INSTITUTE OF
BIOLOGY

# *NATIONAL CENTRE FOR SCIENTIFIC RESEARCH "DEMOKRITOS"*

# *INSTITUTE OF BIOLOGY*

# *2007*
# *ANNUAL REPORT*

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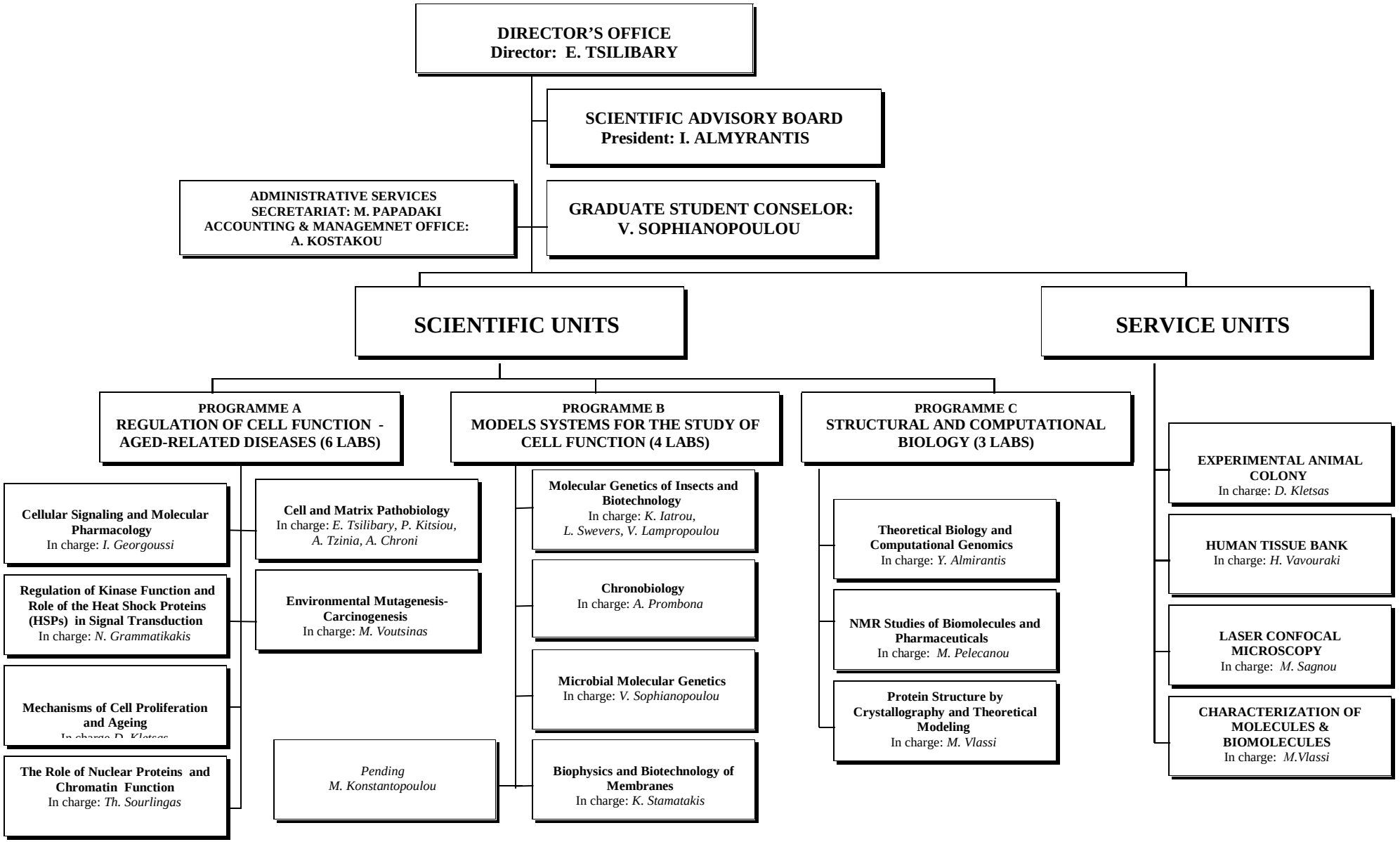

