadopoulou completed her Diploma thesis entitled "Expression of anti-apoptotic genes in senescent cells" in the Department of Biology of the University of Athens, which was accepted and awarded the degree "Excellent". (Scientific Supervisor D. Kletsas).

Other Scientific Activities

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

Member (Special Secretary) of the Board of the Hellenic Society of Free Radicals and Oxidative Stress (up to June 2010) (D. Kletsas)

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

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

Editorial board member for the journals "Biogerontology", "European Spine Journal", "Fibrogenesis and Tissue Repair", "Open Longevity Science", "Open Spine Journal" and "Journal of Dental Biomechanics". (D. Kletsas)

Member of the Scientific Committee of the 20th European Tissue Repair Society Congress. 15-17 September 2010, Gent, Belgium (D. Kletsas)

Reviewing of manuscripts submitted to European Spine Journal, PLoS ONE, Journal of Cellular Physiology, Molecular Cancer Therapeutics, Biogerontology, Journal of Investigative Dermatology, Molecular Carcinogenesis, Mechanisms of Ageing and Development, Cell Biochemistry & Function, Osteoarthritis and Cartilage, Dental Materials, American Journal of Orthodontics and Dentofacial Orthopedics, Journal of Photobiology, Archives of Dermatological Research, Journal of Dental Biomechanics (D. Kletsas)

D. Kletsas (2010). "Cellular senescence: mechanisms and role in tissue homeostasis" Research Seminar in the Gray Institute of Radiation Oncology & Biology, 17 May, 2010, University of Oxford, UK. (invited speaker)

Other Activities for the Institute of Biology

D. Kletsas:

Member of the Bioethics Committee of NCSR "Demokritos"

Member of the Scientific Consultative Board of the Institute of Biology

Scientific Supervisor of the Experimental Animal Colony

Supervisor of the Fluorescence Activated Cell Sorting Facility

H. Pratsinis:

Seminar Supervisor of the post-graduate Ph.D. fellows of the Institute of Biology (up to June 2010)

Member of the examination committee for the selection of the post-graduate Ph.D. fellows of the Institute of Biology (October 2010)

Member of the advisory committee of the post-graduate Ph.D. fellow A. Dimozi

Impact Factors:

D. Kletsas (for 8 publications): 25,425

H. Pratsinis (for 6 publications):14,393

Citations 2010 (without self-citations):

D. Kletsas: 396

H. Pratsinis: 96

Total Citations 2006-2010 (without self-citations):

D. Kletsas: 1790

H. Pratsinis: 378

h-factor:

D. Kletsas: 22

H. Pratsinis: 13

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 2010: 1.441,6 €.

Project 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 2010: 48.333,32€.

Project entitled *Investigation of the effect of neonatal fibroblasts on the pro-inflammatory phenotype of senescent cells*, funded by Organogenesis Inc., Massachusetts, USA with Coordinator Dr. D. Kletsas
Duration: 2010-2011
Total programme funding: 50.000 \$
Funding of the lab for 2010: 11.257,61 \$

Note:

Submitted project proposals under review: one to the EU (Marie Curie project) and three in the framework "Thales" to GSRT.

Research Group: Nuclear Proteins and Chromatin Function

Research Staff

Thomais Sourlingas, Researcher

Kalliope Sekeri, Emeritus Researcher

Marios Xidous, Graduate Student

Paraskevi Salpea, Graduate Student

Research Interests

The research interests of our group are focused on studying the functional role of the histone subtypes and their epigenetic modifications, mainly acetylation, phosphorylation and methylation, of numerous biological processes (see below). We are also studying the effects that histone deacetylase inhibitors have on the acetylation status of histone and non histone proteins and the consequences that these changes have on gene expression and cellular function. The cell systems used are ageing cell systems (fibroblasts and lymphocytes), peripheral blood leucocytes and leukemic cell lines. The specific ongoing projects are:

- (1) We are studying chromatin conformational changes (remodeling) that takes place during aging and during apoptosis and are, amongst other factors, the result of (1) changes in the constitution of the H1 linker histone subtypes and (2) epigenetic post translational modifications, especially phosphorylation of the H1 histone subtypes and acetylation and methylation of the nucleosomal core histones. Specifically, we are investigating the role of the H1 subtype constitution of chromatin and their epigenetic modifications in heterochromatin formation or in the reorganization of eu- and heterochromatin regions during aging and apoptosis in fibroblasts, human peripheral blood lymphocytes and cancer cell lines.
- (2) At the gene level, we are studying the role that changes in epigenetic histone modifications, i.e., acetylation and methylation, have in gene expression levels of age-related genes of peripheral blood leucocytes (lymphocytes, monocytes and dendritic cells).
- (3)** Study of the acetylation and methylation status of the promoter regions of circadian genes in mammalian cell systems and how these epigenetic changes affect their expression.
- (4) Another line of research involves the study of the role of the linker histone H1 subtype composition, as well as their epigenetic changes and changes in the mRNA levels of the H1 histone subtypes and how these affect chromatin conformation in peripheral blood leucocytes from patients with schizophrenia.

2010 Findings

- (1) Study of the epigenetic changes that occur around the DNA loci of age-related genes. Results from this line of work will be compared to the expression levels of these genes. The genes that are under study are *H1.0* and *dfna5*. *H1.0* and *dfna5* are differentiation- and age- related genes. Results have so far shown that:
 - In the *dfna5* gene region, methylation levels of lysine 4 of histone H3 were found to be higher in samples from newborns in relation to those in samples from young donors (20-30 years old). This histone modification is associated with transcriptional activation. This increase agrees with the results obtained for *dfna5* expression levels, which were also found to be increased in newborns in relation to those of young donors.
 - Histone acetylation in the *H1.0* gene region in both activated and non activated lymphocytes were found to be higher in samples from elderly donors versus those from young donors. These results were expected since histone acetylation is associated with transcriptional activation and *H1.0* expression levels increase in aging cell systems.

This study is being carried out by P. Salpea within the framework of her doctoral thesis and is also part of a collaboration 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).

(2) We are also studying the effects that chemical substances which change histone acetylation levels may have on the expression levels of genes of the mammalian biological clock. The agents that were used are trichostatin A, a histone deacetylase inhibitor (HDAC) of class I and II HDACs and nicotinamide, an inhibitor of class III HDACs (sirtuins). With qPCR, we found that the expression levels of the clock genes *per1*, *per2*, but not *cry1*, increase after trichostatin A application. This induced increase in the expression levels of the *per* genes is not a result of *de novo* synthesis, as shown by experiments where cycloheximide (protein synthesis inhibitor) was applied in conjunction with trichostatin A. Moreover, application of nicotinamide in combination with trichostatin A lowers these levels. In order to ascertain whether changes in the acetylation status of histones in the promoter region of the *per1* gene are responsible for the observed changes in its expression levels by these two agents, chromatin immunoprecipitation (ChIP) experiments were undertaken. Initial results showed that there is an increase in histone acetylation in the glucocorticoid response element (GRE) region and in the region of the transcription start site (TSS). This study is being carried out by M. Xidou within the framework of his doctoral thesis. 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) of the Institute of Biology N.C.S.R. "D".

Publications

Ninios, Y.P., Sekeri-Pataryas, K.E. and T.G. Sourlingas. Histone H1 subtype preferences of DFF40 and possible nuclear localization of DFF40/45 in normal and trichostatin A-treated NB4 leukemic cells. *Apoptosis*, 15: 128-138, 2010.

Repouskou, A, Sourlingas, T.G., Sekeri-Pataryas, K.E. and A. Prombona. The circadian expression of c-Myc is modulated by the histone deacetylase inhibitor trichostatin A in synchronized murine neuroblastoma cells. *Chronobiology Int.*, 27(4): 722-741, 2010.

Articles in Press

Repouskou, A, Sourlingas, T.G., Sekeri-Pataryas, K.E., Prombona, A. The circadian expression of c-Myc is modulated by the histone deacetylase inhibitor trichostatin a in synchronized murine neuroblastoma cells. *Chronobiology Int.*, in press. (IF: 3.987).

Educational Activities

Summer School (IB/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, 22 students).

Supervision of the doctoral thesis work of Marios Xidou, biologist, recipient of a 4-year scholarship from NCSR "D". Title of thesis work: "Effect of histone acetylation levels in the regulation of the biological clock: consequences in cellular function".

Supervision of the doctoral thesis work of Paraskevi Salpea, biologist, recipient of a 4-year scholarship from NCSR "D". Title of thesis work: "Study of histone acetylation and of the linker DNA histones in chromatin remodeling events during aging".

Member of the Internal Advisory Committee for the doctoral thesis work of M. Xidou, A. Repouskou, P. Salpea and I. Ninios.

Other Activities for the Institute of Biology

Member of the Education Committee of the IB/NCSR "D" (till June, 2010).

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

Other Scientific Activities

Scientific collaborations:

- With the laboratory of 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 is completing her doctoral thesis work in our lab, received a second scholarship (pro-Fogarty 2009-2010) to work in the lab of Dr. Howard on a project that will be part of her thesis work.
- With Dr. A. Prombona (Laboratory of Chronobiology), Biology Institute, NCSR "D". This research collaboration involves the study of the effects of histone acetylation of genes that regulate the mammalian biological clock (circadian rhythm) and potential consequences to cellular function and carcinogenesis.
- 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. Kozzika Foundation. The aim of the project is to study changes in the histone H1 subtype constitution of chromatin and their mRNA levels of leucocytes from the peripheral blood of patients with schizophrenia.
- With the University of Goettingen, Prof. D. Doenecke. The collaboration involves the analysis of the H1 linker histone subtype constitution by Capillary Zone Electrophoresis (CZE) during aging.

Impact Factors (for 2 publications): 8,056

Citations 2010 (without self-citations): 19

Citations 2006-2010: 79

h-factor: 6

Current External Funding

Collaboration project of our lab with the Neurobiology Research Institute of the Th. Th. Kozzika Foundation, entitled *Study of the changes in the histone H1 subtype constitution of chromatin and their mRNA levels in leucocytes from patients with schizophrenia*.

Duration: 1/1/2007 -

Funding of the lab for 2010: 10.000 €

Note:

The lab has taken part in 2 proposals within the framework of the "Thalis" program.:

- Title: "Study of the signal transduction pathways of the glucocorticoid receptor and its cross-talk with other endocellular factors in the pathophysiology of depression".
- Title: "Study of the genetic heterogeneity of thalassemia syndromes at the genome level: applications for prevention and therapy".

Our lab has also participated in 2 proposals within the framework of the action 'Support of Postdoctoral Researchers'.

- Title: "A systematic analysis of telomere chromatin as a function of telomere length: Changes in histone post translational modifications and the H1 linker histone subtypes".
- Title: "Interaction of the DNA fragmentation factor, DFF40, with the H1 linker histone subtypes during the induction of apoptosis in age-related pathologies: Is there a specificity to cell type and mode of apoptosis?".

Research Group: Cell & Matrix Biochemistry/Pathobiology

Research Staff

Fotini-Effie Tsilibary, Research Director

Athina Tzinia, Senior Researcher

Angelika Chroni, Senior Researcher

Paraskevi Kitsiou, Researcher

Garyfallia Drossopoulou, Lecturer

Apostolia Fragouli, Postdoctoral Fellow

Nikos Tsotakos, Graduate Student

Myrto Kostomiri, Graduate Student

Katerina Kapodistria, Graduate Student

Theodore Koutmos, Graduate Student

Ioannis Daphnis, Graduate Student

Georgios Daniil, Graduate Student

Nefeli Lagopati, Collaborating Graduate Student

Sofia Verouti, Collaborating Graduate Student

Vassilis Photopoulos, Collaborating Graduate Student

Maria Arvaniti, Undergraduate Student

Eleni Kotsopoulou, Research Technician

Letta Argyri, Research Technician

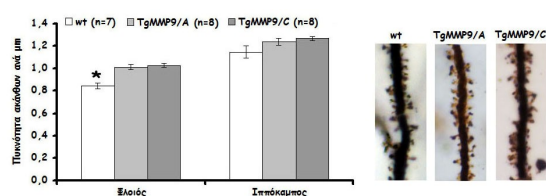
Research Interests

- Diabetes Mellitus **A)** Study of kidney function: Regulation of gene expression in glomerular podocytes: Gene regulation during embryonic development - Gene expression and epigenetic mechanisms in physiological and diabetic conditions. **B)** Renoprotective role of Vitamin D on glomerular podocytes. **C)** Studies in insulin-producing pancreatic beta-cells: Nephrin signalling in pancreatic β -cells: Cross talk between nephrin signalling and insulin survival signaling.
- Neurodegenerative Disorders: **A)** *In vivo* effect of collagenase B (MMP-9) in the brain of transgenic mice (TgMMP9), **B)** *In vitro* effect of MMP-9 in NGF-differentiated PC12 cells, **C)** Role of lipids and lipoproteins in Alzheimer's disease. Insights on the relationship between apolipoprotein E4 and A_{β} metabolism in brain, **D)** effect of Oleuropein in the formation of Alzheimer's amyloid plaques
- Related to Arthropathies: **A)** The effect of neuropeptide Calcitonin in Hyman osteoblastic cells, **B)** Expression of MMP-9 in septic and aseptic Arthritis.
- Application of TiO₂ nanoparticles in cancer treatment (Nanoparticles and cell apoptosis): Biological anticancer activities of photo-activated TiO₂ innovative nanoparticles.
- Related to atherosclerotic disease: Molecular mechanisms of atherosclerosis - Structure function relationship of proteins involved in lipoprotein metabolism pathways

2010 Findings

Study of Neurodegenerative Disorders

A) 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/Tg5XFAD). TgMMP9 mice exhibit improved cognitive abilities, enhanced neuronal functions and neuronal plasticity. This effect might be due to increased secretion the soluble fragment of APP (sAPP α) resulting from the α -secretase activity of MMP9. Even more, in preliminary studies it was shown that double transgenic mice TgMMP9/Tg5XFAD exhibit reduced amyloid plaques in the brain, compared to Tg5XFAD animals.



Increased spine density was observed in transgenic mice overexpressing MMP9 (TgMMP9) as an indication of enhanced neuronal plasticity.

B) Studying the *in vitro* effect of MMP9 in NGF-differentiated PC12 cells it was shown that in the presence of the neurotrophic factor NGF the non amyloidogenic pathway of APP processing was induced. Concomitantly, a reduced expression of b-secretase BACE was observed, whereas the expression of a-secretases, ADAM17 and MMP9 at the most were increased (*J Alzheimers Dis, In Press*). **C)** Regarding the role of apolipoprotein E4, it was shown that specific short apoE4 proteolytic fragments (e.g. apoE4[Δ (166-299)]), produced in the brain of Alzheimer's disease patients during the early steps of disease pathogenesis, may promote intraneuronal accumulation of A β 42 leading to neuronal dysfunction (*J. Neurochem, 2010*). **D)** As an inhibitor of Alzheimer's amyloid beta accumulation, the antioxidant constituent of oil Oleuropein was studied. It was shown that Oleuropein favors the non amyloidogenic pathway of APP processing (*submitted*)

Research related to atherosclerotic disease: **A)** Characterization of function of HDL from family subjects with low HDL syndromes that carry mutations in apoA-I, ABCA1 or LCAT showed that mutations in proteins of the HDL biosynthesis pathway decrease the antioxidant/anti-inflammatory capacity of HDL due to reduced activity of HDL-associated antioxidant enzymes and increased levels of HDL-associated proinflammatory molecules.. These types of analyses could prove valuable in diagnosis of familial low HDL syndromes. **B)** We studied the capacity of HDL obtained from patients with stable coronary artery disease (SCAD) or acute coronary syndrome (ACS) and from control subjects to promote cholesterol efflux from macrophages. No difference was observed between the 3 groups. This study was performed in collaboration with Dr. Ulf Landmesser's group, Cardiovascular Center, University Hospital Zurich. This group showed that in marked contrast to HDL from control subjects (HDL_{control}), HDL from patients with either SCAD or ACS (HDL_{CAD}) inhibited rather than stimulated endothelial NO production and endothelial repair. The capacity of HDL to stimulate endothelial NO production was critical for endothelial anti-inflammatory effects and the promotion of endothelial repair. Notably, HDL_{CAD}, in contrast to HDL_{control}, activated endothelial protein kinase C (PKC) beta-2, which in turn inhibited eNOS-activating pathways and eNOS-dependent NO production. The fact that we did not observe a significant difference in the macrophage cholesterol efflux capacity of circulating HDL from patients with CAD and control subjects is compatible with the concept that the impaired capacity of circulating HDL to stimulate endothelial NO production is rather related to endothelial PKCbeta-2 activation than to a major impairment in cholesterol efflux.

Publications

Venieratos, P., Drossopoulou, G., Tsilibary, E., Kitsiou P. (2010). High glucose induces suppression of insulin signalling and apoptosis via upregulation of endogenous IL-1 β and SOCS-1 in mouse pancreatic beta-cells. *Cell. Signal.* 22, 791-800.

N. Lagopati, N., Kitsiou, P., Kontos A.I., Venieratos, P., Kotsopoulou, E., Kontos A.G, Dionysiou, D., Pispas, S., Tsilibary, E., Falaras, P. (2010). Photo-induced treatment of breast epithelial cancer cells using nanostructured titanium dioxide solution. *J. Photochem. Photobiol. A: Chemistry* 214, 215-223.

Tsagaraki, I, Tsilibary, E.C, Tzinia, A.K.(2010). TIMP-1 interaction with β 1 and α v β 3 integrins contributes to resistance of human osteosarcoma MG63 cell line against TNF- α -induced apoptosis *Cell Tiss. Res.*, 342: 87.

Dafnis I., Stratikos E., Tzinia A., Tsilibary E. C., Zannis V. I. and Chroni A. (2010) An apolipoprotein E4 fragment can promote intracellular accumulation of amyloid peptide beta 42, *J. Neurochem.*, 115, 873-884.

Georgiadou D., Hearn A., Evnouchidou I., Chroni A., Leondiadis L., York, I. A., Rock K. L. and Stratikos E. (2010) Placental Leucine Aminopeptidase Efficiently Generates Mature Antigenic Peptides In Vitro but in Patterns Distinct from Endoplasmic Reticulum Aminopeptidase 1. *J. Immunol*, 185, 1584-1592.

Articles in Press

Fragkouli A, Tzinia AK, Charalampopoulos I, Gravanis A, Tsilibary EC. Matrix Metalloproteinase-9 Participates in NGF-Induced α -Secretase Cleavage of Amyloid- β Protein Precursor in PC12 Cells. *J Alzheimers Dis*. 2011 Feb 14. [Epub ahead of print]. (i.f. 5.1)

Ohnsorg P. M., Rohrer L., Perisa D., Kateifides A., Chroni A., Kardassis D., Zannis V. and von Eckardstein A. (2011) The carboxy-terminus of apolipoprotein A-I (ApoA-I) is necessary for the transport of lipid-free ApoA-I but not pre-lipidated ApoA-I particles through aortic endothelial cells. *J. Biol. Chem.*, (i.f. 5.328) *in press*

Fragkouli A, Papatheodoropoulos C, Georgopoulos S, Stamatakis A, Stylianopoulou F, Tsilibary EC and Tzinia AK: "Elevated soluble APP α levels and enhanced neuronal plasticity in mice over-expressing MMP9" (*submitted*)

Articles in Books and Conference Proceedings

Holleboom A. G., Daniil G., Hovingh G. K., Schimmel A. W., van Miert J. N., Kastelein J. J. P., Stroes E. S. G., Kuivenhoven J. A., Chroni A., Carriers of LCAT Gene Mutations Have High Density Lipoprotein With Decreased Anti-Oxidative Capacity. *Atherosclerosis Supplements*, Vol. 11, Issue 2, p62 (2010)

Karlsson H., Sundberg S., Levels J. H. M., Turkina M., Daniil G., Chroni A., Kuivenhoven J. A., Lindahl M. Mutant apoA-I(L178P) identified in HDL from heterozygotes from a family with endothelial dysfunction and increased arterial wall thickness. *Atherosclerosis Supplements*, Vol. 11, Issue 2, p67 (2010)

Ohnsorg P., Rohrer L., Chroni A., Zannis V., von Eckardstein A. Interactions of apolipoprotein A-I mutants with endothelial cells. *Atherosclerosis Supplements*, Vol. 11, Issue 2, p88 (2010)

Presentations at Scientific Conferences

A Fragkouli, C. Papatheodoropoulos, S. Georgopoulos, A. Stamatakis, F. Stylianopoulou, E. Tsilibary and A. Tzinia (2010) OVEREXPRESSION OF MATRIX METALLOPROTEINASE 9 FACILITATES SYNAPTIC PLASTICITY AND LEARNING AND MEMORY PROCESSES IN VIVO. 7th FENS Forum of European Neuroscience, July 3-7, Amsterdam, Netherlands

Drossopoulou G., Tsoதாகos N. and Tsilibary E. C. (2010). High glucose levels may lead human glomerular epithelial cells to dedifferentiation. 22nd meeting of the European Renal Cell Study Group, Vienna, 2010.