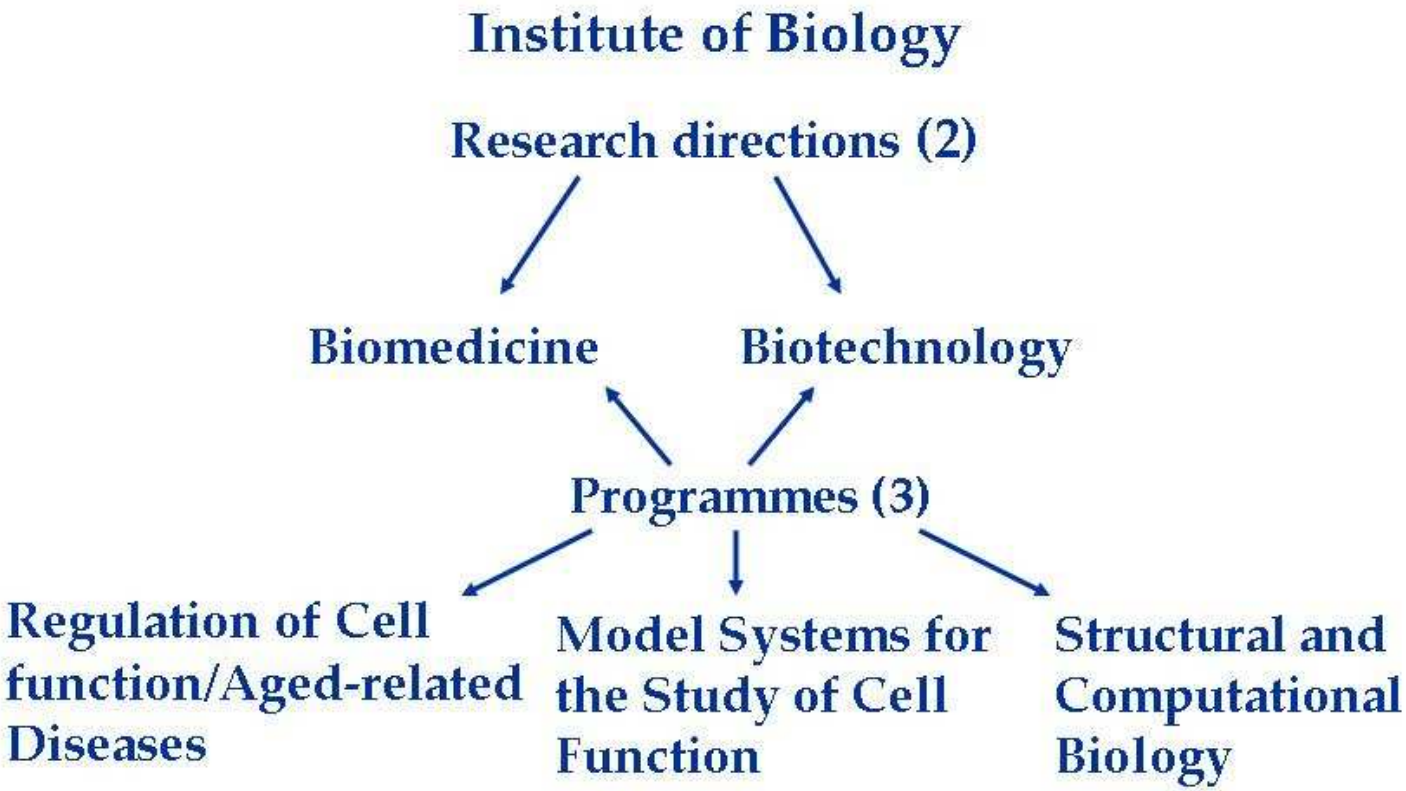
Institute of Biology
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Biotechnology
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Regulation of Cell function/Aged-related Diseases
Model Systems for the Study of Cell Function
Structural and Computational Biology

# P E R S O N N E L

## DIRECTOR

| | |
|---|---|
| Effie Tsilibary | MD, Cell Biologist |

## ACTING DIRECTOR

| | |
|---|---|
| Almyrantis Ioannis | Chemist |

## SCIENTIFIC STAFF

***Research Directors***

| | |
|---|---|
| Almyrantis Ioannis | Chemist |
| Iatrou Kostas | Professor of Biochemistry and Molecular Biology |
| Pelecanou Maria | Pharmacist |
| Sophianopoulou Vassiliki | Biologist |
| Tsilibary Effie | MD, Cell Biologist |

***Senior Researchers***

| | |
|---|---|
| Georgoussi Zafiroula-Iro | Biochemist |
| Grammatikakis Nikolaos | Cell Biologist |
| Kletsas Dimitris | Biologist |
| Prombona Anastasia | Biologist |
| Stamatakis Konstantinos | Biologist |
| Swevers Luc | Biologist |
| Vlassi Metaxia | Physicist-Chrystallographer |

***Researchers***

| | |
|---|---|
| Chroni Aggelika | Biologist |
| Kitsiou Paraskevi | Biologist |
| Konstantopoulou Maria | Biologist |
| Labropoulou Vassiliki | Biochemist |
| Voutsinas Gerassimos | Biologist |
| Sourlingas Thomae | Biologist |
| Tzinia Athina | Biochemist |
| Vavouraki Helen | Radiopharmacist |

***Lecturers***

| | |
|---|---|
| Sagnou Marina | Biologist/ Chemist |

***Technical Specialists***

| | |
|---|---|
| Panagiotopoulou Aggeliki | Biochemist |
| Stefanou Dimitra | Agronomist |

## RESEARCH TECHNICIANS

Avgeris Socrates
Doulgeridis George
Kakkos Stilianos
Kalokiri-Stilianidi Kalliope
Kopanelis Dimitrios
Kotsopoulou Eleni
Pantazi-Mazomenou Anastassia
Tsolomiti-Gourgou Areti
Zafiropoulos Ioannis

## ADMINISTRATIVE STAFF

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

## EMERITUS & COLLABORATING SCIENTISTS

| **Emeritus Sientists** | **LLaboratory** |
|---|---|
| Benaki Dimitra (Dr. Biophysicist)- *Collaborating* | Pelecanou M. |
| Ignatiadou Lydia (Dr. Hydrobiologist)- *Emeritus* | Iatrou K. |
| Mazomenos Vassilios (Dr. Chemical Ecologist)- | *Emeritus* Lab of Chemical Ecology and NaturalProducts |
| Papageorgiou George (Dr. Biochemist)- *Emeritus* | Stamatakis K. |
| Papageorgiou Spyros (Dr. Physicist)- *Emeritus* | Almirantis I. |
| Sekeri Kalliope (Dr. Biochemist) – *Emeritus* | Sourlingas Th. |
| Sideris Eleftherios (Dr. Geneticist)- *Emeritus* | Sophianopoulou V. |
| Stathakos Dimitrios (Dr. Biochemist)- *Emeritus* | Kletsas D. |
| Tsimilli – Michael Meropi (Dr. Biologist) | Stamatakis K. |

## POSTDOCTORAL FELLOWS

| **Fellow** | **Supervisor** |
|---|---|
| Agalou Adamantia | Georgoussi I. |
| Drossopoulou Garifallia | Tsilibary E. |
| Efrose Rodica | Iatrou K. |
| Fourla Danai | Georgoussi I. |
| Fragouli Apostolia | Tsilibary E. |
| Kizis Dimosthenis | Sophianopoulou V. |
| Kotzia Georgia | Iatrou K. |
| Martinou Kelly | Konstantopoulou M. |
| Nicolopoulos G. | Vlassi M. |
| Pratsinis Haris | Kletsas D. |
| Skamnaki Vassiliki | Chroni A. |
| Spyropoulou Antonia | Kletsas D. |
| Vlachakis Dimitris | Vlassi M. |