Georgiadou D., Hearn A., Evnouhidou I., Chroni A., Leondiadis L., York I., Rock K. L., and Stratikos E. PLAP efficiently generates mature antigenic peptides in vitro but in patterns distinct from ERAP1. 6th International Antigen Processing and Presentation Workshop, 29 March - 2 April 2010, Cargèse, Corsica, France

Karlsson H., Levels J. H. M., Hovingh G. K., Holleboom A. G., Vergeer J. J., Kastelein J. J. P., Argyri L., Chroni A., Kuivenhoven J. A., Lindahl M. Protein profiling of HDL and LDL/VLDL from heterozygous carriers of SR-BI mutation P297S; Increased levels of apo L-I in HDL and apo E in LDL/VLDL. 6th International Atherosclerosis Society-sponsored Workshop on High Density Lipoproteins, May 17-20, 2010, Whistler, BC, Canada

Holleboom A. G., Daniil G., Hovingh G. K., Schimmel A. W., van Miert J. N., Kastelein J. J. P., Stroes E. S. G., Kuivenhoven J. A., Chroni A., Carriers of LCAT Gene Mutations Have High Density Lipoprotein With Decreased Anti-Oxidative Capacity. 78th European Atherosclerosis Society Congress, 20-23 June 2010, Hamburg, Germany

Karlsson H., Sundberg S., Levels J. H. M., Turkina M., Daniil G., Chroni A., Kuivenhoven J. A., Lindahl M. Mutant apoA-I(L178P) identified in HDL from heterozygotes from a family with endothelial dysfunction and increased arterial wall thickness. 78th European Atherosclerosis Society Congress, 20-23 June 2010, Hamburg, Germany

Ohnsorg P., Rohrer L., Chroni A., Zannis V., von Eckardstein A. Interactions of apolipoprotein A-I mutants with endothelial cells. 78th European Atherosclerosis Society Congress, 20-23 June, Hamburg, Germany

Koutmos T., Tsoதாகos N., Drossopoulou G. and Tsilibary E. (2010). Downregulation of specialized podocytic components induced by long-term exposure to high glucose levels

indicates de-differentiation of cultured human glomerular epithelial cells. 61st Meeting of the Hellenic Society of Biochemistry and Molecular Biology, 15-17 October 2010, Alexandroupoli.

Verouti S., Drossopoulou G., Fragkopoulou E., Tsilibary E., Dimopoulos K.A and Iatrou X. (2010). VDR is expressed in Human Glomerular Podocytes. Vitamin-D induces upregulation of podocalyxin and VDR expression. 16th Hellenic Nephrology Congress Kos 2010 (Best Poster Award)

Verouti S., Fragkopoulou E., Drossopoulou G., Tsilibary E., Dimopoulos K.A and Iatrou X. (2010). An *in vitro* study of Vitamin D and PAF function on Human Glomerular Podocyte morphology. 4th Hellenic Atherosclerosis Congress, Ioannina 2010.

Kotsopoulou E., Koutmos T., Pachnis V., Tsilibary E. and Drossopoulou G. (2010) Impaired WT1 expression in glomerular podocytes of monoisoformic RET51/51 animals is associated with reduced PCLP and NEPHRIN expression. 61st Meeting of the Hellenic Society of Biochemistry and Molecular Biology, 15-17 October 2010, Alexandroupoli.

Dafnis I., Stratikos E., Tzinia A., Tsilibary E. C., Zannis V. I., Galanopoulou D. and Chroni A. An apolipoprotein E4 fragment can promote intracellular accumulation of amyloid peptide beta 42. Meeting of Postgraduate Students Association of University of Athens Chemistry Department, 28—29 May 2010, Athens

Daniil G., Phedonos A. A. P., Argyri L., Kuivenhoven J. A., Mavri-Vavayianni M. and Chroni A. Antioxidant/anti-inflammatory properties and apoA-I-containing subpopulations of HDL from family subjects with monogenic low HDL syndromes. Meeting of Postgraduate Students Association of University of Athens Chemistry Department, 28—29 May 2010, Athens

Daniil G., Holleboom A. G., Kuivenhoven J. A. and Chroni A. HDL of carriers of LCAT gene mutations has decreased antioxidant/anti-inflammatory properties. 13th Meeting of the Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease, 7-9 October 2010, Athens.

Daniil G., Phedonos A. A. P., Argyri L., Kuivenhoven J. A., Chroni A. Characterization of antioxidant/anti-inflammatory properties and subpopulations of HDL from family subjects with monogenic low HDL syndromes. 61st Meeting of the Hellenic Society of Biochemistry and Molecular Biology, 15-17 October 2010, Alexandroupoli.

Other Scientific Activities

EC Tsilibary: i) Member COST Action BM1001-ECMnet (representative from Greece), ii) Member COST Action 0702- Eurokup (Urine Kidney Proteomics, iii) Member of the committee for abstract selection for the congress: ERA-EDTA (European Renal Association/European Dialysis & Transplant Association, XLVII Annual Meeting), iv) evaluator for the scientific journals: PLOS, PLOS-One, Cells-Tissues-Organs, Dialysis & Transplantation.

P. Kitsiou: i) Person in charge for the writing of the part (*Expected impacts listed in the work programme, Spreading excellence, exploiting results, disseminating knowledge in FP7-REGPOT-2011-1* ii) Reviewer for scientific journals *Kidney International, Recent Patents on Endocrine, Metabolic & Immune Drug Discovery*

A. Tzinia: Member of COST Action BM1001-ECMnet (Greek representative)

A. Chroni: i) Member of COST Action BM0904-HDLnet (Member of the Management Committee), ii) Participated in the preparation of Institute of Biology's research proposal "CEMADis: Center of Excellence for Multidisciplinary Approaches to Identify Targets and Tools for Interfering with Disease" that was submitted for funding by Research Potential FP7-REGPOT-2011-1 Coordination and support actions (Supporting)., iii) Reviewer for peer-reviewed journal *Atherosclerosis*

Other scientific presentations/lectures

1) E.C. Tsilibary, invited speaker "Stem Cell transplantation: A scientific approach" IEK XYNI, March 1st, 2010, Athens

Other Distinctions and Awards

- 1) A. Chroni: Research proposal award from the Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease
- 2) Best poster award: Daniil G., Holleboom A. G., Kuivenhoven J. A. and Chroni A. HDL of carriers of LCAT gene mutations has decreased antioxidant/anti-inflammatory properties. 13th Meeting of the Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease, 7-9 October 2010, Athens.
- 3) Best poster award: Verouti S., Drossopoulou G., Fragkopoulou E., Tsilibary E., Dimopoulos K.A and Iatrou X. (2010). VDR is expressed in Human Glomerular Podocytes. Vitamin-D induces upregulation of podocalyxin and VDR expression. 16th Hellenic Nephrology Congress Kos 2010

Educational Activities

- 1) E.C. Tsilibary: i) Neurodegenerative diseases (Alzheimer's disease) and new research developments, Summer school, NCSR "Demokritos", July 5-16, 2010 (1 hour); ii) "Pathogenetic mechanisms and therapeutic implications of diabetes mellitus", March 23, 2010, Graduate course "Pathobiochemistry", Dept. Biology, University of Athens (3-hour lecture-14 graduate students); iii) "The process of cell apoptosis in pathological conditions: desired or non-desired process?" Graduate course in Molecular and Applied Physiology, Medical School, University of Athens, November 18, 2010 (3-hour lecture-27 graduate students); iv) supervisor of two MSc dissertations, Graduate course in Molecular and Applied Physiology, Medical School, University of Athens (M. Leandrou: "Overview in Alzheimer's Disease: Molecular Mechanisms and Therapeutic Potentials" & I. Melissovas: "Overview of Tumor suppressor protein p53 and its mutations in breast cancer"; member of the 7-member graduate examination committee of PhD candidate I. Daphni, Dept. Chemistry, University of Athens.
- 2) A. Chroni: i) Member of the Institute of Biology Advisory Committee of PhD students N. Tsotakos, I. Vaggelatos, G. Daniil and M. Kostomoiri, ii) Presentation: "Lipids and apolipoproteins: From cardiovascular disease to Alzheimer's disease". NCSR Demokritos Summer School, 5-16 July 2010 (1 hour), iii) Presentation in round table "Postgraduate studies in NCSR Demokritos - Possibilities and Prospects". NCSR Demokritos Summer School, 5-16 July 2010, iv) Invited talk: The role of apolipoprotein E4 in the pathogenesis of Alzheimer's disease. Seminar Series, Chemistry Department, University of Athens, February 8, 2010, Athens., v) "Lipids and apolipoproteins: From cardiovascular disease to Alzheimer's disease". Guest lecture in graduate course "Clinical Chemistry II", Clinical Chemistry Graduate Program, Department of Chemistry, University of Athens. May 13 2010 (2h -10 students), vi) "Lipoprotein metabolism pathways and atherosclerosis. The association between atherosclerosis and Alzheimer's disease." Guest lecture in graduate course "Human Biochemistry", Biochemistry Graduate Program, Department of Chemistry, University of Athens 25 May 2010 (3 hours- 7 students).
- 3) P. Kitsiou: Member of the Advisory Committee of Ph.D. students N. Tsotakos, P. Salpea and Theodore Koutmos

Other Activities for the Institute of Biology

A. Chroni:

- 1) Person in charge for education issues in IB. Representative of IB in the Education Committee of NCSR Demokritos
- 2) Person in charge for the operation of FPLC

P. Kitsiou:

- 1) Person in charge for the organization of scientific lectures (period 2009-2010) with invited lecturers.

Photini-Effie C. Tsilibary:

- 1) Head, Institute of Biology,
- 2) Member, Executive committee, NCSR "Demokritos",
- 3) Member, Scientific sub-committee of the executive committee, NCSR "Demokritos",
- 4) Head of the organizing committee organizing the celebration of "Demokritos: 50 years of contribution to science, education and technology"
- 5) Head, Committee on Bioethics, NCSR "Demokritos",

Impact Factors (for 5 publications): 18,975

Citations 2010 (without self- citations): 196

EC Tsilibary: 62, A. Tzinia: 16, P. Kitsiou:8, A. Chroni: 58, G. Drossopoulou: 52.

Total Citations 2006-2010 (without self- citations): 967

EC Tsilibary: 364, A. Tzinia: 63, P. Kitsiou: 37, A. Chroni: 272, G. Drossopoulou: 231.

h-factor: EC Tsilibary: 30, A. Tzinia: 8, P. Kitsiou: 5, A. Chroni: 12, G. Drossopoulou: 8

Current External Funding

Program entitled *Functional genomics of inborn errors and therapeutic interventions in high density lipoprotein (HDL) metabolism* funded by the European Union with the Principal Investigator for NCSR "Demokritos": A. Chroni,
Duration: 2007-2009

Total funding (lab): 294.000€

Funding of the lab for 2010: 34.629€.

Program entitled *Study of functional interactions between HDL and cholesterol transporter ABCG1 - Role in inflammation and atherosclerosis* funded by the Hellenic Society of Lipidology, Atherosclerosis and Vascular Disease with the Principal Investigator for NCSR "Demokritos": A. Chroni,

Duration: 2010-2011

Total funding (lab): 8.000€

Funding of the lab for 2010: 0€.

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 with the Principal Investigator for NCSR "Demokritos": A. Chroni,

Duration: 18/2/2011-17/2/2012

Total funding (lab): 100.400€

Funding of the lab for 2010: 0€.

"HERAKLITOS" (Principal Investigators: P. Papazafiri from The Faculty of Biology, UOA, and E. Tsilibary from NCSR "D"), Title of Ph.D. Thesis: "Photo-induced anticancer activity of TiO₂ nanoparticles: Mechanisms and applications" (Ph.D. Student: N. Lagopati)

Program Duration: 10/2010-12/2012

Total funding: 45.000 €

2010 funding: 0 €

Abbot Hellas (Donation for Research and Development)

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

Total funding: 20.000 €

2010 funding: 8.000 €

COST program ECMNet-BM1001: *“Brain Extracellular Matrix in Health and Disease”*, funding from the European Science Foundation - COST Action; E. Tsilibary, member of the Management Committee and (representative from Greece).
Duration: 15/12/2010 - 14/12/2014.

COST program ECMNet-BM1001: *“Brain Extracellular Matrix in Health and Disease”*, funding from the European Science Foundation - COST Action; A.Tzinia, representative from Greece.
Duration: 15/12/2010 - 14/12/2014.

European Science Foundation - COST Action BM0904
Title: *“HDL - From Biological Understanding to Clinical Exploitation.”*
Member of the Management Committee A. Chroni, 8/6/2010 - 7/6/2014

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

- 1) *“THALIS”*
Title: *«Early renal alterations in chronic kidney disease»* (Co-ordinator A. Charonis BRFAA., Principal Investigator for NCSR *“Demokritos”*: G. Drossopoulou, Participated Investigator for NCSR *“Demokritos”*: EFK. Tsilibary)
- 2) AMERICAN HEALTH ASSISTANCE FOUNDATION
Title: *«Role of MMP-9 over-expression in Alzheimer's disease mouse models»*,
Principal Investigator: A. Tzinia
- 3) Supporting Postdoctoral Researchers, Title: *«Evaluation of possible effects of salmon Calcitonin selected for osteoporosis in terms of atherosclerosis progression»* Principal Investigator: A. Tzinia (Post-Doctoral Investigator: I. Tsagkaraki)
- 4) Supporting Postdoctoral Researchers, Title: *Combating Alzheimer's disease: The protective role of matrix enzyme MMP9”* Principal Investigator: EFK Tsilibary, Participated Investigator: A. Tzinia (Post-Doctoral Investigator: A. Fragkouli)
- 5) Supporting Postdoctoral Researchers, Title: *“Interaction / association of the DNA fragmentation factor, DFF40, with the H1 linker histone subtypes during the induction of apoptosis in age-related pathologies: Is there a specificity to cell type and mode of apoptosis?”* Principal Investigator: P. Kitsiou, Participated Investigator: T. Sourlingas (Post-Doctoral Investigator: I. Ninios)
- 6) Research Potential FP7-REGPOT-2011-1 Coordination and support actions (Supporting).
“CEMADis: Center of Excellence for Multidisciplinary Approaches to Identify Targets and Tools for Interfering with Disease”, Principal Investigator: E.C. Tsilibary

Research Group: Environmental Mutagenesis -Carcinogenesis

Research Staff

Gerassimos Voutsinas, Senior Researcher

Panagiotis Karkoulis, Graduate Student

Dimitra Anastasiou, Collaborating Graduate Student

Stefanos Kachrilas, Collaborating Graduate Student

Eumorphia Konstantatou, Collaborating Graduate Student

Eleutheria Peristeri, Collaborating Graduate Student (*MSc*)

Angeliki-Stephania Bassoyianni, Undergraduate 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

2010 Findings

1. Mutational and Immunohistochemical Study of the PI3K/Akt Pathway in Papillary Thyroid Carcinoma in Greece

PI3K/Akt signaling pathway plays critical role in many cell processes. There is indication that enhanced activation of PI3K/Akt cascade is implicated in thyroid tumors. Aim of this study was to evaluate the mutational status and expression of PI3K/Akt pathway mediators in papillary thyroid carcinoma in Greece. We evaluated the presence of mutations in PIK3CA (exons 9 and 20), AKT1 (exons 6-11), AKT2 (exons 6-11), AKT3 (exons 5-10), PTEN (exons 3-8), and PDPK1 (exons 4-10) genes in 83 papillary thyroid carcinomas by DNA sequencing. The expression levels of phospho-Akt and insulin-like growth factor I receptor (IGF-IR) were evaluated by immunohistochemistry. PIK3CA mutations were found in three samples. The analysis of AKT1 revealed one silent mutation in exon 9 (G726A) in 16 samples. One specimen carried an AKT3 mutation. One missense mutation was found in one sample in PTEN. No mutations were found in AKT2 and PDPK1. Increased levels of phosphorylated total Akt and IGF-IR were identified in some papillary cancers. Our findings indicate that PI3K/Akt signaling pathway is activated in some papillary tumors. However, mutations in genes coding most mediators of the pathway have not been proven to be the major modus of enhanced activation. These data suggest a potential role for PI3K/Akt-mediated signaling in papillary thyroid tumors.

2. Allelic Imbalance of Expression and Epigenetic Regulation within the Alpha-Synuclein Wild-Type and p.Ala53Thr Alleles in Parkinson Disease

Genetic alterations in the alpha-synuclein (SNCA) gene have been implicated in Parkinson Disease (PD), including point mutations, gene duplications, and sequence variations within the promoter. Such alterations may be involved in pathology through structural changes or overexpression of the protein leading to protein aggregation, as well as through impaired gene expression. It is, therefore, of importance to specify the parameters that regulate SNCA expression in its normal and mutated state. We studied the expression of SNCA alleles in a lymphoblastoid cell line and in the blood cells of a patient heterozygous for p.Ala53Thr, the first mutation to be implicated in PD pathogenesis. Here, we provide evidence that: (1) SNCA shows monoallelic expression in this patient, (2) epigenetic silencing of the mutated allele involves histone modifications but not DNA methylation, and (3) steady-state mRNA levels deriving from the normal SNCA allele in this patient exceed those of the two normal SNCA alleles combined, in matching, control individuals. An imbalanced SNCA expression in this patient is thus documented, with silencing of the p.Ala53Thr allele and upregulation of the wild-type-allele. This phenomenon is demonstrated for a first time in the SNCA gene, and may have important implications for PD pathogenesis.

3. 17-Allylamino-17-demethoxygeldanamycin induces downregulation of critical Hsp90 protein clients and results in cell cycle arrest and apoptosis of human urinary bladder cancer cells

Background: 17-Allylamino-17-demethoxygeldanamycin (17-AAG), a benzoquinone ansamycin antibiotic, specifically targets heat shock protein 90 (Hsp90) and interferes with its function as a molecular chaperone that maintains the structural and functional integrity of various protein clients involved in cellular signaling. In this study, we have investigated the effect of 17-AAG on the regulation of Hsp90-dependent signaling pathways directly implicated in cell cycle progression, survival and motility of human urinary bladder cancer cell lines. **Methods:** We have used MTT-based assays, FACS analysis, Western blotting, semi-quantitative RT-PCR, immunocytochemistry and scratch-wound assay in RT4, RT112 and T24 human urinary bladder cancer cell lines. **Results:** We have demonstrated that, upon 17-AAG treatment, bladder cancer cells are arrested in the G1 phase of the cell cycle and eventually undergo apoptotic cell death in a dose-dependent manner. Furthermore, 17-AAG administration was shown to induce a pronounced downregulation of multiple Hsp90 protein clients and other downstream effectors, such as IGF-IR, Akt, IKK-a, IKK-b, FOXO1, ERK1/2 and c-Met, resulting in sequestration-mediated inactivation of NF- B, reduced cell proliferation and decline of cell motility. **Conclusions:** In total, we have clearly evinced a dose-dependent and cell type-specific effect of 17-AAG on cell cycle progression, survival and motility of human bladder cancer cells, due to downregulation of multiple Hsp90 clients and subsequent disruption of signaling integrity.

Publications

Sozopoulos E., H. Litsiou, G. Voutsinas, N. Mitsiades, N. Anagnostakis, T. Tseva, E. Patsouris and S. Tseleni-Balafouta (2010) Mutational and immunohistochemical study of the PI3K/Akt pathway in papillary thyroid carcinoma in Greece, *Endocr Pathol* 21, 90-100.

Voutsinas G.E., E.F. Stavrou, G. Karousos, A. Dasoula, A. Papachatzopoulou, M. Syrrou, A.J.M.H. Verkerk, P. van der Spek, G.P. Patrinos, R. Stöger and A. Athanassiadou (2010) Allelic imbalance of expression and epigenetic regulation within the alpha-synuclein wild-type and p.Ala53Thr alleles in Parkinson disease, *Hum Mutat* 31, 685-691.

Karkoulis, P.K., D.J. Stravopodis, L.H. Margaritis and G.E. Voutsinas (2010) 17-Allylamino-17-demethoxygeldanamycin induces downregulation of critical Hsp90 protein clients and results in cell cycle arrest and apoptosis of human urinary bladder cancer cells, *BMC Cancer* 10:481.

Articles in Press

Stravopodis, D.J., P.K. Karkoulis, E.G. Konstantakou, S. Melachroinou, A. Thanasopoulou, G. Aravantinos, L.H. Margaritis, E. Anastasiadou and G.E. Voutsinas (2011) Thymidilate synthase inhibition induces p53-dependent and p53-independent apoptotic responses in human urinary bladder cancer cell lines, *J Cancer Res Clin Oncol* 137, 359-374. (IF=2.261)

Lampidonis, A.D., E. Rogdakis, G.E. Voutsinas and D.J. Stravopodis (2011) The resurgence of Hormone-Sensitive Lipase (HSL) in mammalian lipolysis, *Gene* [Epub ahead of print: 7 January 2011]. (IF=2.721)

Educational Activities

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 practical laboratory exercises, in the American College of Greece (Deree College), Aghia Paraskevi Attikis, Greece.

Other Scientific Activities

Participation in Greek and International scientific bodies and organizations:

1. Reviewer for Molecular and Cellular Biochemistry, and Tumor Biology
2. Reviewer for the Union for International Cancer Control (International Union Against Cancer)
3. Greek Alliance for Rare Diseases (Treasurer)
4. Greek Alliance for Rare Diseases (PESPA) (Member of the Scientific Committee)
5. Tuberous Sclerosis Association of Greece (EEOS) (Member of the Scientific Committee)
6. Meeting of the Council of Alliances of Eurordis, 13 May 2010, Krakow, Poland.
7. Eurordis Membership Meeting 2010, 14-15 May 2010, Krakow, Poland.
8. Southern European Regional Workshop "Patients Partnering in Clinical Research", 24-25 March 2010, Athens, Greece.
9. Facilitator in the Greek Conference in the frame of the Europlan Project of EU-Eurordis, organized by PESPA, 26-27 November 2010, Athens, Greece.
10. Final Workshop "Patients Partnering in Clinical Research", 7-8 December 2010, Brussels, Belgium.
11. Meeting of the Council of Alliances of Eurordis, 12 December 2010, Paris, France.
12. 13th Workshop of Eurordis, Round Table of Companies, "Patients' Access to OMPs, Innovative Pricing Schemes and National Measures in a Global Financial and Economic Crisis Environment", 13 December 2010, Paris, France.