## GRADUATE STUDENTS

| Student | Supervisor |
|---|---|
| Aliberti Sofia | Grammatikakis N. |
| Billini Maria | Stamatakis K. |
| Bouzarelou Dimitra | Sophianopoulou V. |
| Danil George - *MSc* | Chroni A. |
| Dafnis Ioannis - *MSc* | Chroni A. |
| El Hamitie Abraam | Grammatikakis N. |
| Georganta Irene | Georgoussi I. |
| Georgomanolis Theodoros | Swevers L. |
| Handris Panagiotis | Kletsas D. |
| Ioannides Konstantinos | Iatrou K. |
| Karkoulis Panagiotis | Voutsinas G. |
| Leontiadis Leonidas | Georgoussi I. |
| Magkrioti Christiana | Iatrou K. |
| Manta Maria | Tsilibary E. |
| Michas George - *MSc* | Chroni A. |
| Mavrogonatou Eleni | Kletsas D. |
| Nikas Vassilios | Voutsinas V. |
| Ninios Ioannis | Sourlingas Th. |
| Papakonstantinou Maria | Georgoussi I. |
| Repouskou Anastasia | Prombona A. |
| Roumelioti katerina | Sophianopoulou V. |
| Salpea Paraskevi | Sourlingas Th. |
| Talamagas Anargiros | Tsilbary E. |
| Tsagaraki Ioanna | Tzinia A. |
| Tsotakos Nikos | Tsilibary E |
| Tzanopoulou Stamatia | Pelekanou M. |
| Vaggelatos Ioannis | Sophianopoulou V. |
| Venieratos Panagiotis | Tsilibary E. |
| Xedous Marios | Sekeri K. |

## GRADUATE RESEARCH ASSOCIATES

| Fellow | Supervisor |
|---|---|
| Klimopoulos Alexandros *(MSc)* | Almirantis I. |
| Makris Konstantinos *(MD)* | Vavuraki H. |

## COLLABORATING GRADUATE STUDENTS

| Student (University) | Supervisor |
|---|---|
| Anastassiou Dimitra (Univ. of Athens) | Voutsinas G. |
| Bombori Anna (Univ. of Athens, MSc) | Sofianopoulou V. –**MSc obtained in 2007** |
| Chrissouli Stefania (Univ. of Athens, MSc) | Kletsas D. |
| Gioni Vassiliki (Univ. of Athens, MSc) | Kletsas D. |
| Kachrilas Stafanos (Univ. of Athens) | Voutsinas G. |
| Konstantakatou Evmorphia (Univ. of Athens) | Voutsinas G |
| Kostomoiri Mirto (Univ. of Athens) | Tsilibary E./Tzinia A. |
| Lagopati Nefeli (Athens Polytechnic School)) | Tsilibary E. |

| | |
|---|---|
| Lampidonis Antonis (Agricultural Univ. of Athens) | Voutsinas G. |
| Litsiou Eleni (University of Athens) | Voutsinas G. |
| Malakassis Anastassios (Univ. of Athens, MSc) | Kletsas D. |
| Melachrinou Sofia (University of Athens) | Voutsinas G. |
| Oikonomopoulos Spiros (Univ. of Athens, MSc) | Kletsas D. |
| Papadopoulou Adamantia (Univ. of Athens, MSc) | Kletsas D. |
| Vatsi Stamatia (Univ. of Athens, MSc) | Vlassi M. |
| Vestaki Katerina (Univ. of Athens, MSc) | Voutsinas G. |
| Villias Giorgos (Univ. of Athens, MSc) | Vlassi M. |