Other Activities for the Institute of Biology

Head of the Laboratory for "Molecular Diagnosis of Genetic Diseases", rendering genetic testing services for Tuberous Sclerosis and Neurofibromatosis

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 Biology, NCSR "Demokritos".

Member of 3 internal advisory committees of scholars working on their theses (A. Repouskou, M. Xydous, P. Karkoulis)

Impact factors (for 3 publications): 11,044

Number of citations for 2010 (without self-citations): 52

Number of citations 2006-2010 (without self-citations): 207

h-factor: 11

Current External Funding

Research project entitled *Structural and functional analysis of genes involved in the PI3K signal transduction pathway in urinary bladder cancer: effects on prognosis and therapy*, financed by the Ministry of Health and Social Solidarity. Project Coordinator: G.E. Voutsinas

Duration: 1/9/2008-31/8/2010

Total funding (laboratory): 12.000 €

Laboratory funding for 2010: 4.000 €.

Research project entitled *Decoding of the apoptotic potential of the specific inhibitor of proteasome activity Bortezomib (Velcade) in targeted chemotherapy of human urinary bladder cancer*, financed by the Association of Greek Oncologists Pathologists (ΕΟΠΕ). Project Coordinator: D.J. Stravopodis (Dept of Biology, University of Athens)

Duration: 1/12/2009 - 31/11/2010

Total funding (laboratory): 2.500 €

Laboratory funding for 2010: 1.250 €.

Research project entitled *Hsp90 inhibition as a new tool against human urinary bladder cancer*, financed by the Miltiades Empeirikos Public Welfare Foundation. Project Coordinator: D.J. Stravopodis (Dept of Biology, University of Athens)

Duration: 1/7/2010 – 31/6/2011

Total funding (laboratory): 6.000 €

Laboratory funding for 2010: 3.000 €.

Research project entitled *Molecular diagnosis of Neurofibromatosis type 1*, financed by the American College of Greece. Project Coordinator: G.E. Voutsinas

Duration: 1/11/2010 – 31/10/2011

Total funding (laboratory): 10.000 €

Laboratory funding for 2010: 3.000 €.

Note: Four (4) research proposals which have been submitted for funding are under evaluation:

1. «Non-ionizing electromagnetic radiations: biologic effects», Research proposal for funding submitted to the Ministry of Education, Life-long Learning and Religion.
2. «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 for funding submitted to the Ministry of Education, Life-long Learning and Religion.
3. «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 for funding submitted to the Ministry of Education, Life-long Learning and Religion.
4. «Pharmacological management of the Tuberous Sclerosis Complex», Research proposal for funding submitted to the 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, Senior Researcher

Vassiliki Labropoulou, Senior Researcher

Lydia Ignatiadou, Emeritus Scientist

Rodica Efroze, Postdoctoral Fellow

Konstantinos Koussis, Postdoctoral Fellow

Panagiota Tsitoura, Postdoctoral Fellow

Konstantinos Ioannidis, Graduate Student

Christiana Magrioti, Graduate Student

Ruben De Wilde, Visiting Graduate Student

Dimitris Raptopoulos, Special Collaborator

Dimitra Stefanou, Technical Specialist

Dimitris Kopanelis, Research Technician

Research Interests

- 1. Regulatory mechanisms controlling insect physiological functions.** (a) Oogenesis in lepidopteran insects. (b) Mechanisms of immunosuppression in lepidopteran insects following parasitization by hymenopteran endoparasitoids. (c) Mechanisms controlling olfactory function in the malaria mosquito vector *Anopheles gambiae*.
- 2. Molecular biology and genetic manipulation of insect nuclear polyhedrosis viruses.** (a) Recombinant viruses as vectors for insect genetic transformation. (b) Modified viruses as vectors for human gene therapy and cellular immunization applications.
- 3. Functional genomics.** (a) Systems for production of proteins of economic importance in lepidopteran insect and mammalian cell lines. (b) 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).

2010 Findings

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

To investigate the expression of proteins involved in the uptake and processing of dsRNA, expression profile studies were conducted and it was found that the R2D2 factor, an essential co-factor of Dicer-2, and the RNA-binding protein Translin, are possible limiting factors for an effective RNAi response in tissues of the silkworm (collaboration with Dr. Guy Smagghe, University of Ghent, Belgium). Over-expression of *Tribolium* R2D2 in Bm5 cells showed that R2D2 can play a role in the regulation of developmental processes. It was also found that an alkaline nuclease of the silkworm that is secreted in the gut and is involved in the digestion of nucleic acids, is expressed in many tissues of the silkworm and therefore could also be a limiting factor in gene silencing through RNAi. The ORF of the enzyme was cloned in insect expression vectors while the study of its subcellular localization and its functional properties is in progress.

Parasitization of lepidopteran insects - the interference of CcBV Ank proteins with the transcription of immune response genes in the host

Our studies of the functional characterization on the effect of CcBV Ank protein expression on the two major insect immune signalling pathways, Imd and Toll, have been completed. All the studied Ank proteins inhibited the transcription of the microbial peptide gene cecropin B1 of the silkworm (*Bombyx mori*) by 30-60% with the exception of Ank1. In contrast, only one Ank protein, Ank2, had an inhibitory effect on the Toll pathway. Immunostaining experiments that determined the subcellular localization of the expressed Ank proteins clearly showed that nearly all Ank proteins are found both in the cytoplasm and the nucleus. In fact, it was shown that Ank4 and Ank8 colocalize partially in the nucleus with the active BmRelish1d2 transcription factor and interact with the inactive

precursor BmRelish1 in pull-down assays. Therefore, based on our functional experiments, we conclude that the expression of Ank proteins targets predominantly the Imd pathway.

Ligands of olfactory proteins as repellents

Our initial studies on the screening for identification of molecules with repellent action against the mosquito vector of the malaria parasite, *Anopheles gambiae*, in essential oils prepared from Greek plants (collaboration with Dr. Maria Konstantopoulou, Laboratory of Chemical Ecology and Natural Products, IB), have been completed. The essential oils and fractions thereof were analyzed for (i) the presence of substances that bind to odorant-binding proteins (OBPs) of the mosquito; (ii) biological activity (electrophysiological responses in whole antennae and repellent action against mosquitoes *in vivo*; collaboration with Dr. Patrick Guerin, University of Neuchatel, Switzerland); (iii) identification of candidate active compounds and (iv) confirmation of their bio-activity in electrophysiological and behavioural experiments. From the essential oils of 59 species of plants analyzed, 28 contained substances binding to mosquito OBPs. From the 28 active oils, 12 compounds with significant repellent activity have been identified. The most active repellent compounds are evaluated by collaborators for absence of neurotoxicity in mammals, an essential prerequisite for the undertaking of their testing as repellents under field conditions in Africa where malaria is an endemic disease.

Engineering of baculovirus vectors for transduction of mammalian cells and insect transformation

Additional characterization was carried out on baculoviruses that have incorporated a piggyBac transposition system with mammalian expression capacity. Following infection of HEK293 cells, the sites of chromosomal integration of the transposition cassette were determined. Further studies are focused on the generation of cell lines over-expressing the viral transcriptional regulator IE-1, and a subunit of the viral RNA polymerase, Lef-8, for the rescue of the respective deficient baculoviruses. The studies showed that cells expressing IE-1 are producing fewer viral particles than normal cells. The reasons for the unexpected behaviour of the IE1-expressing cells are probed through comparisons of the transcriptome profiles of IE-1 expressing cells and cells that do not express this viral regulator.

Screening systems for substances with molting-accelerating activity and endocrine disruptors

It has been proposed that many polluting substances in the sea can influence the life-cycle of many sea organisms, including crustaceans (Crustacea). To investigate this possibility, a cell-based system was developed for the detection of interference with the function of the ecdysone receptor of the shrimp *Crangon crangon* (collaboration with Dr. Guy Smagghe, University of Ghent, Belgium). The cell-based system is currently being evaluated for detection of factors that can cause endocrine disruptions.

Screening systems for substances with serotonergic activity.

To discover lead compounds with serotonergic activity, specific functional screening platforms were developed, in collaboration with the laboratory of Molecular Pharmacology of the IB (Dr. Z. Georgoussi), which are based on insect cell lines that over-express the human serotonergic receptors 5HT-3A (K/Ca ion channel) and 5HT-4A (G protein-coupled receptor). With assays that measure the release of intracellular calcium and cAMP in the presence of serotonin and known partial agonists and antagonists, the functionality of the two systems was confirmed. The available platforms are currently being used for the screening of plant extracts with the aim of identifying lead compounds for new pharmaceuticals that activate or inhibit the function of the specific serotonergic targets.

Publications

Soin, T., De Geyter, H., Mosallanejad H., Iga, M., Martín, D., Ozaki, S., Shigeki Kitsuda, S., Harada, T., Miyagawa, H., Stefanou, D., Kotzia, G., Efrose, R., Labropoulou, V., Geelen, D., Iatrou, K., Nakagawa, Y., Janssen, C.R., Smagghe, G., and Swevers, L. (2010). Assessment of species specificity of molting accelerating compounds in Lepidoptera: comparison of activity between *Bombyx mori* and *Spodoptera littoralis* by *in vitro* reporter and *in vivo* toxicity assays. *Pest Manag Sci* **66**, 526–535.

Verhaegen, Y., Parmentier, K., Swevers, L., Rougé, P., Soin, T., De Coen, W., Cooreman, K., and Smagghe, G. (2010). The brown shrimp (*Crangon crangon* L.) ecdysteroid

receptor complex: Cloning, structural modeling of the ligand-binding domain and functional expression in an EcR-deficient *Drosophila* cell line. *Gen. Comp. Endocr.* **168**; 415-423.

Biessmann, H., Andronopoulou, E., Biessmann, M.R., Douris, V., Dimitratos, S.D., Eliopoulos, E., Guerin, P.M., Iatrou, K., Justice, R.W., Kröber, T., Marinotti, O., Tsitoura, P., Woods, D.F., and Walter, M.F. (2010). The *Anopheles gambiae* Odorant Binding Protein 1 (AgamOBP1) mediates indole recognition in the antennae of female mosquitoes. *PLoS ONE* **5**, e9471.

Soin T, Swevers L, Kotzia G, Iatrou K, Janssen CR, Rougé P, Harada T, Nakagawa Y, Smagghe G. (2010). Comparison of the activity of non-steroidal ecdysone agonists between dipteran and lepidopteran insects, using cell-based EcR reporter assays. *Pest Manag Sci.* **66**, 1215-29.

Efrose, R., Swevers, L. and Iatrou, K. (2010). Baculoviruses deficient in *ie1* gene function abrogate viral gene expression in transduced mammalian cells. *Virology* **406**, 293-301.

Lavdas, A., Efrose, R., Douris, V., Gaitanou, M., Swevers, L., Thomaidou, D., Iatrou, K., Matsas, R. (2010). Soluble forms of the cell adhesion molecule L1 produced by insect and baculovirus-transduced mammalian cells enhance Schwann cell motility. *J. Neurochem.* **115**, 1137-1149.

Tsitoura, P., Andronopoulou, E., Tsikou, D., Agalou, A., Kotzia, G.A., Labropoulou, V., Swevers, L., Georgoussi, Z., Iatrou, K. (2010). Expression and membrane topology of *Anopheles gambiae* odorant receptors in lepidopteran insect cells. *PLoS One* **5**(11):e15428.

Qiao, H., He, X., Schymura, D., Ban, L., Field, L., Romana Dani, F., Elena Michelucci, E., Caputo, B., Della Torre, A., Iatrou, K., Jing-Jiang Zhou, J., Krieger, J., and Pelosi, P. (2010). Binding assays reveal cooperative interactions between odorant-binding proteins of *Anopheles gambiae*. *Cell. Mol. Life Sci.* DOI 10.1007/s00018-010-0539-8.

Schymura, D., Forstner, M., Schultze, A., Kröber, T., Swevers, L., Iatrou, K. and Krieger, J. (2010). Antennal expression pattern of two olfactory receptors and an odorant binding protein implicated in host-odor detection by the Malaria vector *Anopheles gambiae*. *Int. J. Biol. Sci.* **6**, 614-626.

Mosallanejad, H., Badisco, L., Swevers, L., Soin, T., Knapen, D., Vanden Broeck, J., and Smagghe, G. (2010). Ecdysone signaling and transcript signature in *Drosophila* cells resistant against methoxyfenozide. *J. Insect Physiol.* **56**, 1973-1985.

Ignatiades, L. and Gotsis-Skretas, O. (2010.) A review on toxic and harmful algae in Greek coastal waters. *Toxins*, **2**, 1019-1037; doi :10.3390/toxins2051019.

Articles in Press

Terenius, O., Papanicolaou, A., Garbutt, J.S., Eleftherianos I., Huvenne, H., Sriramana, K., Albrechtsen, M., An, C., Aymeric, J.-L., Barthel, A., Bebas, P., Bitra, K., Bravo, A., Chevalier, F., Collinge, D.P., M. Crava, C.M., de Maagd, R.A., Duvic, B., Erladson, M., Faye, I., Felföldi, G., Fujiwara, H., Futahashi R., Gandhe, A.S., Gatehouse, H.S., Laurence N. Gatehouse, L.N., Giebultowicz, J., Gómez I., Grimmelikhuijzen C.J., Groot A.T., Hauser, F., Heckel, D.G., Hegedus, D.D., Hrycaj, S., Huang, L., Hull, J.J., Iatrou, K., Iga, M., Kanost, M.R., Kotwica, J., Li, C., Li, J., Liu, J., Lundmark, M., Matsumoto, S., Meyering-Vos, M., Millichap, P.J., Monteiro, A., Mrinal, N., Niimi, T., Nowara, D., Ohnishi, A., Oostra, V., Ozaki, K., Papanikolaou, M., Popadic, A., Rajam, M.V., Suzanne Saenko, S., Simpson R.M., Soberón M., Strand M.R., Tomita S., Toprak, U., Wang, P., Wee, C.W., Whyard, S., Zhang, W., Nagaraju, J., French-Constant, R.H., Herrero, S., Gordon, K., Swevers, L., Smagghe, G. (2011). RNA interference in Lepidoptera: an overview of successful and unsuccessful studies and implications for experimental design. *J. Insect Physiol.* **57**, 231-245. (IF 2.235).

Articles in Books and Conference Proceedings

Gotsis-Skretas, O., Ignatiades, L. 2010. Phytoplankton carbon-biomass in the Mediterranean Sea. *Rapp. Comm. Int. Mer Medit.*, **39**:369.

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. *Marine Strategy Framework Directive*, April 2010, JRC European Commission, EUR 24338 EN-2010.

Presentations at Scientific Conferences

H. Biessmann, A.C. Maranhao, E. Andronopoulou, M.R. Biessmann, V. Douris, S.D. Dimitratos, E. Eliopoulos, P.M. Guerin, K. Iatrou, R.W. Justice, T. Kröber, O. Marinotti, P. Tsitoura, D.F. Woods, and M.F. Walter (2010). The *Anopheles gambiae* Odorant Binding Protein 1 (AgamOBP1) mediates indole recognition in the antennae of female mosquitoes. Keystone Symposia Global Health Series: Molecular Targets for Control of Vector-Borne Diseases - Bridging Lab and Field Research. April 11-16, 2010, Copper Mountain, Colorado, USA.

K Koussis, H Biessmann, F Dani, S Dimitratos, P Guerin, M Konstantopoulou, T Kroeber, P Pelosi, L Swevers, P Tsitoura, M Walter, DF Woods and K Iatrou (2010). Volatile constituents of plant origin bind selectively various odorant binding proteins of the malaria vector *Anopheles gambiae* and act as strong repellents capable of interfering with the vector's host seeking and blood feeding activities. Keystone Symposia Global Health Series: Molecular Targets for Control of Vector-Borne Diseases - Bridging Lab and Field Research. April 11-16, 2010, Copper Mountain, Colorado, USA.

Y. Verhaegen, Parmentier, K., Soin, T., Swevers, L., Cooreman, K., De Coen, W., Robbens, J., and Smagghe, G. (2010). Use of a transformed insect cell line to identify crustacean endocrine disrupters *in vitro*. Innovation for Sustainable Production - Bruges 18-21 April 2010

G. Smagghe, Christiaens, O., Iga, M., Verlarde, R.A., Rougé, P., Nakagawa, Y., and Swevers, L. (2010). Nuclear receptors in caterpillars and aphids: towards selective targets for pest control. 12th IUPAC International Congress of Pesticide Chemistry. Melbourne 4th-8th July 2010. In disrupters *in vitro*. Innovation for Sustainable Production - Bruges 18-21 April 2010

G. Smagghe, Soin, T., Kotzia, G., Iatrou, K., Janssen, C.R., Rougé, P., Harada, T., Nakagawa, Y., and Swevers, L. (2010). Comparison of activity of non-steroidal ecdysone agonists between dipteran and lepidopteran insects using cell-based EcR reporter assays. 18th International Ecdysone Workshop, České Budějovice, Czech Republic, 19-23 July, 2010.

G. Smagghe, De Geyter, E., Geelen, D., Soin, T., and Swevers, L. (2010). Exposure of insect cells to *Quillaja saponaria* saponin caused an anti-ecdysteroid action that may be explained by cytotoxicity and permeation. 18th International Ecdysone Workshop, České Budějovice, Czech Republic, 19-23 July, 2010.

Y. Verhaegen, Parmentier, K., Swevers, L., Rougé, P., Soin, T., De Coen, W., Cooreman, K., and Smagghe, G. (2010). The brown shrimp (*Crangon crangon* L.) ecdysone receptor complex: Cloning, structural modeling of the ligand-binding domain and functional expression in an EcR-deficient *Drosophila* cell line. 18th International Ecdysone Workshop, České Budějovice, Czech Republic, 19-23 July, 2010.

Y., Verhaegen, Renders, E., Parmentier, K., Swevers, L., Rougé, P., De Coen, W., Cooreman, K., and Smagghe, G. (2010). The RXR receptor: a target of endocrine disruption in the brown shrimp (*Crangon crangon* L.) 18th International Ecdysone Workshop, České Budějovice, Czech Republic, 19-23 July, 2010.

G. Smagghe, De Geyter, E., Geelen, D., Soin, T., and Swevers, L. (2010). *Quillaja saponaria* saponin is causing an anti-ecdysteroid action in insect cells that may be explained by cytotoxicity and permeation. 25th Conference of European Comparative Endocrinologists, Pécs, Hungary, 31 August - 4 September, 2010.

G. Smagghe, Verhaegen, Y., Parmentier, K., Swevers, L., Rougé, P., Soin, T., De Coen, W., and Cooreman, K. (2010). The brown shrimp (*Crangon crangon* L.) ecdysone receptor complex: Cloning, structural modeling of the ligand-binding domain and functional expression in an EcR-deficient *Drosophila* cell line. 25th Conference of European Comparative Endocrinologists, Pécs, Hungary, 31 August - 4 September, 2010.

G. Smagghe, Verhaegen, Y., Renders, E., Parmentier, K., Swevers, L., Rougé, P., De Coen, W., and Cooreman, K. (2010). The RXR receptor: a target of endocrine disruption in the

brown shrimp (*Crangon crangon* L.) 25th Conference of European Comparative Endocrinologists, Pécs, Hungary, 31 August - 4 September, 2010.

L. Swevers, Georgomanolis, T., and Iatrou, K. (2010). Induction of the transition from vitellogenesis to choriogenesis by decline in ecdysone signalling in the ovary of the silkworm, *Bombyx mori*: a possible regulatory role for the orphan nuclear receptor BmE75C. 25th Conference of European Comparative Endocrinologists, Pécs, Hungary, 31 August - 4 September, 2010.

L. Swevers, R. Efrose, P. Tsitoura and K. Iatrou (2010). Functional expression platforms for research and added-value applications. International Silkworm Genome Symposium and Annotation Workshop November 8-11, 2010 Tsukuba, Japan.

L. Swevers, Terenius, O., and Smagghe, G. (2010). RNAi in Lepidoptera: Successes and failures and implications for experimental design. Entomology 2010: 58th Annual Meeting Entomological Society of America (ESA). Symposium: RNA-interference Insect Management: Real-world Applications. SanDiego, California, USA, 12-15 December, 2010.

L. Swevers, R. Efrose, P. Tsitoura, K. Kousis, C. Magrioti, V. Labropoulou, K. Ioannidis and K. Iatrou (2010). Gene expression platforms for lepidopteran cells as tools for functional annotation of genome sequences, basic research and added-value applications. International Silkworm Genome Symposium and Annotation Workshop, November 8-11, 2010, Tsukuba, Japan.

39th CIESM Congress, Venice, Italy, 10-14 May, 2010 (L. Ignatiadou).

K.E. Tsitsanou, C.E. Drakou, A. Thiraiou, E. Eliopoulos, K. Iatrou and S.E. Zographos (2010). AgamOBP1 is a molecular target for the development of novel insect repellents. Fifth Conference of the Hellenic Crystallographic Association, September 24-25, 2010, Larissa, Greece.

P. Tsitoura, Lioupis, A., Swevers, L., Georgoussi, Z. and Iatrou, K. (2010). Expression of transmembrane receptors in insect cells: biochemical properties and functional assays. 61st Conference of the Hellenic Society for Biochemistry and Molecular Biology, October 15-17, 2010, Alexandroupolis, Greece.

C. Magrioti, Iatrou K., Labropoulou V. (2010) The Ankyrin-repeat proteins of the polydnavirus CcBV and their interference in lepidopteran immunity signaling pathways. 61st Conference of the Hellenic Society for Biochemistry and Molecular Biology, October 15-17, 2010, Alexandroupolis, Greece.

R. Efrose, Swevers, L., Iatrou, K. (2010). Stable transformation of mammalian cells using insect viruses encompassing a *piggyBac* transposition system. 61st Conference of the Hellenic Society for Biochemistry and Molecular Biology, October 15-17, 2010, Alexandroupolis, Greece.

K. Koussis, Kröber T., Konstantopoulou, M., Dani, F.R., Pelosi, P., Guerin P.M., Iatrou, K. (2010). Volatile constituents of aromatic plants as repellents for the African malaria mosquito vector *Anopheles gambiae*. 61st Conference of the Hellenic Society for Biochemistry and Molecular Biology, October 15-17, 2010, Alexandroupolis, Greece.

L. Swevers, Stefanou, D., Liu, J., Huvenne, H., and Smagghe, G. (2010). Studies of RNAi pathway in the silkworm-derived Bm5 cell line. 61st Panhellenic Conference of the Hellenic Society of Biochemistry and Molecular Biology, Alexandroupolis, 15-17 October.

Other Scientific Activities

Member, Evaluation Committee for promotion of scientific personnel at the Hellenic Pasteur Institute (K. Iatrou).

Member, Committee for recruitment of 2 external collaborators at the Institute of Microelectronics, NCSR "Demokritos", to support the scientific program "MiNaSys-CoE-Micro and NanoSystems Center of Excellence (REGPOT)" (L. Swevers).

Member, EU Committee for the drafting of the directive for quality management of the European Marine Environment. Title of the Directive: "Marine Strategy Framework Directive" Eutrophication Quality Descriptor, (2009-2010). (L. Ignatiadou).

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

Member, Editorial Boards of "Sericologia", "Insect Biochemistry and Molecular Biology", "Archives of Insect Biochemistry and Physiology", "Open Biotechnology Journal" and "Journal of Biomedicine and Biotechnology" (K. Iatrou).

Member, Editorial Board of "Archives of Insect Biochemistry and Physiology" (L. Swevers).

Member, Editorial Board of "Mediterranean Marine Science Journal" (L. Igantiadou).

Organizer of the 2nd Joint Symposium of EC/FP7-F Projects for Malaria Vector Biology and Control, Kolymbari, Crete, Greece, July 24, 2010 (K. Iatrou).

Referee of scientific articles for the journals "Insect Biochemistry and Molecular Biology", "Insect Molecular Biology", "Journal of Insect Science", "Sericologia", "PLoS ONE", "BMC Developmental Biology", "Journal of Insect Physiology", "Journal of Biomedicine and Biotechnology", "Developmental Biology", "Molecular Biology Reports", "Journal of Agricultural and Food Chemistry", "Parasites & Vectors", "Comparative and Functional Genomics", "Journal of Virological Methods" (K. Iatrou).

Referee of scientific articles for the journals "Insect Biochemistry and Molecular Biology", "Insect Molecular Biology", "BMC Genomics", "Archives of Insect Biochemistry and Molecular Biology", "Journal of Biomedicine and Biotechnology", "Journal of Insect Physiology", "Pest Management Science", "PLoS ONE" (L. Swevers).

Referee of scientific articles for the journal "Virulence" (V. Labropoulou).

Referee of scientific articles for the journals "Marine Ecology", "Mediterranean Marine Science Journal", "Limnology and Oceanography" (L. Ignatiadou)

Educational Activities

One hour lecture entitled "Malaria in the third world: targeting the mosquito vector of the parasite to limit the spread of the disease" at the Summer School of NCSR "Demokritos" (K. Iatrou).

One hour lecture entitled "Alternative methods of insect pest control: growth regulators and environmental RNAi" at the Summer School of NCSR "Demokritos" (L. Swevers).

Other Activities for the Institute of Biology

Substitute member of the committee for recruitment of researchers (K. Iatrou).

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

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

Supervision of Ph.D. thesis of Christiana Magkrioti, IB graduate student (University of Athens) (K. Iatrou, V. Labropoulou).

Member of the Examination Committee for the recruitment of new graduate students at the IB (English language) (L. Swevers).

Member of the Internal Committee for supervision of graduate students with scholarship from NCSR "Demokritos" at the IB: Christiana Magkrioti, Konstantinos Ioannidis, Maria Papanstandinou (L. Swevers).

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

Member of the Internal Committee for supervision of graduate students with scholarship from NCSR "Demokritos" at the IB: Christiana Magkrioti, Konstantinos Ioannidis (V. Labropoulou).

Responsible for tours and public relations of the Institute of Biology, NCSR "Demokritos" from September 2010 (V. Labropoulou).

Impact Factors (for 10 publications): 34,045

Citations 2010 (without self-citations):

Iatrou K. (including citations with L. Swevers and V. Labropoulou): 85

Swevers L. (including 41 citations with K. Iatrou): 57
Labropoulou V. (including 14 citations with K. Iatrou and L. Swevers): 45
Ignatiadou L.: 76

Total Citations 2006-2010 (without self- citations):

Iatrou K. (including citations with L. Swevers and V. Labropoulou): 335
Swevers L. (including 175 citations with K. Iatrou): 194
Labropoulou V. (including 24 citations with K. Iatrou and L. Swevers): 181
Ignatiadou L.: 268

h-factor:

22 (K. Iatrou)
15 (L. Swevers)
8 (V. Labropoulou)
16 (L. Ignatiadou)

Current External Funding

PENED 2005 project entitled *Mechanisms of immunosuppression in lepidopteran insects following parasitization by hymenopteran endoparasitoids: the role of the interactions between proteins produced by hymenopteran endosymbiotic polydnaviruses and hemocyte proteins of the lepidopteran hosts*, funded by GSRT (Coordinator K. Iatrou).

Duration: 3/2006-3/2009

Total funding (lab): 57.600 €

Funding of the lab for 2009: 0 €.

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

Duration: 12/2008-12/2012

Total funding (consortium): 2.500.000 €

Total funding (lab): 563.000 €

Funding of the lab for 2010-11: 219.217,38 €

Co-funding of the lab for 2010: 2.612,19

Note: The following proposals have been submitted and are under evaluation for the program "Thalis" as "Central Research Group":

1. Title: Prostaglandin receptors during insect development – study of signal transduction and isolation of agonists and antagonists in natural products. Coordinator: S. Dedos, University of Athens (Coordinating IB scientist: K. Iatrou).
2. Title: Role of the insulin signal transduction cascade and the FOXO transcription factor in the regulation of lifespan and metabolism during diapause of lepidopteran insects. Coordinator: A. Kourti, Agricultural University of Athens (Coordinating IB scientist: K. Iatrou).
3. Title: Genomic and functional approach to understand the resistance of insects and mites against insecticides and development of applications for its management. Coordinator: J. Vontas, University of Crete (Coordinating IB scientist: K. Iatrou).

The laboratory also participates in the "Thalis" program as member of a "Central Research Group": Regulation of cellular signalling by autophagy and heat-shock proteins after exposure to ionizing radiation. Coordinator: E. Sivridis, University of Thrace (Coordinating IB scientist: N. Grammatikakis) (V. Labropoulou).

Finally, in the framework of the program "New Insecticides for Malaria Control discovery research program" of the Foundation of the National Institutes of Health (Bill & Melinda Gates Foundation), was submitted a project with title "*Sensory appendage proteins as targets for control of the African malaria mosquito vector Anopheles gambiae*" (Coordinator: Dan Woods, Inscent, Inc., Irvine, US; coordinator in Greece: K. Iatrou ; participation: L. Swevers, V. Labropoulou).

Research Group: *Pending (Collaboration with the laboratory of Molecular Genetics of Insects and Biotechnology - In charge: Dr. K. Iatrou)*

Research Staff

Maria Konstantopoulou, Researcher

Kelly Martinou, Collaborating Scientist

Anatsassia Pantazi – Mazomenou, Research Technician

Rafaela Panteleri, Undergraduate Student

Research Interests

- Chemical ecology: isolation and identification of biologically active substances, relating to insect chemical communication and to 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 chemical attractants for insects and technologies for their application in pest population control.
- 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 incorporate them in insect population management.

2010 Findings

In the context of the EC funded project ENAROMaTIC (a collaboration with the Laboratory of "Insect Molecular Genetics and Biotechnology", Prof. K. Iatrou), the collection of endemic botanical species was continued and our current collection comprises of over 250 species belonging to 65 plant families. Steam distillation technique in a modified Clevenger apparatus is employed to extract essential oils. The crude extracts are tested with the high throughput screening assay for OBPs ligand identification. Extracts exhibiting positive response are fractionated by means of solid phase extraction using increasing polarity solvents. Fractions are evaluated for biological activity. Gas chromatography is employed to identify differences in eluates between crude and their derived fractions. Tentative identification of biologically active compounds is performed using Mass Spectroscopy. The experimental procedure is currently under way aiming to isolate and identify substances with repellent action.

Methods of Integrated Pest Management were deployed against insect pests causing significant economical damage, as the Tomato Leaf Miner (*Tuta absoluta*) and the Red Palm Weevil (*Rhynchophorus ferrugineus*). These methods were based on the use of entomopathogenic fungi, entomopathogenic nematodes and the development of volatile attractants in conjunction with specialized dispensers for their controlled release for optimum efficacy.

The Mating Disruption method for control of the Pine Processionary Moth (*Thaumetopoea pityocampa*) was optimized and deployed in a small scale field application in "Attikon Alsos", in collaboration with Benaki Phytopathological Institute and the Prefecture of Attiki. A specific amorphous polymer was used as an inert matrix for formulation of the insect's pheromone. The polymer chosen is endowed with specific properties that provide adequate protection to the pheromone molecules against adverse environmental condition (extreme temperatures, humidity, UV etc.). In addition it is non-toxic, biodegradable and does not pollute the environment. Concurrently with the field application the insect's flight was monitored in a number of suburban areas in Attiki including the campus of "Demokritos".

The larvicidal action of chemical compounds of natural origin was studied on mosquitoes (*Culex pipiens*). Aiming to develop biotechnical methods for the control of mosquito populations the given compounds were microencapsulated in specific substrates. Toxicity levels were investigated according to the standards laid by WHO. Toxicity and mutagenesis of chemical compounds of natural origin was also studied on other dipterous insects.

Publications

Hegazi, E.M., W.E. Khafagi, M.A Konstantopoulou, D. Raptopoulos, S. Shweilb, S. Abd El-Rahman , A. Atwa , S.E. Ali and H. Tawfik, (2010). Suppression of Leopard Moth, *Zeuzera pyrina* L. (Lepidoptera: Cossidae) Populations in Olive Trees in Egypt Through Mating Disruption. J. Econ. Entomol. 103(5), 1621-1627.

Milonas P.G., D.Ch. Kontodimas, A. Michaelakis, D.G. Raptopoulos, M.A. Konstantopoulou, (2010). Optimization of pheromone trapping method for click beetles, (*Agriotes* sp.) in Greece. Phytoparasitica 38, 429-434.

Articles in Press

Hegazi M.E., M.A. Konstantopoulou, A. Herz, W.E. Khafagi, E. Agamy , S. Showiel, A. Atwa, G.M., Abd El-Aziz, and S.M. Abdel-Rahman, (2011). Seasonality in the occurrence of two lepidopterous olive pests in Egypt. Insect Sci. (in press) (I.F.=1.118).

Presentations at Scientific Conferences

Koussis, K, H Biessmann, F Dani, S Dimitratos, P Guerin, M Konstantopoulou, T Kroeber, P Pelosi, L Swevers, P Tsitoura, M Walter, DF Woods and K Iatrou (2010). Volatile constituents of plant origin bind selectively various odorant binding proteins of the malaria vector *Anopheles gambiae* and act as strong repellents capable of interfering with the vector's host seeking and blood feeding activities. Keystone Symposia Global Health Series: Molecular Targets for Control of Vector-Borne Diseases - Bridging Lab and Field Research. April 11-16, 2010, Copper Mountain, Colorado, USA. Abstract: 153.

Koussis, K, T Kroeber, M Konstantopoulou, F Dani, P Pelosi, P Guerin, and K Iatrou (2010). Volatile constituents of aromatic plants as repellents for the African malaria mosquito vector *Anopheles gambiae*. 61o Panhellenic Congress of Hellenic Society of Biochemistry and Molecular Biology, 15-17 October, Alexandroupolis. Abstract: 13.

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. Tshipidou-Mavragani).

Participation in submitting a research proposal for the financial support of doctoral thesis of postgraduate student, in collaboration with the Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki (Assoc. Prof. P. Tshipidou-Mavragani). The proposal is under evaluation for funding by the «Onasis Foundation».

Member of the evaluation committee for recruitment of two external co-workers for the Institute of Informatics and Telecommunication in the framework of an international project funded by the European Commission.

Member of the committee for evaluation of candidates for TWAS (The Academy of Science for developing world) awards 2010 in the field of Agrobiotechnology

Reviewer of the following international scientific journals (I.F. 2008): Chemosphere (I.F.= 3.253), Journal of Agricultural and Food chemistry (I.F.= 2.469), Entomologia Experimentalis et Applicata (I.F.= 1.568), Bulletin of Insectology (I.F.=0.448), Journal of Applied Entomology (I.F.= 1.436), Crop Protection (IF=1.331) and Insect Science (I.F.=1.118).

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

Other Activities for the Institute of Biology

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

Member of the committee for reasoned opinion of IB researchers regarding the election of the Institute Director.

Member of the evaluation and examination Committee of the postgraduate students' exams in IB.

Impact Factors (for 2 publications): 1,873

Citations 2010 (without self- citations): 35

Total Citations 2006-2010 (without self- citations): 126

h-factor: 7

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 (2010) for the laboratory derive through the total Coordinator's funds

Note:

Three proposals were submitted in the action of "Thales" - Enhancing interdisciplinary and multidisciplinary research and innovation with the potential to attract high level researchers from abroad by conducting basic and applied research excellence. Ministry for Education Lifelong Learning and Religion:

- Title: *Molecular mechanisms of florescence and bioactive secondary metabolites production of *Eruca sativa**. Coordinator: K. Papadopoulou, University of Thessaly (Research partner from Institute of Biology: M. Konstantopoulou)
- Title: *Prostaglandin receptors for insect development*. Coordinator: D. Skarlatos, National and Kapodistrian University of Athens (Research partner from Institute of Biology: Prof. K. Iatrou)
- Title: *Genomic and functional analysis for insect and acari resistance to insecticides* Coordinator: J. Vontas, University of Crete (Research partner from Institute of Biology: Prof. K. Iatrou)

Two proposals were submitted 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, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)*.
- *Development of ecological and innovative technologies for the population management of pine processionary moth, *Thaumetopoea pityocampa*, in urban and suburban environments*.

Research Group: Chronobiology

Research Staff

Anastassia Prombona, Senior Researcher

Anastasia Repouskou, Graduate Student

Aggeliki Galeou, Graduate Student

Research Interests

Investigation of the biological clock function in plants

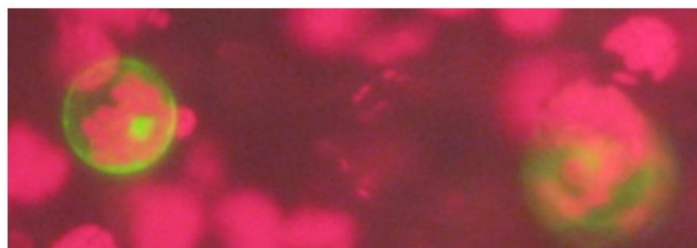
Study of rhythmically expressed genes in *Phaseolus vulgaris*. Regulation of rhythmic gene expression by input light signals and photoperiodism leading to the synchronization of the biological clock. Investigation of the role of clock proteins in the central oscillator function.

Involvement of the biological clock function in carcinogenesis

Regulation of cell cycle and cell proliferation by the biological clock in mouse fibroblasts and cancer cell lines. Study of the effects of modulated acetylation levels of histones and clock proteins on the biological clock function and the cell cycle. Elucidation of the role of the circadian time in proliferation of cancer cells during application of drugs (chronotherapy).

2010 Findings

Investigation of the biological clock function in plants



Bean protoplasts transformed with green fluorescent protein (GFP). Under UV light the chlorophyll of the chloroplasts fluoresces red.

A. Galeou has isolated during her master's research a fragment of 1250 bp from the 5' upstream region of the PvTOC1 ORF. In silico analysis of the sequence revealed putative promoter regulatory motifs. Cloning work of truncated promoter fragments is in progress, in order to determine the contribution of the theoretically identified motifs in

expression levels and rhythmicity. Expression will be tested in transformed *Phaseolus* protoplasts.

Involvement of the biological clock function in carcinogenesis

The PhD work of A. Repouskou investigates the interaction of the biological clock function with carcinogenesis. The focus during this year was on studying the regulation of rhythmic c-MYC protein accumulation in clock-synchronized N2A mouse neuroblastoma cell cultures. Our results demonstrated that circadian time is important for onco-protein synthesis and degradation. Moreover, application of trichostatin A (TSA, a specific inhibitor of histone deacetylases) disrupted the time-dependent protein accumulation at specific circadian times. This project is carried out in collaboration with Dr.T. Sourlingas ('Nuclear Proteins and Chromatin Function' group).

Publications

A. Repouskou, T.G. Sourlingas, K.E. Sekeri-Pataryas and A. Prombona. The Circadian Expression of c-MYC Is Modulated by the Histone Deacetylase Inhibitor Trichostatin A in Synchronized Murine Neuroblastoma Cells. *Chronobiology International* 27(4), 722-741, 2010.

Other Activities for the Institute of Biology

Member of the Scientific Advisory Committee of IB

Member of the advisory board of the PhD students A. Repouskou, A. Galeou, M. Xidou

Impact Factors (for 1 publication): 3,495

Citations 2010 (without self- citations): 5

Total Citations 2006-2010 (without self- citations): 33

h-factor: 5

Note: participation to the following proposal has been submitted and is under evaluation:

- *Non-ionizing electromagnetic radiations: biological impact*, University of Athens (Greek Ministry of Education, Lifelong Learning and Religious Affairs)

Research Group:

Microbial Molecular Genetics

Research Staff

Vassiliki Sophianopoulou, Research Director

Eleftherios Sideris, Emeritus Scientist

Dimitra Bouzarelou, Graduate Student- *Phd obtained in 2010*

Ioannis Vaggelatos, Graduate Student

Alexandros Athanassopoulos, Graduate Student

George Krezias, Undergraduate Student

Vassiliki, Pantazopoulou, Undergraduate Student

Konstantina Ioannidi, Training Student

Research Interests

Our group is primarily interested in several aspects concerning i) the expression, function, cell biology and evolution of transport proteins and ii) membrane organization. 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.

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 factors that regulate directly or indirectly the activity of amino acid transporters ie *trans*-acting molecular determinants involved in topogenesis/recycling/endocytosis of amino acid transport systems (eisosomal proteins, CKI kinases, aldolases) and c) studies on structure-function relationships of amino acid transporters.

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. Study of the molecular mechanisms involved in modification of fungal cell wall

Activities: a) identification and regulation of the expression of genes encoding putative expansin-like protein(s) and cell wall enzymes in *Aspergillus nidulans* b) functional, developmental and physiological characterization of their products.

Intermediate and long-term objectives: novel biotechnological means with fungicidal activity, development of novel enzymatic applications, industrial applications in food industry and environmental sciences (bio-furling agro-waste treatment).

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** topogenesis, function and specificity

b) Identifying the pathways and molecular mechanisms involved in the **membrane trafficking and endocytosis** of specific transporters in response to various physiological, developmental and genetic signals

c) Studying the role of **eisosomal proteins** in fungal membrane organization in respect to membrane trafficking and endocytosis of specific transporters, cell cycle progression and animal/human infections

2010 Findings

Transporters of medical, pharmacological and agricultural importance

1) In the context of the investigation of amino acid transport mechanisms, complementation of the hypomorphic *fbaA1013* mutation -one in a total of 22 UV isolated mutations affecting utilization of amino acids as sole nitrogen sources- with an *A. nidulans* genomic library resulted in cloning of a gene encoding an essential 1,6 biphosphate aldolase (FBA/FbaA). We showed that a functional FbaA protein is necessary for plasma membrane localization of the AgtA acidic amino acid (L-glutamate/L-aspartate) transporter, as the *fbaA1013* mutation results in targeting to and presumably subsequent degradation of AgtA in the vacuole. To our knowledge, our results showed for the first time, that FBA -an enzyme involved in both glycolysis and gluconeogenesis- is also involved in plasma membrane localization of amino acid transporters (AgtA and proline transporter PrnB) (see **Roumelioti et al., 2010; Figure 1D**).

2) In an attempt to find possible substrates of the AauZ CkiA (casein kinase I) kinase of *A. nidulans* -alleles of which result in disruption of amino acid transporter targeting to plasma membrane- we have cloned and studied homologues of *Saccharomyces cerevisiae* eisosomal proteins stated to be involved in plasma membrane and lipid internalization. We identified in *A. nidulans* (and in all the Pezizomycotina) two homologues of Pil1/Lsp1 yeast core eisosomal proteins, PilA and PilB, originating from a duplication independent from that extant in the Saccharomycotina and one strict orthologue of Sur7, the transmembrane protein of eisosomes. In *A. nidulans* conidiospores the three proteins colocalise at the cell cortex and form tightly packed punctate structures that appear different from the clearly distinct eisosome patches observed in *S. cerevisiae*. In mycelia, punctate structures are present, but they are composed only of PilA, while PilB is diffused in the cytoplasm and SurG is located in vacuoles and endosomes. Deletion of each of the genes does not result in any obvious growth phenotype, except for a moderate resistance to itraconazole (see **I. Vangelatos et al., 2010; Scazzocchio et al., 2011; Figure 1B**).

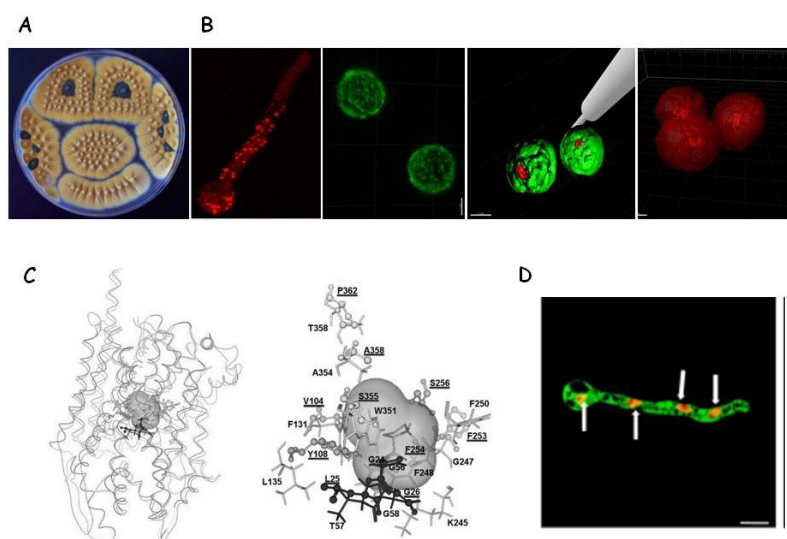


Figure 1: A. *Aspergillus nidulans* B. Eisosomal proteins labeled with GFP and mRFP. C. Amino acids of the major proline transporter PrnB binding site indicated by molecular modelling D. 1,6 biphosphate aldolase labeled with GFP

3) Our molecular modelling study of PrnB transporter (see **I. Vangelatos et al., 2009**) provide compelling evidence that the amino acid binding pocket of PrnB is located in the vicinity of the unwound part of two broken helices and allows predictions concerning residues that might be crucial in determining the specificity profile of APC (Amino acid-Polyamine-Organocation) members and presented data on which amino acid residues are key elements for protein stability and trafficking to the plasma membrane. In the context of structure/function relationships of amino acid transporters, based on this analysis and on novel mutational data concerning PrnB, we have *in vitro* mutagenised G247, F248, 335 and Ser142 residues of the predicted proline binding pocket of PrnB, in order to investigate their role in determining the specificity profile of APC members (**Figure 1C**).

2010 Publications

Roumelioti, K., Vangelatos, I., and Sophianopoulou, V. (2010). A cryptic role of a glycolytic-gluconeogenic enzyme (aldolase) in amino acid transporter turnover in *Aspergillus nidulans* *Fungal Genet. Biol.* 47: 254-267 [5TU](http://www.ncbi.nlm.nih.gov/pubmed/20026236)
<http://www.ncbi.nlm.nih.gov/pubmed/20026236>

Vangelatos, I., Roumelioti, K., Gournas, Ch., Suarez, T., Scazzocchio, C., and Sophianopoulou, V. (2010). Eisosomes organization in the filamentous ascomycetes *Aspergillus nidulans* *Eukaryotic Cell* 9(10):1441-54
<http://www.ncbi.nlm.nih.gov/pubmed/20693301>

Articles in Press

Scazzocchio, C., Vangelatos, I., and Sophianopoulou, V. (2011). Eisosomes and membrane compartments in the ascomycetes: a view from *Aspergillus nidulans*. Invited Addendum Article. *Commun. Integr. Biol.* Vol. 4(1): 64-68
<http://www.ncbi.nlm.nih.gov/pubmed/21509182>

Articles in Books and Conference Proceedings

Vangelatos, I., Scazzocchio, C., and Sophianopoulou, V. (2010). Eisosome organization in the filamentous fungus *Aspergillus nidulans*. Abstract of the IMC9 "The Biology of Fungi". A4: Endocytosis and Exocytosis.

Vangelatos, I., Scazzocchio, C., and Sophianopoulou, V. (2010). Eisosomes in the filamentous ascomycete *Aspergillus nidulans*. Abstract III.4 page 22.

Presentations at Scientific Conferences

Vangelatos, I., Scazzocchio, C., and Sophianopoulou, V.* (2010). Eisosomes in the filamentous ascomycete *Aspergillus nidulans*. Abstract III.4 page 22. Leopoldina Workshop on Plasma Membrane Domains in Fungi and Plants. Patchy Prague 2010. Prague Czech Republic 10-13 October (Oral presentation*-Financial support by the Germany Academy of Science Leopoldina).

Vangelatos, I., Scazzocchio, C., and Sophianopoulou, V.* (2010). Eisosome organization in the filamentous fungus *Aspergillus nidulans*. Abstract of the IMC9 "The Biology of Fungi". A4: Endocytosis and Exocytosis. Edinburgh, UK, 1-6 August (Selected for oral presentation*).

Other Scientific activities

Editorial Advisor Board Member of "The Open Mycology Journal" (Bentham Science Publishers) (V. Sophianopoulou).

Referee for Molecular Genetics and Genomics and Comparative Biochemistry and Physiology (V. Sophianopoulou).

Invited speaker at the "Leopoldina Workshop on "Plasma Membrane Domains in Fungi and Plants". "Patchy Prague 2010". Prague Czech Republic, 10-13 October 2010 (V. Sophianopoulou).

Member of the Evaluation Committee for the PhD. Thesis of D. Bouzarelou, National and Kapodistrian University of Athens (V. Sophianopoulou).

Member of the Advisory Committee for the PhD thesis of I. Vangelatos, University of Ioannina (V. Sophianopoulou).

Member of the Advisory Committee for the PhD thesis of A. Athanasopoulos, National and Kapodistrian University of Athens (V. Sophianopoulou).

Educational Activities

D. Bouzarelou **defended her PhD** thesis with title: «Characterisation of plant expansin homolog genes in *Aspergillus nidulans*». Department of Biology, EKPA, 18/5/2010. Grade: Excellent (Supervisor: V. Sophianopoulou).

Four-hour lectures on "Microbial Biotechnology-Model Systems of Molecular Microbiology" a Post-Graduate Course at University of Athens Department of Biology (V. Sophianopoulou).

Coordinator of the Post-Graduate Course on “Chromatin structure and regulation of gene expression” at IB (V. Sophianopoulou).

Presentation with title: “Eisosomes: gates or blocks of endocytosis?” at the summer school of NCSR “Demokritos” Abstract book 123-125 (V. Sophianopoulou).

Participation in the FEBS Workshop on Biochemistry Education, Institut Pasteur, Athens 14 May 2010 (V. Sophianopoulou).

Retreat IB: Presentation with title: «Scientific directions of the Molecular Microbial Genetics Lab-Research progress and achievements 2005-2010» 29/06/10.

Invited Speaker Institute of Radioisotopes and Radiodiagnostic Products (IRRP) NCSR «Δ». Presentation with title: «Regulation of function/topogenesis of transporters involved in neurotransmission: CKI kinases and Eisosomal Proteins» 11/03/10.

Other Activities for the Institute of Biology

V. Sophianopoulou:

- Member of the Scientific Consulting Board at IB (resignation 06/2010)
- President of the Education Committee at IB (until 07/2010)
- Member of the Education Committee at NCRS Demokritos (until 07/2010)
- Supervision of the PhD thesis of the graduate student D. Bouzarelou at the IB (University of Athens). Defence May 2010. Grade “Excellent”.
- Supervision of the PhD. thesis of the graduate student I. Vangelatos at the IB (University of Ioannina)
- Supervision of the PhD thesis of the graduate student A. Athanasopoulos at the IB (University of Athens)
- Supervision of Diploma Thesis of the undergraduate student V. Pantazopoulou at the IB (University of Athens)
- Supervision of Diploma Thesis of the undergraduate student G. Krezias at the IB (University of Athens)
- Member of the Internal Committee for supervision of graduate students with scholarship from NCSR “Demokritos” at the IB: N. Sdralia
- Member of the Examination Committee for the recruitment of new graduate students at the IB (Biology)

Impact Factor (for 2 publications):.7

Citations 2010 (without self-citations): 42

Total Citations 2006-2010 (without self-citations): 178

h-factor: 12

Current External Funding

EU programme (FP7-PEOPLE-2009-RG Marie Curie Actions—European Re-integration Grants -ERG) entitled *Eisosomal proteins in Aspergillus nidulans: regulators of endocytosis, cell wall synthesis, membrane sub-domain organization and cell cycle*.

Responsible Scientist: V. Sophianopoulou

Duration: 2011-2013

Total funding (lab): 45.000€

Funding of the lab for 2010: 0 €.

Note:

The following proposal has been passed to the 2nd round of evaluation for the program “Thalis” as “Central Research Group”:

Title: *Structure-function relations of bacteria transporters and their eukaryotic homologues* Coordinator: S. Frilingos, University of Ioannina (Coordinator of Central Research Group: V. Sophianopoulou).

The laboratory also participates in the following proposal passed to the 2nd round of evaluation as member of a “Central Research Group”

Title: *Development of genetic and genomic tools with Minos transposon and their applications in model organisms.* Coordinator: Ch. Savakis, Biomedical Sciences Research Center Al. Fleming (Coordinating IB scientist: V. Sophianopoulou).

Research Group: Biophysics and Biotechnology of Membranes

Research Staff

Kostas Stamatakis, Senior Researcher

George Papageorgiou, Emeritus Scientist

Meropi Tsimilli - Michael, Collaborating Scientist

Elias Georgakopoulos Soares, Summer Student

Research Interests

We investigate the time-dependent changes (induction) of chlorophyll a (Chl a) fluorescence in model cyanobacteria and higher plants, with emphasis on the role of carotenoids as photon collectors. In contrast to chlorophylls and phycobilins, the light harvesting mechanism of carotenoids and the supply of electronic excitation from them to the Chls a of the reaction centers of photosystems I and II (PSI, PSII) has not been described in satisfactory detail. 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.

Studies on the photosynthetic Hydrogen production.

2010 Findings

In the research project of 2010 we excited the β -Cars in the PSII_{CC} and PSI_{CC} of cyanobacterium *Synechococcus* sp PCC7942 cells selectively and we examined its effect on the oxidoreduction state of intersystem set of electron carriers (PQ pool) by means of state transitions phenomenology. Our results show that excited β -Cars drive an oxidation of the PQ pool, which suggests an over performing PSI and an underperforming or non-performing PSII. Further steady state measurements of photoinduced electron transport across PSII and PSI suggest a non-performing PSII under conditions of selective β -Car excitation.

Articles in Books and Conference Proceedings

Kana R, Komarek O, Kotabova E, Papageorgiou GC, Govindjee, Prasil O (2010): Light-induced State 2-to-State-1 Transition is mainly responsible for the S-to-M Rise in the Chlorophyll a Fluorescence Induction of the Cyanobacterium *Synechocystis* sp. PCC 6803. In *Advances in Photosynthesis Research, Proceedings of the 22nd International Congress of Photosynthesis*, 22-22 August 2010, Beijing, China (in press).

Presentations at Scientific Conferences

D. Benaki, K. Stamatakis, E. Mikros, and M. Pelecanou (2010) Interaction of β -amyloid peptide with potential inhibitors. 18th EuroQSAR EAST -NMR Satellite Meeting, 24th -25 th September, 2010.

D. Benaki, K. Stamatakis, E. Mikros, M. Pelecanou(2010) Spectroscopic investigation of the interaction of curcumin with β -amyloid peptide Neuroscience. Days of the Hellenic Society for Neuroscience, October 1-2, 2010.

Other Scientific Activities

Chapters in Books :

Papageorgiou GC and Stamatakis K (2010) Chlorophyll a Fluorescence: A Sensitive Reporter of Osmotic Properties and Permeability Properties of Cyanobacterial Cells. In *Protocols on Algal and Cyanobacterial Research*, Bagchi SN, Kleiner D & Mohanty P (eds) Narosa Publishing House, New Delhi.

Papageorgiou GC and Govindjee (eds, 2010) Chlorophyll Fluorescence: A Signature of Photosynthesis. In *Advances in Photosynthesis* (series editor Govindjee) vol. 19, pp 818 + xxxii, ISBN 1-4020-3217-X (HB), soft cover edition, Springer, The Netherlands.

Papageorgiou GC (2010) Fluorescence Emission from the Photosynthetic Apparatus. In: Eaton-Rye J and Tripathy B (eds) *Photosynthesis: Plastid Biology, Energy Conversion and Carbon Assimilation*. Springer, Netherlands (in press).

Editorial Board Member

Papageorgiou GC Associate Editor & Editorial Board Member of *Photosynthetica*

Scientific manuscript reviewer:

Papageorgiou GC: Biochim Biophys. Acta (Bioenergetics), Phtosynth. Research, Photosynthetica, J. of Fluorescence, Proceed. Natl. Acad. Sci. USA, etc.

Citations 2010 (without self-citations): 7

Total citations 2006-2010 (without self-citations): 48

h-factor: 6

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 2010: 0€

Note:

The following proposals have been submitted and are under evaluation:

- *Photosynthetic Hydrogen production in green algae and cyanobacteria* (Greek Ministry of Education, Lifelong Learning and Religious Affairs, Supporting Postdoctoral Researchers)
- *Primary productivity estimation of Greek natural ecosystems based on ecophysiological measurements, modeling and satellite images* (Greek Ministry of Education, Lifelong Learning and Religious Affairs)
- *Photosynthetic Hydrogen production* (Empeirikion Foundation)

PROGRAMME C:
STRUCTURAL AND
COMPUTATIONAL BIOLOGY

Research Group: Theoretical Biology and Computational Genomics

Research Staff

Yannis Almirantis, Research Director

Spyros Papageorgiou, Emeritus Scientist

Alexandros Klimopoulos, Collaborating Graduate Student (*MSc*) - *MSc obtained in 2010*

Labrini Athanassopoulou, Collaborating Graduate Student (*MSc*) - *MSc obtained in 2010*

Yannis Tsiagas, Collaborating Graduate Student (*MSc*)

Dimitris Polichronopoulos, Collaborating Graduate Student (*MSc*)

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.

2010 Findings

Statistical methods, including block entropy based approaches, have already been used in the study of long-range features of genomic sequences seen as symbol-series, either considering the full alphabet of the four nucleotides or the binary purine/pyrimidine character set. We explored the alternation of short protein-coding segments with long non-coding spacers in entire chromosomes, focussing on the scaling properties of block entropy.

In previous studies, it has been shown that the sizes of non-coding spacers follow power-law like distributions in most chromosomes of eukaryotic organisms from distant taxa. We have developed a simple evolutionary model based on well-known molecular events (segmental duplications followed by elimination of most of the duplicated genes) which reproduces the observed linearity in log - log plots.

The scaling properties of block entropy $H(n)$ have been studied in several works. Their findings suggest that linearity in semi-logarithmic scale characterises symbol sequences which exhibit fractal properties and long-range order, while this linearity has been shown in the case of the logistic map at the Feigenbaum accumulation point.

In our recent work, we started with the observation that the block entropy of Cantor-like binary symbol series scales in a similar way. Then, we perform the same analysis for the full set of human chromosomes and for several chromosomes of other eukaryotes. A similar but less extended linearity in semi-logarithmic scale, indicating fractality, is observed, while randomly formed surrogate sequences clearly lack this type of scaling. Genomic sequences always present entropy values much lower than their random

surrogates. Symbol sequences produced by the aforementioned evolutionary model follow the scaling found in genomic sequences, thus corroborating the conjecture that “segmental duplication – gene elimination” dynamics may have contributed to the observed long-rangeness in the coding / non-coding alternation in genomes.

Publications

L.Athanasopoulou, S.Athanasopoulos, K.Karamanos, & Y.Almirantis. Scaling properties and fractality in the distribution of coding segments in eukaryotic genomes revealed through a block entropy approach. *Physical Review E* (2010) 82, 051917.

Articles in Press

Papageorgiou S. Physical forces may cause Hox gene collinearity in the primary and secondary axes of the developing vertebrates. *Development, Growth & Differentiation*. (Impact Factor: 2,28)

Presentations at Scientific Conferences

D.Polychronopoulos, D.Sellis, Y.Almirantis (2010).A study of the genomic distribution of conserved noncoding elements in several organisms: global genomic organization expressed in the form of power laws. Hellenic Society for Computational Biology & Bioinformatics Conference, 2010.

Educational Activities

Masters’ theses that have finished in 2010 under the supervision of Y. Almirantis:

1. Labrini Athanasopoulou, Physicist.

Project title: “Study of the scale properties of the human genome using block entropy” in the framework of the "Post Graduate Specialisation Diploma" “Mathematical modeling in modern technologies”, N.T.U.A.

2. Alexandros Klimopoulos, Electronic Engineer.

Project title: “Study of the distribution of repeated elements in a variety of genomes and its relation with genomic function and evolution” in the framework of the Post Graduate Specialisation Diploma in Bioinformatics, Biology Department, U.O.A.

- 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 Biology

Vice director of the Institute of Biology, until July 2010 (Yannis Almirantis)

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

Other Distinctions and Awards

The “CONFERENCE POSTER” D.Polychronopoulos, D.Sellis, Y.Almirantis: “A study of the genomic distribution of conserved noncoding elements in several organisms: global genomic organization expressed in the form of power laws”. (Hellenic Society for Computational Biology & Bioinformatics Conference,2010) has been selected from the Faculty of 1000 (F1000) and ranked in the “top 2% of published articles in biology and medicine”: <http://f1000.com/8034967>.

The PRE article: L.Athanasopoulou, S.Athanasopoulos, K.Karamanos, & Y.Almirantis. “Scaling properties and fractality in the distribution of coding segments in eukaryotic genomes revealed through a block entropy approach”. *Physical Review E* (2010) **82**, 051917, has been selected to be included in the 15th November issue of the “Virtual Journal of Biological Physics Research”: <http://www.vjbio.org>

Impact Factors (for 1 publication): 2,40

Citations 2010 (without self- citations): 16

Total Citations 2006-2010 (without self- citations): 91

h-factor: 11

Research Group: NMR Studies of Biomolecules and Pharmaceuticals

Research Staff

Maria Pelekanou, Research Director

Marina Sagnou, Lecturer

Demetra Benaki, Postdoctoral Fellow

Angeliki Panagiotopoulou, Technical Specialist

Myrta Kostomoiri, Graduate Student (MSc)

Research Interests

Studies of the **structure, interactions** and **structure-function relationship** of bioactive compounds of pharmacological interest for the diagnosis and/or therapy of various diseases. We focus on two major types of compounds:

- Peptides and small proteins
- Bioactive organic compounds designed for targeted biological/pharmacological action either plain or after proper complexation/labelling

The areas of application of our work are mainly Alzheimer's disease and cancer, but also other diseases of the central nervous system, like epilepsy. Our main tools are NMR spectroscopy and circular spectropolarimetry (CD) for the structural studies in combination with methodologies of organic synthesis, radiolabelling, and in vitro and in vivo biological assessment.

2010 Findings

In 2010 our efforts in the development of suitable pharmacophores aiming at tissue-targeting for diagnostic/therapeutic applications, as well as for mechanistic studies, resulted in two publications. The first publication describes the synthesis and evaluation of a complex of the anticancer agent 2-(4'-aminophenyl)benzothiazole with technetium-99m (^{99m}Tc) as potential radiodiagnostic agent for selective tumor detection; indeed, biodistribution studies of the complex in SCID mice bearing MCF-7 xenografts showed appreciable tumor uptake that allowed delineation of the tumor by gamma-camera. In the second publication the synthesis and characterization of Re and ^{99m}Tc complexes of curcumin, a natural product with wide spectrum of pharmacological activity, is reported. The complexes, despite the direct attachment of curcumin to the metal core, retain the affinity of the mother pharmacophore for β -amyloid plaques, the pathological hallmark of Alzheimer's disease (AD). The biological evaluation of the curcumin complexes as diagnostic tools for AD is in progress with stability experiments in the presence of histidine and cysteine, stability in plasma, biodistribution experiments in healthy mice, etc. In the same area, our group actively participated in the characterization of Re and ^{99m}Tc complexes of the antibiotics ciprofloxacin and norfloxacin for the discrimination of active infection from aseptic inflammation, as well as of complexes of synthetic derivatives of dextrans for imaging of the sentinel lymph node, crucial in cancer diagnosis. Moreover, complexes of the anticancer agent 2-(4'-aminophenyl)benzothiazole with ruthenium, palladium, and platinum were designed and synthesized aiming at the development of potential anticancer agents with combined mode of action. CD and fluorescence studies in progress show that the complexes interact with DNA.

Within the framework of the FP7 program EURIPIDES in which our team is responsible for the design, synthesis and biological evaluation of novel I-125 or Tc-99m SPECT tracers for the radiodiagnostic imaging of drug resistant epilepsy, about twenty novel compounds were successfully synthesized. Eight of them were designed as derivatives of the clinically used P-glycoprotein inhibitor, tariquidar, two are derivatives of the natural product curcumin, and about ten compounds belong to the thiosemicarbazone category of isatin derivatives. *In vitro* results on the Pgp inhibitory activity revealed the potential of the iodinated urea analogue of tariquidar and the curcumin derivative as tracers for

Pgp function. Radioiodination reactions resulted in the formation of stable tracers *in vitro*, which in biodistribution studies in healthy mice were shown to be equally stable *in vivo* and were excreted mainly via the hepatobiliary route.

In the area of peptides, the interaction of the β -amyloid peptide (β -AP) of AD with curcumin was investigated in the search of potential inhibitors of the aggregation of β -AP and the formation of toxic amyloid plaques. Studies were performed with fluorescence spectroscopy, CD and NMR in aqueous solution and in the presence of methanol (50% and 100%) and the affinity of curcumin for β -AP was demonstrated. In the humanin family of neuroprotective peptides, three new derivatives were designed and synthesized to serve as molecular probes for mechanistic investigations. Suitable labeling moieties like the fluorescent moiety FITC, the streptavidin-counterpart biotin, and the ^{99m}Tc -radiometal chelating unit dimethylGly-Ser-Cys, were attached to colivelin the most active member of the family. These derivatives will be employed in *in vitro* cell survival assays and cellular component binding studies, as well as in *in vivo* biodistribution experiments in suitable mouse-models. The structural characterization of colivelin with NMR and CD is completed by our group in water and trifluoethanol solutions. Similar structural studies will be performed for the new colivelin derivatives in order to assure that derivatization of colivelin has not significantly affected its solution structure.

Publications

Tzanopoulou, S., Sagnou, M., Paravatou-Petsotas, M., Gourni, E., Loudos, G., Xanthopoulos, S., Lafkas, D., Kiaris, H., Varvarigou, A., Pirmettis, I.C., Papadopoulos, M., Pelecanou, M. (2010). Evaluation of Re and ^{99m}Tc complexes of 2-(4'-aminophenyl) benzothiazole as potential breast cancer radiopharmaceuticals. *J. Med. Chem.* 53 (12), 4633-4641.

Sagnou, M., Tsoukalas, C., Triantis, C., Raptopoulou, C.P., Terzis, A., Pirmettis, I., Pelecanou, M., Papadopoulos, M. (2010). A new tricarbonyl fac-[M(acac)(isc)(CO)₃] complex (M = Re, ^{99m}Tc) with acetylacetonate (acac) and isocyanide (isc) in a 2+1 combination *Inorg. Chim. Acta* 363, (8), 1649-1653.

Papagiannopoulou, D., Makris, G., Tsoukalas, C., Raptopoulou, C.P., Terzis, A., Pelecanou, M., Pirmettis, I., Papadopoulos, M.S. (2010) Rhenium(I) and technetium(I) fac-M(NSO)(CO)₃ (M = Re, ^{99m}Tc) tricarbonyl complexes, with a tridentate NSO bifunctional agent: Synthesis, structural characterization, and radiochemistry. *Polyhedron* 29 (2), 876-880.

Articles in Press

Fernández Núñez, E.G., Linkowski Faintuch, B., Teodoro, R., Pereira Wiecek, D., da Silva, N.G., Papadopoulos, M., Pelecanou, M., Pirmettis, I., de Oliveira Filho, R.S., Duatti, A., Pasqualini, R. (2011) Parameters optimization defined by statistical analysis for cysteine-dextran radiolabeling with technetium tricarbonyl core. *Applied Radiation and Isotopes* 69 (4), 663-669; IF: 1.101

Kyprianidou, P., Tsoukalas, C., Chiotellis, A., Papagiannopoulou, D., Raptopoulou, C.P., Terzis, A., Pelecanou, M., Papadopoulos, M., Pirmettis, I. (2011). First example of well-characterized Re and ^{99m}Tc tricarbonyl complexes of ciprofloxacin and norfloxacin in the development of infection-specific imaging agents. *Inorg. Chim. Acta*, in press DOI: 10.1016/j.ica.2011.01.060; IF: 2.233

Sagnou M., Benaki D., Triantis C., Tsotakos Th., Psycharis V., Raptopoulou C. P., Pirmettis I., Papadopoulos M., Pelecanou, M. (2011). Curcumin as the OO bidentate ligand in "2 + 1" complexes with the [M(CO)₃]⁺ (M = Re, ^{99m}Tc) tricarbonyl core for radiodiagnostic applications. [Inorg. Chem.](#) 50 (4), 1295-1303 IF 4.657

Chiotellis, A., Tsoukalas, C., Pelecanou, M., Pirmettis, I., Papadopoulos, M. (2011) New $^{99m}\text{Tc}(\text{CO})_3(\text{NNO})$ complexes in the development of 5HT_{1A} receptor imaging agents. *Radiochimica Acta* doi: 10.1524/ract.2011.1835; IF: 1.459

Articles in Books and Conference Proceedings

Triantis, C., Tsoukalas, C., Tsotakos, T., Raptopoulou, C., Terzis, A., Pelecanou, M., Pirmettis, I., Papadopoulos, M. New fac-M(acac)(P)(CO)₃ and M(acac)(P)₂(CO)₂ complexes

(M=Re, ^{99m}Tc). In "Technetium and other radiometals in Chemistry and Medicine" (U. Mazzi, W. Eckelman, W. Volkert eds.), pp. 41-46, SGE Editoriali Padova, 2010.

Tsotakos, T., Morais, M., Correia, J. D. G., Santos, I., Martins, M., Pereira, S., Pelecanou, M., Papadopoulos, M., Pirmettis, I. New $^{99m}\text{Tc}(\text{CO})_3$ mannosylated dextrans bearing s-derivatives cysteine chelator. In "Technetium and other radiometals in Chemistry and Medicine" (U. Mazzi, W. Eckelman, W. Volkert eds.), pp. 69-72, SGE Editoriali Padova, 2010.

Tsotakos, T., Tsoukalas, C., Papadopoulos, A., Pelecanou, M., Papadopoulos, M., Pirmettis, I. Synthesis and biodistribution in mice of new $^{99m}\text{Tc}(\text{CO})_3$ labeled fatty acids. In "Technetium and other radiometals in Chemistry and Medicine" (U. Mazzi, W. Eckelman, W. Volkert eds.), pp. 101-2, SGE Editoriali Padova, 2010.

Chotzagiannoglou, V., Kyprianidou, P., Tsotakos, T., Tsoukalas, C., Saso, L., Pelecanou, M., Papadopoulos, M., Pirmettis, I. $^{99m}\text{Tc}(\text{CO})_3$ ciprofloxacin dithiocarbamate complexes as infection imaging agents. In "Technetium and other radiometals in Chemistry and Medicine" (U. Mazzi, W. Eckelman, W. Volkert eds.), pp. 103-4, SGE Editoriali Padova, 2010.

Panagiotopoulou, A., Tsoukalas, C., Raptopoulou, C., Terzis, A., Pirmettis, I., Papadopoulos, M., Pelecanou, M. New "2+1" mixed ligand *fac*-tricarbonyl complexes of the general formula $\text{Re}(\text{OO})(\text{L})(\text{CO})_3$. In "Technetium and other radiometals in Chemistry and Medicine" (U. Mazzi, W. Eckelman, W. Volkert eds.), pp. 105-6, SGE Editoriali Padova, 2010.

Lazopoulos, A., Traintis, C., Drakopoulos, A., Tsoukalas, C., Tsotakos, T., Raptopoulou, C., Terzis, A., Pelecanou, M., Pirmettis, I., Papadopoulos, M. Synthesis and characterization of the new *fac*- $\text{Re}(\text{NO})(\text{P})(\text{CO})_3$ complexes. In "Technetium and other radiometals in Chemistry and Medicine" (U. Mazzi, W. Eckelman, W. Volkert eds.), pp. 107-8, SGE Editoriali Padova, 2010.

Sagnou, M., Benaki, D., Paravatou-Petsotas, M., Pirmettis, I., Papadopoulos, M., Pelecanou, M. "2+1" curcumin complexes with *fac*- $[\text{Re}(\text{CO})_3]^+$ core. In "Technetium and other radiometals in Chemistry

Presentations at Scientific Conferences

C. Triantis, C. Tsoukalas, T. Tsotakos, C. Raptopoulou, A. Terzis, M. Pelecanou, I. Pirmettis, M. Papadopoulos (2010). New *fac*- $\text{M}(\text{acac})(\text{P})(\text{CO})_3$ and $\text{M}(\text{acac})(\text{P})_2(\text{CO})_2$ complexes (M=Re, ^{99m}Tc). International Symposium on Technetium and other Radiometals in Chemistry and Medicine, September 8-11, 2010, Bressanone, Italy

T. Tsotakos, M. Morais, J. D. G. Correia, I. Santos, M. Martins, S. Pereira, M. Pelecanou, M. Papadopoulos, I. Pirmettis (2010). New $^{99m}\text{Tc}(\text{CO})_3$ mannosylated dextrans bearing S-derivatives cysteine chelator. International Symposium on Technetium and other Radiometals in Chemistry and Medicine, September 8-11, 2010, Bressanone, Italy

T. Tsotakos, C. Tsoukalas, A. Papadopoulos, M. Pelecanou, M. Papadopoulos, I. Pirmettis (2010). Synthesis and biodistribution in mice of new $^{99m}\text{Tc}(\text{CO})_3$ labeled fatty acids. International Symposium on Technetium and other Radiometals in Chemistry and Medicine, September 8-11, 2010, Bressanone, Italy

V. Chotzagiannoglou, P. Kyprianidou, T. Tsotakos, C. Tsoukalas, L. Saso, M. Pelecanou, M. Papadopoulos, I. Pirmettis (2010). $^{99m}\text{Tc}(\text{CO})_3$ ciprofloxacin dithiocarbamate complexes as infection imaging agents. International Symposium on Technetium and other Radiometals in Chemistry and Medicine, September 8-11, 2010, Bressanone, Italy

A. Panagiotopoulou, C. Tsoukalas, C. Raptopoulou, A. Terzis, I. Pirmettis, M. Papadopoulos, M. Pelecanou (2010). New "2+1" mixed ligand *fac*-tricarbonyl complexes of the general formula $\text{Re}(\text{OO})(\text{L})(\text{CO})_3$. International Symposium on Technetium and other Radiometals in Chemistry and Medicine, September 8-11, 2010, Bressanone, Italy

A. Lazopoulos, C. Traintis, A. Drakopoulos, C. Tsoukalas, T. Tsotakos, C. Raptopoulou, A. Terzis, M. Pelecanou, I. Pirmettis, M. Papadopoulos (2010). Synthesis and characterization

of the new *fac*-Re(NO)(P)(CO)₃ complexes. International Symposium on Technetium and other Radiometals in Chemistry and Medicine, September 8-11, 2010, Bressanone, Italy

M. Sagnou, D. Benaki, M. Paravatou-Petsotas, I. Pirmettis, M. Papadopoulos, M. Pelecanou, "2+1" curcumin complexes with *fac*-[Re(CO)₃]+core (2010). International Symposium on Technetium and other Radiometals in Chemistry and Medicine, September 8-11, 2010, Bressanone, Italy

D. Benaki, K. Stamatakis, E. Mikros, and M. Pelecanou (2010). Interaction of β -amyloid peptide with potential inhibitors. 18th EuroQSAR EAST -NMR Satellite Meeting, September 24-25, 2010, Rhodes, Greece (Oral, D. Benaki).

M. Sagnou, S. Tzanopoulou, M. Paravatou-Petsotas, E. Gourni, G. Loudos, S. Xanthopoulos, D. Lafkas, H. Kiaris, A. Varvarigou, I. C. Pirmettis, M. Papadopoulos, M. Pelecanou. (2010) Biological evaluation of Re and ^{99m}Tc complexes of 2-(4'-aminophenyl) benzothiazole as breast cancer radiopharmaceuticals. 14th Hellenic Symposium of Medicinal Chemistry, April 23-25, Thessaloniki. (Oral, M. Sagnou)

M. Sagnou, S. Tzanopoulou, R. Alberto, C. Raptopoulou, A. Terzis, I. Pirmettis, M. Papadopoulos, M. Pelecanou (2010) Potential diagnostic imaging agents for Alzheimer's disease. 14th Hellenic Symposium of Medicinal Chemistry, April 23-25, Thessaloniki (Oral, M. Sagnou).

M. Sagnou, D. Benaki, E. Gourni, A. Kostopoulos, M. Pelecanou, D. Dickens, A. Owen, A. D. Varvarigou (2010) The development of P-glycoprotein imaging probes as epilepsy diagnostic tools. 14th Hellenic Symposium of Medicinal Chemistry, April 23-25, Thessaloniki.

D. Benaki, K. Stamatakis, E. Mikros, M. Pelecanou (2010) Spectroscopic investigation of the interaction of curcumin with β -amyloid peptide (2010) Neuroscience Days of the Hellenic Society for Neuroscience, October 1-2, NCSR "D", Athens.

D. Benaki, C. Zikos Christos, A. Evangelou Alexandra, T. Elbert, J. Slaninova, P. Bouziotis, M. Paravatou-Petsotas, M. Papadopoulos, I. Pirmettis, M. Pelecanou Maria, E. Livianiou (2010) *In vitro*, *in vivo*, and structural studies of colivelin, the most active derivative of the humanin family of neuroprotective peptides (2010) Neuroscience Days of the Hellenic Society for Neuroscience, October 1-2, NCSR "D", Athens.

Educational Activities

"Introduction to NMR spectroscopy" teaching session within the framework of the graduate course of "Protein Biotechnology", Department of Biology, University of Crete (5 hours, 6 students), M. Pelecanou

"Applications of NMR in Medicine" in the framework of the graduate course "Introduction to Research Methodology", School of Medicine, Univ. of Athens (3 hours, 20 students), M. Pelecanou

Other Scientific Activities

Member (M. Pelecanou) of the NCSR "D" team with scientific responsible M. Vlassi, that participates in the program "Network to optimize use of European Scientific Infrastructure X-FEL by the Greek Research Community" financed by GSRT. Within that framework "Workshop on X-FEL Radiation & its Applications" was organized and held in NCSR "D", June 18-19 2010

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

Member (M. Pelecanou) of the Greek network of participation in the program INSTRUCT: An Integrated Structural Biology Infrastructure for Europe financed by the EU/ ESFRI/ FP7 with coordinator Dr. D. Stuart, University of Oxford.

Other Activities for the Institute of Biology

M. Pelecanou:

- Member of the Scientific Advisory Board of the Institute of Biology

- Responsible 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 the Institute of Biology

M. Sagnou:

- Responsible for the operation of the Confocal microscope
- Deputy for educational affairs
- Responsible for graduate student seminars
- Member of the examination committee for the award of graduate scholarships

D. Benaki:

- Responsible for the NCSR “D” Blood Bank

Impact Factors (for 3 publications): 9,021

Citations 2010 (without self- citations):

M. Pelecanou: 37

M. Sagnou: 10

Total Citations 2006-2010(without self- citations):

M. Pelecanou: 197

M. Sagnou: 56

h-factor:

M. Pelecanou: 14

M. Sagnou: 4

Current External Funding

Program entitled *Diagnostic imaging of amyloid plaques of Alzheimer’s disease*, funded by the Public Benefit Foundation John S. Latsis. Coordinator Dr. M. Pelecanou

Duration: 1/4/2010-31/12/2010

Total funding (lab): 12.000 €

Funding of the lab for 2010: 12.000 €

Program entitled *Development of ^{99m}Tc radiopharmaceuticals for sentinel node detection and cancer diagnosis*, funded by the International Atomic Energy Agency (IAEA). Coordinator Dr. I. Pirmettis (IRRP, NCSR “D”)

Duration: 2008-2010

Total funding (lab): 3.000 €

Funding of the lab for 2010: 1.500 €

Program entitled European Research initiative to develop Imaging Probes for early In-vivo Diagnosis and Evaluation of response to therapeutic Substances, funded by EU. Coordinator Dr. A. Varvarigou (IRRP, NCSR “D”)

Duration: 2008-12

Total funding for Greece: 318.000 €

Total funding (lab): 16.000 €

Funding of the lab for 2010: 13.000 €

Note:

They have been submitted and are under evaluation 4 projects within “THALIS” framework:

1. *Biosynthesis and genetic identification of cyclic peptide with potential therapeutic properties against Alzheimer’s disease: inhibitors of protein aggregation* (Coordinator: Dr. E. Gonos, Laboratory of Molecular and Cellular Aging, Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens)

2. *Synthesis of pyrrolo-quinoline and pyrrolo-isoquinoline nucleosidic derivatives with potential antiviral and antitumour properties* (Coordinator: D. Komiotis, Department of Biochemistry & Biotechnology, University of Thessaly, Greece)
3. *Innovative imaging techniques using THz frequencies for biomedical applications* (Coordinator: H. Avramopoulos, School of Electrical and Computer Engineering, National Technical University of Athens, Greece)
4. *Novel osteophilic ^{99m}Tc and $^{186/188}\text{Re}$ based radiopharmaceuticals for imaging/therapy of bone metastatic tumours and inflammation* (Coordinator: N. Efsthopoulos, 2nd Department of Orthopaedics, Faculty of Medicine, University of Athens, Greece)

Additional grant submissions under evaluation:

1. *Tumor selective rhenium and technetium probes with dual imaging potential*
Greek-Romanian Joint Research and Technology Programmes 2011-2012
 October 2010
 Scientific coordinator for the Greek team: M. Pelecanou
2. *Development of novel targeted diagnostic rhenium and technetium tools with dual tumor imaging potential.*
 Empirikion Foundation
 November 2010
 Scientific coordinator: M. Pelecanou

Research Group: Protein Structure and Molecular Modeling

Research Staff

Metaxia Vlassi, Research Director

Dimitris Vlachakis, Postdoctoral Fellow (until 10/2010)

Diamadis Sellis, Graduate Associate

Our current research activities focus on

- 1) Structural studies of proteins with emphasis on sequence repeat-containing proteins aiming to elucidate sequence/structure relationships and the structural determinants of sequence repeat mediated protein interactions. The approach followed mainly includes structural bioinformatics techniques such as *in silico* 3D-modelling (homology/comparative modelling & threading), docking, molecular dynamics simulations etc.
- 2) Studying the dynamics of protein structure by means of molecular dynamics (MD) simulations and development of related bioinformatics tools.
- 3) 3D-modelling of enzymes of mainly medical interest and of potential inhibitors towards a structure-based drug design.

2010 Findings

1) Molecular Dynamics Simulations on Arginine-Serine (RS) repeat-containing peptides

Arginine-Serine (RS) repeats are found in a large number of proteins with various functions and have been implicated in protein interactions. In a previous work (see annual report 2009), and with the aim to elucidate the role of serine phosphorylation in the conformation of RS repeats, we have performed Molecular Dynamics (MD) simulations on the unphosphorylated and phosphorylated RS domain of lamin b receptor (LBR) consisting of four (human orthologue) and five RS consecutive repeats (chicken orthologue).

In 2010, we repeated the MD simulations (in both implicit and explicit solvation) by using various, improved force-fields in order to produce reliable MD data. These MD simulations were performed using the recently developed version (4.5.x.) of the program Gromacs-4, through a new version of our GUI, Gromita (see Sellis et. al, *Bioinformatics & Biology Insights*, 2009), which we developed in our lab in 2010 (see below).

A grant application for similar studies on other aminoacid repeats and their modifications has been resubmitted to the GSRT in the framework of the "THALIS" program (see the *Grant Applications* section).

2) Development of a bioinformatics tool for Molecular Dynamics (MD) simulations of proteins in solution.

In a previous work, and in the framework of the DEMOEREVNA program (financed by NCSR "Demokritos", coordinator: M. Vlassi) we have developed the computer program, Gromita, which is a Graphical User Interface (GUI) to version 4 of the MD program, Gromacs. The related article (Sellis et al. 2009) in *Bioinformatics and Biology Insights* is highly visible (3059 views within 58 weeks after publication) as indicated by the views statistics of the journal (see <http://la-press.com/t.php?i=stats>). In addition, in 2010, more than 30 potential Gromita users have applied for a license to use the GUI.

In 2010, we developed a new Gromita version (v. 109), which is compatible with the recently developed Gromacs versions 4.5.x. These improved versions of Gromacs allow, among others, usage of almost all known force fields, including newly developed and more accurate force fields for MD simulations of proteins in aqueous solutions. The development of a manual as well as updating of the related website (<http://bio.demokritos.gr/gromita>), are under development.

3) Molecular Modelling of inhibitors of enzymes of medical importance

3a) In a previous work (see annual report 2009), by means of molecular docking techniques, in combination with MD simulations, we have shown that a series of synthetic fluoro-pyranosyl nucleosides can efficiently dock into the active site of the human poly(A)-specific ribonuclease (PARN). Our *in silico* data implied that such compounds can be the basis for the development of new PARN inhibitors.

In 2010, we performed *in silico* docking experiments on a new series of synthetic nucleosides into the active site of PARN. The specialized program Glide (Schrödinger suite) was used for this purpose. The *in silico* data predicted the inhibitory activity of some of the compounds tested and indicated a potential, slow, but very potent PARN inhibitor. The predictions were verified experimentally by our collaborators, using *in vitro* enzymatic activity tests. This work is submitted for publication.

3b) Aiming at a structure-based design of inhibitors of the RNA-helicase of the Dengue virus (type II), in 2010, we performed *in silico* docking experiments of 60000 compounds (from the Asinex data base) into the active site of the enzyme. High throughput docking techniques, through the program Glide and the known crystal structure of the enzyme, were used for this purpose.

Presentations at Scientific Conferences

Sellis, D., Vlachakis, D., Tartas, A. and Vlassi, M. (2010) Molecular dynamics simulations of proteins in aqueous solutions: I. Development of the Gromita software. II. The example of a repeat-containing protein of known structural dynamics. 61st Conference of the Hellenic Society for Biochemistry & Molecular Biology, 15-17 October, 2010, Alexandroupolis, Greece.

Chatzigeorgiou, V., Vlachakis, D., Zervakaki, V., Manta, S., Komiotis, D, Vlassi, M., Stathopoulos, K. and Balatsos, N. (2020) Inhibition mechanism of poly(A)-specific ribonuclease (PARN) by nucleoside analogues. 61st Conference of the Hellenic Society for Biochemistry & Molecular Biology, 15-17 October, 2010, Alexandroupolis, Greece.

Other Scientific Activities

- Member of the national network BE/OPT-XFEL (Network to Optimize use of the European X-FEL by the Greek Research Community)
- Organizer of the workshop entitled: “Workshop on XFEL Radiation & its Applications”, 18-19 June, NCSR “Demokritos”.

The workshop was organized in the framework of the BE/OPT-XFEL Network, (financed by the Greek General Secretariat for Research & Technology: see Current Grants section), a Greek initiative aiming at adequately preparing the Greek scientific community for the research opportunities created by the European X-FEL facility. The speakers (13) were invited scientists (from Europe, USA, Japan), experts in X-Ray Free Electron Lasers (XFELs), including members of the European X-FEL scientific advisory board and users of similar infrastructures (CFEL/Germany, SLAC/Stanford/USA, Spring-8 Center/Japan). The presentations covered basic principles of XFEL operations and scientific applications in a wide range of fields that were inaccessible prior to XFEL. 80 students as well as senior scientists from Universities and other Research Center of Greece, as well as from the private sector, attended the workshop. More information can be found at www.xfel.gr

- Member of the national network “INSTRUCT” (“INSTRUCT: An Integrated Structural Biology Infrastructure for Europe).
- Member of the network “Center for Crystallographic Studies of Macromolecules”

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)

Other Activities for the Institute of Biology

Member of the Scientific Advisory Board of IB (since Oct. 2010)

Vice Director of IB (since Nov. 2010)

Surrogate Member of the Scientific Advisory Committee of NCSR “D”

President of the Committee for computing and network support of IB

Member of the committee for consumables delivering at IB

Member of the committee for instrumentation delivering at IB in the framework of EPAN

Responsible scientist for the use of common instrumentation (i.e. shaking incubator etc)

Member of the Committee for examining the level of knowledge of the Greek language of EU citizens

Citations 2010 (without self- citations): 22

Total Citations 2006-2010 (without self- citations): 133

h-factor: 11

Current External Funding

Project entitled *BE/OPT-XFEL Network to Optimize use of the European X-FEL by the Greek Research Community*, funded by GSRT” (approved: 5-1-2010).

Duration: 20/1/2010-31/3/2011

Total budget: 125.000 €

Total funding (lab): 13388 € (income of 13220 €, 17/1/2011)

Note: Part of the budget corresponds to the organization cost of the BE/OPT XFEL workshop (Main Organizer: M. Vlassi see above).

Note: Grant applications submitted

- (i) A grant application entitled “*Intrinsically disordered proteins: A complete in vitro, in vivo and in silico analysis by a combination of state-of-the art technique*”s has been re-submitted (in English) to the GSRT in the framework of the THALIS program (Oct 2010)
- (ii) A grant application entitled “*Signaling in bacteria: from understanding the biochemical and molecular pathways of the AtoS/AtoC system to developing specific antimicrobials*” has been re-submitted (in English) to the GSRT in the framework of the THALIS program (Oct. 2010)
- (iii) A grant application entitled “*Dysfunction of mitochondria in neurodegenerative diseases*” has been re-submitted to the GSRT in the framework of the THALIS program (Oct. 2010).

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

- *HUMAN TISSUE BANK*
- *EXPERIMENTAL ANIMAL COLONY*
- *LASER CONFOCAL MICROSCOPY*
- *CHARACTERIZATION OF
PROTEINS AND BIOACTIVE
MOLECULES*

HUMAN TISSUE BANK

Research Staff

Helen Vavouraki, Researcher

Anastasios Mamalis, Collaborating Scientist

Minas Leventis, Collaborating Graduate Student

Stilianos Kakkos, Research Technician

Vassilki Stefou, Assistant Technician (until 9/2010)

Lab Description - Research Interests

Human Tissue Bank, one of the first legislated laboratories of NCSR "DEMOKRITOS", collects tissues of human origin, it process them and produces grafts to be used in reconstructive surgery. Its operation follows the European Directives 23/2004, 17/2006 and 86/2006 as well as the recommendations of IAEA.

Member of the European Association of Tissue Banks, it is the unique in Greece applying its "knowhow" in the processing of a great variety of human tissues. During the almost 40 years of continuous functioning it has delivered more than 42000 tissue preparations without any problem relevant to their quality. All procedures taking place in the Bank are fully computerized and accredited according to ISO 9001/2008. We are constantly care for the quality control issues, and our compliance with the Greek and European stds

The grafts preparations are delivered to hospitals, health clinics and laboratories, all over the country.

The scientific research interests of the Bank 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. There are therefore, collaborations with university medical and other departments in order to promote Public Health.

2010 Graft production - Findings

For our collaboration with the private sector which promotes and delivers our grafts to the hospitals, we processed tissues from **127 donors**, producing **330 tissue preparations**. **285 grafts** of them have been delivered and used in the Orthopedic Surgery. We have delivered also various other preparations for scientific collaboration purposes.

Publications

'Platelet- Rich plasma combined with Demineralized Freeze-dried bone allograft in the therapy of periodontal endosseous defects: report of two clinical cases.' Markou, N., Pepelassi, E., Kotsovilis, S., Vavouraki, H., et al. Journal of the American Dental Association 2010 ,141 (8), pp. 967-978.

The effect of autologous, platelet-rich plasma on the osteoinductive potential of allogenic demineralized bone matrix. An experimental pilot study in rabbits. Minas LEVENTIS, Efsthios ELEFTHERIADIS, Stavros PAPANIKOLAOU, Panagiota OIKONOMOPOULOU, Helen VAVOURAKI, Lubna KHALDI, Konstantinos TOSIOS, Constantinos ALEXANDRIDIS, Ismene DONTAS- Hellenic Archives of Oral & Maxillofacial Surgery (2010) 3, 141-156 .

Articles in Press

'Attachment and Proliferation of Human Osteoblast-Like Cells on Guided Bone Regeneration (GBR) Membranes in the Absence or Presence of Nicotine: An In Vitro Study' Papaioannou, K., Markopoulou, C., Gioni V., Mamalis A. Vavouraki H., et al. International Journal of Oral and Maxillofacial Implants (IntJOMI), (in press). (I.F 1,972)

'The effect of Platelet-Rich Plasma (PRP) combined with a bone allograft on human Periodontal Ligament (PDL) cells' Pantou A.L, Markopoulou C.E., Dereka X.E., Vavouraki H., Mamalis A., Vrotsos I.A. Cell and Tissue Banking, (in press). (I.F 2,204)

Presentations at Scientific Conferences

“The effect of autologous Platelet Rich Plasma on the osteoconductive potential of allogenic demineralised bone matrix. An experimental study in rabbits. M. Leventis, E. Eleftheriadis, P. Oikonomopoulou, E. Vardas, H. Vavouraki, I. Dontas, L. Khaldi, I. Eleftheriadi, C. Alexandridis. XXth Congress of European Association of Cranio-Maxillo-Facial Surgery, Bruges, September 2010, p 1301-1302.

Other Activities for the Institute of Biology

Responsible of Quality Process according ISO 9001/2008.

Students training in the Bank activities

Other Scientific Activities

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

Impact Factors (for 1 publication): 1,961

Citations 2010 (without self- citations): 4

Total Citations 2006-2010 (without self- citations): 40

h-factor: 5

Note:

Participation in 2 project-propositions in **Thales Project** as member of the main research group

EXPERIMENTAL ANIMAL COLONY

Research Staff

Dimitris Kletsas, Research Director

Ioannis Zafiroopoulos, 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 and ETB
- Rabbits, strain NZW ALBINO
- Mice, strain SCID

During 2010 the Animal Facility provided the following animals:

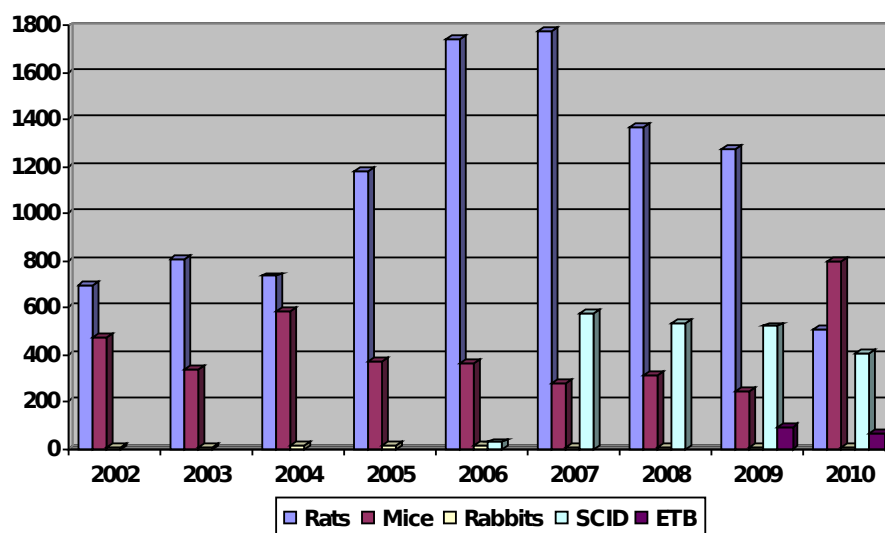
| <i>Users</i> | <i>Rats WISTAR</i> | <i>Rats ETB</i> | <i>Mice</i> | <i>Rabbits NZW</i> | <i>Mice SCID</i> |
|-----------------------------------------------|------------------------|---------------------|-------------|------------------------|----------------------|
| Institute of Biology | 0 | 0 | 111 | 0 | 17 |
| Institute of Radioisotopes & Radiodiagnostics | 10 | 0 | 671 | 5 | 392 |
| External Users | 308 | 65 | 0 | 0 | 0 |
| External Users (maintenance) | 193 | 0 | 19 | 0 | 0 |
| Total | 511 | 65 | 801 | 5 | 409 |

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. Two new colonies of transgenic animals, one expressing MMP9 in neurons (TgMMP9) and one being a model for Alzheimer disease (5xFADD) 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. In addition, a new office building has been created.

DISPOSAL OF LABORATORY ANIMALS 2002-2010



LASER CONFOCAL MICROSCOPY

Research Staff

Marina Sagnou, Lecturer

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.

2010 Findings

During the year 2010, there seemed to be a rather increased demand for the the study of cellular, molecular and biochemical phenomena using confocal microscopy imaging techniques by both the Local Institute of Biology researchers, and those from the University of Athens, The Agricultural University, the Technical University as well as some Hospital Units.

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

CHARACTERIZATION OF PROTEINS & BIOACTIVE MOLECULES

Research Staff

Metaxia Vlassi, Research Director

Maria Pelecanou, Research Director

Aggeliki Panagiotopoulou, Technical Specialist

Description

The service unit for Characterization of Molecules and Biomolecules (CMB) has been established in 2003 and comprises two pre-existing laboratories: 1) the Centre for Crystallographic Studies of Macromolecules (CCM) and 2) the Nuclear Magnetic Resonance laboratory (NMR).

- CCM consists of a) a state-of-the-art X-ray system for diffraction experiments on macromolecules and b) a Circular Dichroism (CD) spectropolarimeter. CCM has been financed by the General Secretariat for Research and Technology (GSRT, EPET II program) as a network of related to molecular structure research groups from "Demokritos" and from other Research/Academic Institutions of Greece. CCM operates at NCSR "Demokritos" since fall 1998.
- The NMR laboratory consists of a) a 250 MHz NMR spectrometer and b) a 500 MHz ADVANCE DRX NMR spectrometer. The latter has been funded in the framework of a GSRT program entitled: 'Up-grading the infrastructure of NCSR "Demokritos" and is shared by the Institutes of Physical Chemistry, Biology and Radioisotopes & Radiodiagnostic Products.

2010 Findings

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

EDUCATIONAL ACTIVITIES

EDUCATION

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

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

During the year 2010, 10 scientists were trained at the postdoctoral level at our Institute. Furthermore, 27 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 2010, 2 of our graduate students finished their thesis work and became PhDs.

Moreover, 7 students from Greek Universities are carrying out their pre-graduate project thesis work at the Institute. Additionally, during 2010, 2 graduate students from abroad visited IB and 2 students (one from abroad and one greek) did practical job training in laboratories at the Biology Institute.

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

- *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)*
- *Lecture 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)*
- *Lecture 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 "7TM Receptors and G proteins 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 "Cellular senescence and tissue homeostasis" in the framework of the postgraduate course "Biochemistry" (Dr. D. Kletsas, Department of Chemistry, University of Athens)*
- *Lecture entitled "Cellular senescence and tissue homeostasis" in the framework of the postgraduate course "Physiology" (Dr. D. Kletsas, Medical School, University of Athens)*
- *Teaching in the framework of the postgraduate programme "Application of Biology in Medicin", 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 and Medical School, University of Athens).

- Lecture entitled "*Pathigenetic mechanisms and therapeutic implications of diabetes mellitus*" in the framework of the course "*Pathobiochemistry*" (**Dr. E. Tsilibary**, Department of Biology, University of Athens)
- Lecture entitled "*The process of cell apoptosis in pathological conditions: desired or non-desired process?*" 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 "*Model Systems of Molecular Microbiology, Microbial Biotechnology*" (**Dr. V. Sophianopoulou**, Department of Biology, 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. Vlasi**, Dept. of Biology, National & Kapodistrian 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)
- Lecture entitled "*Introduction to NMR spectroscopy*" in the framework of the graduate course "*Protein Biotechnology*" (**Dr. M. Pelecanou**, Department of Biology, University of Crete)

During July 2010, the Summer School of NCSR "Demokritos" was held and had included talks from the researchers of the Institute of Biology and of invited speakers coming from other Greek Institutions and abroad. The seminars of Biology 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 (2010) are presented analytically in the following pages.

Finally, the educational endeavours of the Biology Institute also include those accomplished by **Dr. K. Stamatakis** and **Dr. V. Labropoulou**, who give informative seminars to High School, University and Military School students.

**COMPLETION/AWARD
OF DOCTORAL THESES IN 2010**

| GRADUATE STUDENT | TITLE OF DOCTORAL THESIS | ADVISOR (in Institute of Biology) | UNIVERSITY |
|-------------------------------|---------------------------------------------------------------------------------------|------------------------------------------------|-----------------------------------------------------|
| Dimitra Bouzarelou | Characterisation of plant expansin homolog genes in <i>Aspergillus nidulans</i> | V. Sophianopoulou | Department of Biology University of Athens |
| Panagiotis Handris | Alterations in the structure and function of cell nucleus during ageing | D. Kletsas | Medical School University of Athens |

**LECTURE CONTRIBUTIONS TO
THE 2010 SUMMER SCHOOL
OF THE NCSR "DEMOKRITOS"**

(July 2010)

| DATE | SPEAKER | TITLE |
|-------------|----------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|
| 9/7/10 | Dr. K. Iatrou Institute of Biology, NCSR "Demokritos" | Malaria in the third world: targeting the mosquito vector of the parasite to limit the spread of the disease |
| 9/7/10 | Dr. I. Georgoussi Institute of Biology, NCSR "Demokritos" | Seven Transmembrane Receptors and G proteins: Health, pathogenesis and development of new drugs |
| 12/7/10 | Dr. L. Swevers Institute of Biology, NCSR "Demokritos" | Alternative methods of insect pest control: growth regulators and environmental RNAi |
| 12/7/10 | Dr. D. Kletsas Institute of Biology, NCSR "Demokritos" | Cell senescence and tissue homeostasis |
| 13/7/10 | Dr. Th. Sourlingas Institute of Biology, NCSR "Demokritos" | Histone Variants and Post Translational Modifications: Fundamental Factors in Chromatin Remodeling Events during Aging and Apoptosis |
| 14/7/10 | Dr. E. Tsilibary Institute of Biology, NCSR "Demokritos" | Neurodegenerative diseases (Alzheimer's disease) and new research developments |
| 14/7/11 | Dr. V. Sophianopoulou Institute of Biology, NCSR "Demokritos" | Eisosomes: gates or blocks of endocytosis? |
| 14/7/10 | Dr. A. Chroni Institute of Biology, NCSR "Demokritos" | Lipids and apolipoproteins: From cardiovascular disease to Alzheimer's disease |

**SEMINAR PROGRAMME 2010
INSTITUTE OF BIOLOGY**

| DATE | SPEAKER | TITLE |
|-------------|---------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 8/1/10 | Prof. S. Georgatos Medical School, University of Ioannina | Trailing along the nuclear envelope |
| 13/1/10 | P. Karkoulis Institute of Biology, NCSR "Demokritos" | Inhibition of multiple signal transduction pathways by 17-allylamino-17-demethoxygeldanamycin in human urinary bladder cancer cells |
| 27/1/10 | N. Tsoதாகos Institute of Biology, NCSR "Demokritos" | Gene regulation of components that regulate the podocytic phenotype |
| 29/1/10 | Dr. G. Simos Medical School, Univ. of Thessaly | Hypoxia-Inducible Factor 1 α (HIF-1 α): Cellular Function and Regulation by Phosphorylation and Nuclear Transport |
| 3/2/10 | E. Georganta Institute of Biology, NCSR "Demokritos" | STAT5B transcription factor forms a multi-component functional complex with the δ -opioid receptor and G proteins |
| 10/2/10 | A. Armatas Institute of Biology, NCSR "Demokritos" | The study of the proliferative effect of the transforming growth factor (TGF- β 1) on fetal and adult lung fibroblasts |
| 24/2/10 | I. Dafnis Institute of Biology, NCSR "Demokritos" | A carboxy-terminal truncated form of apolipoprotein E4 promotes the intracellular accumulation of Abeta42 |
| 3/3/10 | Ap. A. Dityatev Istituto Italiano di Tecnologia | Synaptic functions of the neural cell adhesion molecule NCAM |
| 3/3/10 | Ch. Magrioti Institute of Biology, NCSR "Demokritos" | Interactions between proteins of the endosymbiotic virus of the Hymenopteran endoparasitoid <i>Cotesia congregata</i> and proteins from the hemocytes of the Lepidopteran host, <i>Manduca sexta</i> |
| 10/3/10 | Th. Georgomanolis Institute of Biology, NCSR "Demokritos" | Characterization of protein interactions and functional role of the SH3 protein of the silkworm <i>Bombyx mori</i> |
| 11/3/10 | Dr. V. Sophianopoulou Institute of Biology, NCSR "Demokritos" (Lecture Series of the IRRP) | Regulation of topogenesis/function of neurotransmission transporters: CKI kinases and eisosomal proteins. |
| 17/3/10 | M. Papakonstantinou Institute of Biology, NCSR "Demokritos" | Interactions of opioid receptors with cytoplasmic proteins. Identification of new signaling pathways |
| 19/3/10 | Dr. D. Vassilakopoulou Dpt. of Biology, Univ. of Athens | Human L- Dopa decarboxylase cellular topology and expression patterns |
| 28/4/10 | A. Repouskou Institute of Biology, NCSR "Demokritos" | The expression and the response of c-MYC to trichostatin A are regulated by the circadian clock. |
| 5/5/10 | Th. Koutmos Institute of Biology, NCSR "Demokritos" | Factors that regulate the expression of genes responsible for the differentiation and function of renal podocytes |
| 5/5/10 | K. Kapodistria Institute of Biology, NCSR "Demokritos" | The role of nephrin signalling in PI3K-Akt survival signalling pathway in mouse pancreatic beta-cells. |
| 12/5/10 | A. Papadopoulou Institute of Biology, NCSR "Demokritos" | Cancer treatments and cellular senescence |
| 12/5/10 | A. Dimozi Institute of Biology, NCSR "Demokritos" | Role of oxidative stress on stress-induced senescence, proliferation and apoptosis of intervertebral disc cells |
| 25/5/10 | Dr. G. Panagiotou | Proteomic analysis of signal transduction in |

| | BSRC "Al. Fleming | cancer |
|----------|-------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|
| 9/6/10 | A. Galeou Institute of Biology, NCSR "Demokritos" | Study of the putative circadian clock element TOC1 in <i>Phaseolus vulgaris</i> |
| 10/9/10 | Prof. K. Cunningham Dept. of Pharmacology and Toxicology Univ. of Texas Medical Branch | Selective disruption of the serotonin 5-HT _{2C} receptor protein complex: Implications in addiction pharmacotherapy |
| 7/10/10 | Prof. C. Chatziantoniou Tenon Hospital, Paris, France | Novel Insights in the mechanisms controlling progression and reversal of Chronic Kidney Disease |
| 25/11/10 | Dr. Z. Kournia National Foundation of Biomedical Research, Academy of Athens | Computer-aided drug design for the discovery of novel anti-cancer agents |
| 2/12/10 | Dr. K. Iatrou Institute of Biology, NCSR "Demokritos" ("The Road of Ideas" Lecture Series of the IB) | Targeting the olfactory functions of Anopheline mosquitoes for malaria control in the third world: achievements and challenges |
| 16/12/10 | Dr. Th. Agalioti BSRC "Al. Fleming" | Targeting the DNA methylation apparatus to the Mouse |

COLLECTIVE DATA

FINANCIAL REPORT 2010

1. INTERNAL FUNDING FROM THE SPECIAL ACCOUNT DEPARTMENT AND FUNDING FROM GSRT (COORDINATOR: E. TSILIBARY, HEAD OF IB)

| INCOME | PROGRAMMES | | | | | PROGRAMMES FROM SERVICES |
|------------------------------------------|-------------------|------------------------------------|---------------|-----------------|--------------|----------------------------------------|
| | 464 | 1240 (IB, IPC, IMS) | 1269 | 1333 | 1397 | [1334 - 1475 - 1164 - 1507] |
| CARRIED OVER FROM 2008 | 25.764,37 | 2.324,21 | 964,83 | 0,00 | 92,45 | -32.312,43 |
| FUNDING FROM NCSR "D" | 40.000,00 | 44,05 | | | | 0,00 |
| MATCHING FUNDS | 43.014,96 | | | | | 0,00 |
| INCOME FROM SERVICES | | | | | | 79.643,57 |
| DONATIONS FROM COMPANIES | | | | | | 0,00 |
| TRANSFER FROM OTHER SOURCES | 5.294,89 | | | 2.826,60 | | 22.651,72 |
| <u>TOTAL INCOME</u> | 114.074,22 | 2.368,26 | 964,83 | 2.826,60 | 92,45 | 69.982,86 |
| <u>EXPENSES</u> | | I.B. | | | | |
| EQUIPMENT | 8.455,81 | | | | | 9.376,64 |
| SUPPLIES | 20.066,94 | | | | | 16.976,94 |
| SALARIES | 2.100,00 | | | | | 19.660,00 |
| TRAVELS | 113,31 | | | | | 935,00 |
| OTHER EXPENSES | 18.774,56 | | | | | 20.602,72 |
| COMMITTED | 3.288,67 | | | | | 3.845,87 |
| TRANSFER FROM OTHER SOURCES | 8.225,00 | 2.368,26 | 964,83 | 2.826,60 | | 5.587,80 |
| <u>TOTAL EXPENSES</u> | 61.024,29 | 2.368,26 | 964,83 | 2.826,60 | 0,00 | 76.984,97 |
| <u>2. GOVERNMENTAL FUNDING</u> | | | | | | |
| LIQUID NITROGEN | 0,00 | | | | | |
| ANIMAL CHOW | 4.999,73 | | | | | |
| <u>TOTAL GOVERNMENTAL FUNDING</u> | 4.999,73 | | | | | |

3. ***EXTERNAL FUNDING FROM THE PROGRAMMES OF THE INSTITUTE (Programmes that are coordinated by the Head of IB are included)***

| SOURCE OF FUNDING (number of programmes) | FUNDING (in EUROS) | | | |
|------------------------------------------------------|---------------------------|------------------------|------------------------|------------------|
| | Programme A | Programme B | Programme C | INSTITUTE |
| European Union (7) | 153.102 | 221.830 | 13.000 | 387.932 |
| General Secretariat for Research & Technology (1) | - | - | 13.388 | 13.388 |
| Ministry of health & Social Solidarity (1) | 4.000 | - | - | 4.000 |
| International Atomic Energy Agency (IAEA) (1) | - | - | 1.500 | 1.500 |
| John S. Latsis Public Benefit Foundation (1) | - | - | 12.000 | 12.000 |
| Empeirikion Foundation (1) | 3.000 | - | - | 3.000 |
| Greek Society of Oncologists (1) | 1.250 | - | - | 1.250 |
| Kotsikas Foundation (1) | 10.000 | - | - | 10.000 |
| American College of Greece (1) | 3.000 | - | - | 3.000 |
| Organogenesis Inc. (1) | 7.724 | - | - | 7.724 |
| Abbot Hellas (1) | 8.000 | - | - | 8.000 |
| TOTAL | 190.076 | 221.830 | 39.888 | 451.794 |

COLLECTIVE DATA ON PRODUCTIVITY OF SCIENTIFIC PROGRAMMES

| | P R O G R A M M E | | | I N S T I T U T E |
|------------------------------------------------------------------------------------|-----------------------------|-----------------------------|----------------------------|-------------------------------------|
| | A | B | C | |
| Researchers | 11 | 7 | 4 | 23* |
| Technical Specialist | - | 1 | 1 | 2 |
| Emeritus & Collaborating Scientists | 2 | 5 | 1 | 9** |
| Postdoctoral Fellows | 5 | 3 | 2 | 10 |
| Graduate Students | 17 | 7 | 1 | 27% |
| Collaborating Graduate Students | 8 | - | 4 | 13 ^{!!!} |
| Graduate Research Associates | - | 1 | 1 | 2 |
| Undergraduate Students | 5 | 6 | - | 11 |
| Research Technicians | 3 | 2 | - | 9 @ |
| Administrative Staff | - | - | - | 2 |
| Total Personnel | 51 | 32 | 14 | 108 |
| Publications in Peer-Reviewed Journals | 21 | 15 | 4 | 39[#] |
| Publications (Average) in Peer-Reviewed Journals per Scientist | 1.909 | 2.14 2 | 1 | 1.695 |
| Cumulative Impact Factor in Peer-Reviewed Journals (number of publications) | 74.0 21 (21) | 46.4 13 (15) | 11.42 1 (4) | 125.475[#] (39) |
| Average Impact Factor in Peer-Reviewed Journals | 3.524 | 3.09 4 | 2.855 | 3.217 |
| Cumulative Impact factor per Scientist | 6.729 | 6.63 0 | 2.855 | 5.455 |
| Proceedings to Conferences | 6 | 5 | 7 | 18 |
| Proceedings (Average) per Scientist | 0.545 | 0.71 4 | 1.75 | 0.782 |
| Total Publications | 27 | 20 | 11 | 57[#] |
| Publications (Average) per Scientist | 1.285 | 2.85 7 | 2.75 | 2.478 |
| Citations | 787 | 352 | 85 | 1228* |
| International Patents | - | - | - | - |
| Greek Patents | - | - | - | - |
| Presentations to International Conferences | 15 | 20 | 7 | 42[£] |
| Presentations (Average) per Scientist to International Conferences | 1.363 | 2.85 7 | 1.75 | 1.826 |
| Presentations to Greek Conferences | 24 | 8 | 5 | 36[£] |
| Presentations (Average) per Scientist to Greek Conferences | 2.181 | 1.14 2 | 1.25 | 1.565 |
| Total Presentations to Conferences | 39 | 28 | 12 | 78 |
| Presentations (Average) per Scientist to Conferences | 3.545 | 4 | 3 | 3.391 |

* 1 Scientist of Human Tissue Bank is included

** 1 Collaborating Scientist of Human Tissue Bank is included

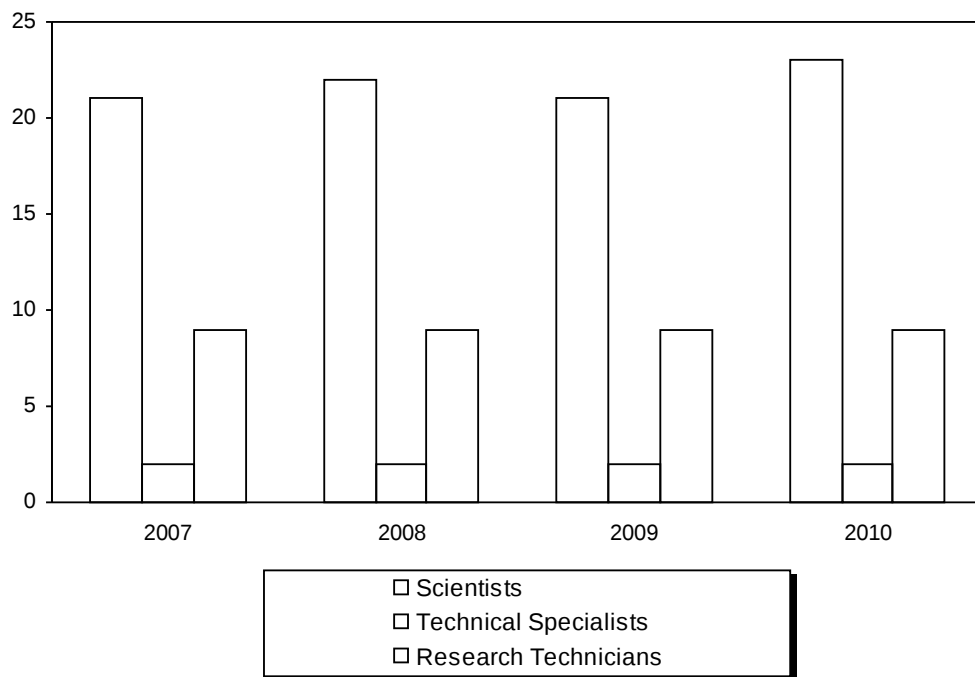
% 2 students in rotation are included

!!! 1 Collaborating Graduate Student of Human Tissue Bank is included

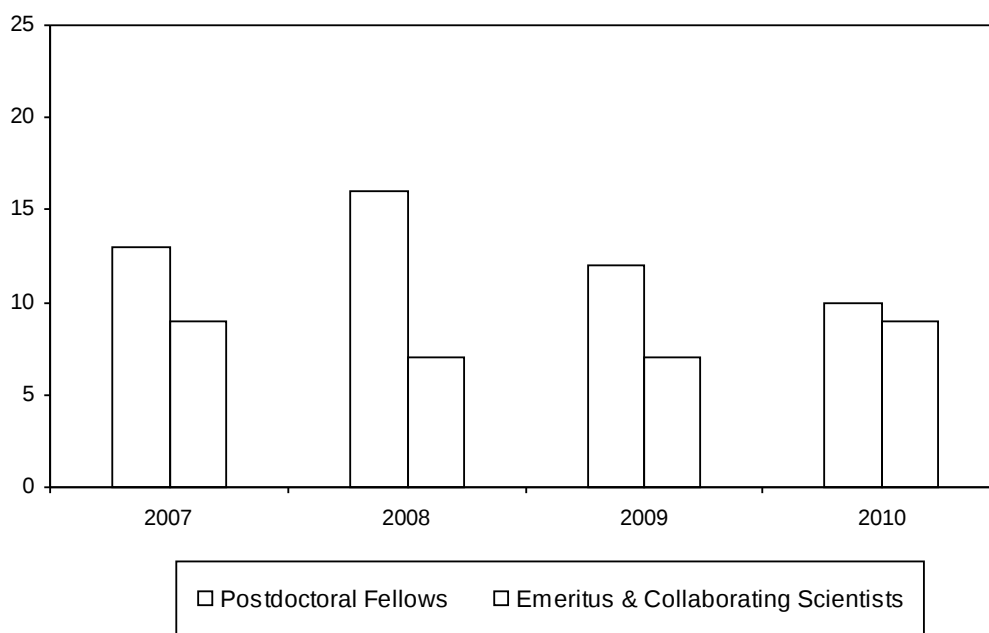
*@ 2 Research Technicians who are occupied in Experimental Animal Colony and 1 Research Technician who is occupied in Human Tissue Bank are included
1 publication of Human Tissue Bank is included and 2 common publications of A and B programme
£ 1 presentation to International Conference of Human Tissue Bank is included and 2 common presentation of B and C programme
&There is 1 common presentation to Greek Conference of B and C programme*

CHANGES OF IB STAFF DURING 2007-2010

"TENURED EMPLOYEES"

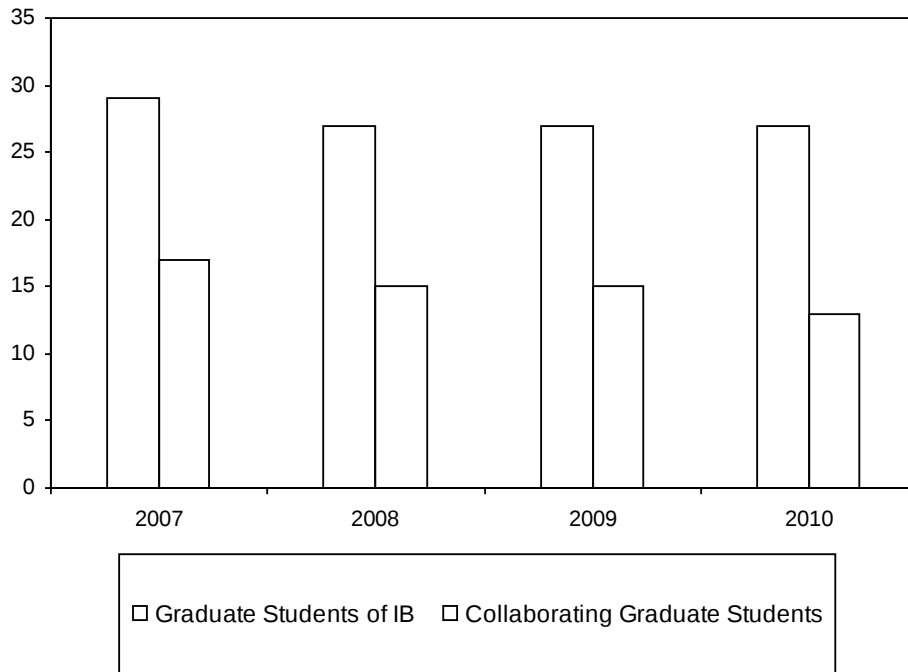


"POSTDOCTORAL FELLOWS and EMERITUS & COLLABORATING SCIENTISTS"

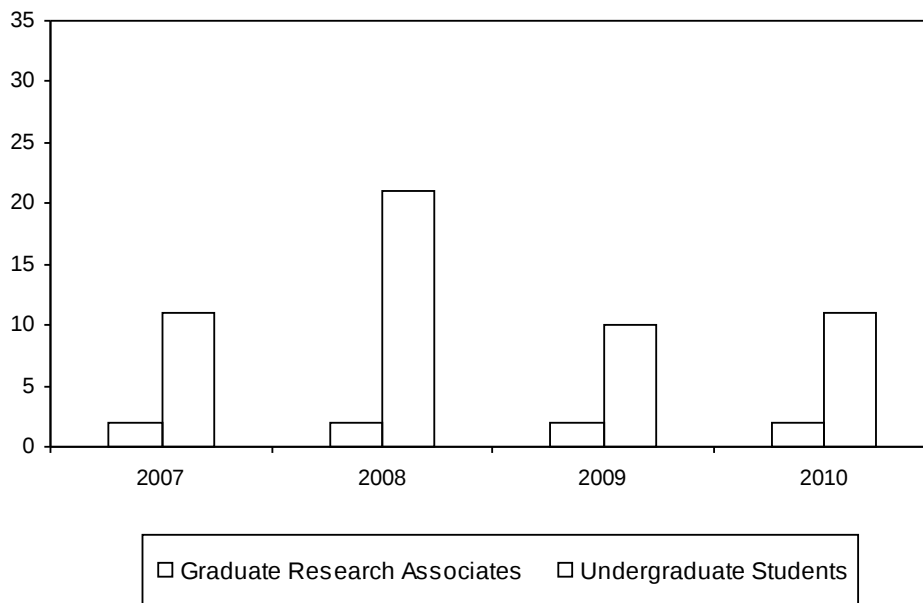


CHANGES OF IB STAFF DURING 2007-2010

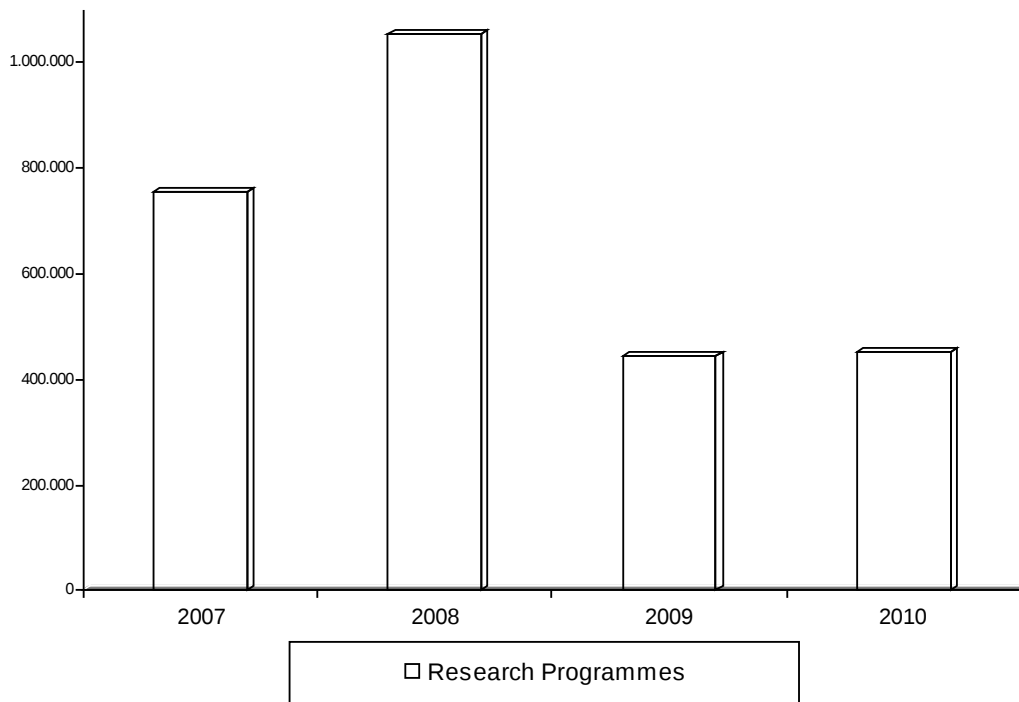
"GRADUATE STUDENTS"



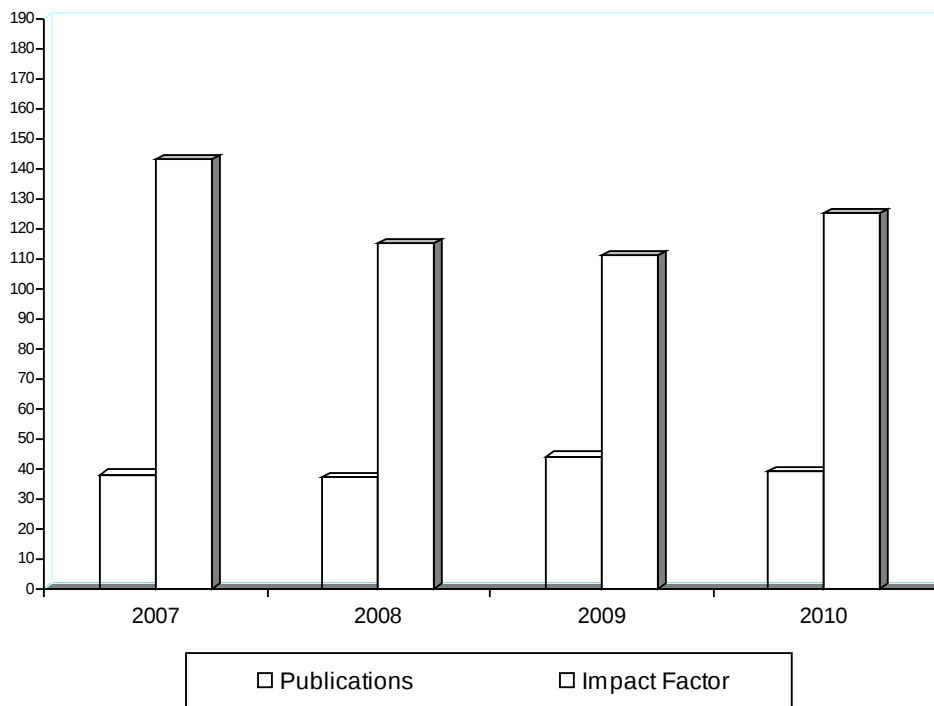
"GRADUATE RESEARCH ASSOCIATES AND UNDERGRADUATE STUDENTS "



**CUMULATIVE EXTERNAL FUNDING OF THE INSTITUTE
DURING 2007-2010
(EURO)**



**PUBLICATIONS IN PEER-REVIEWED JOURNALS
AND CUMULATIVE IMPACT FACTOR DURING 2007-2010**



CITATIONS OF THE INSTITUTE DURING 2007-2010