## UNDERGRADUATE STUDENTS AND OTHER IN TRAINING

| **Student (University)** | **Supervisor** |
|---|---|
| Antoniou Dafni (Univ. of Athens) | Prombona A. |
| Galeou Aggeliki (Univ. of Athens) | Prombona A. |
| Dragoumani Konstantina (Univ. of Creta) | Vlassi M |
| Kapi Marianna (Univ. of Athens) | Prombona A. |
| Laspa Marina (Univ. of Athens) | Sophianopoulou V. |
| Nomikou Irene (University of Athens) | Prombona A. |
| Pittis Alexandros (Univ. of Athens) | Sophianopoulou V. |
| Sarris Michalis (University of Athens) | Georgoussi I. |
| Tambakis Ioannis (Agricultural Univ. of Athens) | Sophianopolou V. |
| Voreas Tilemachos (Univ. of Athens) | Stamatakis K. |
| Zagari Eleni (Agricultural Univ. of Athens) | Stamatakis K. |

## INTRODUCTION

The Institute of Biology (IB) is one of eight (8) different Institutes of the National Centre for Physical Sciences "Demokritos". The Centre is unique in Greece for combining different sciences and for collaborations among different disciplines and fields of expertise; these collaborative efforts aim at an optimal result in the promotion of research and technology, in thematic areas of complementary expertise and research interests of researchers from different institutes.

The IB has 21 research members, after the incorporation of three (3) specialized scientists who belonged previously in the category of "specific technical scientists". Moreover two new scientists were elected as "Lecturers" (Researchers D') and are expected to join the ranks of IB researchers in 2008. An application has also been submitted for new research positions in 2008, and it is hoped that the new candidates will rank in quality, as high as the ones for this year. During 2007 Drs. Kletsas and Vlassi were promoted to the rank of "Research Director" (Researcher A'), fully deserving warm congratulations for their promotion.

The IB has been gradually obtaining new equipment with the GSRT Program "EPAN for infrastructures": During 2007 the total amount for equipment purchased was ~250.000 €, and the process continues, together with an effort to upgrade the existing equipment. The process of ISO certification was completed for the Laboratory of Human Tissues ("Human Tissue Bank"), whish started to sell bone fragments, thus securing one more source of funds for the IB. The process of ISO certification for the Laboratory of experimental animals ("Experimental Animal Colony") is pending, and at the same time this facility has multiplies its income with the coordinated efforts of the facility supervisor, Dr. Kletsas and other members of the facility.

Retired ("honorary") researchers were proven active once again with peer-reviewed publications, seminars, participation in research, etc, and in general had a valuable contribution to the progress of the IB.

My continuous effort to upgrade the IB was supported and reinforced by all the members of the Scientific Advisory Board: Drs. Almyrantis, Vlassi, Kletsas, Prombona, and Sophianopoulou, all contributed substantially for successfully completing many administrative tasks, including making difficult decisions in several instances, and helping to solve various problems. Sincere thanks are also extended to the Education Committee of the IB, whose members took care of matters related to graduate students, and to all researchers who participated in different committees. Special thanks to the Vice-Director Dr. Almyrantis who invariably stood by my side and played a pivotal role for maintaining a smooth and unperturbed function of the IB.

During 2007 the external research seminars continued with success, with the coordination of Dr. Prombona.

Despite many obstacles and difficulties, the loyalty and support offered to me by the majority of researchers is the main source of optimism and confidence in the successful outcome of the goals for upgrading the IB. It is my firm belief that our continuous effort and a spirit of unity will promote the IB as an internationally competitive institute. There is ample trust in the scientific potential of the body of researchers who continuously improve and upgrade themselves thus substantially contributing to the goals of the IB. Wishes for good luck in their continuous efforts are extended to all members.

Special thanks are also extended to the Accountant, Ms. Kostakou and the Secretary of the IB, Ms. Papadaki.

**Effie C. Tsilibary, MD, PhD**

**Director of IB**
February 2008

# *PROGRAMME A:*

# *REGULATION OF CELL FUNCTION*

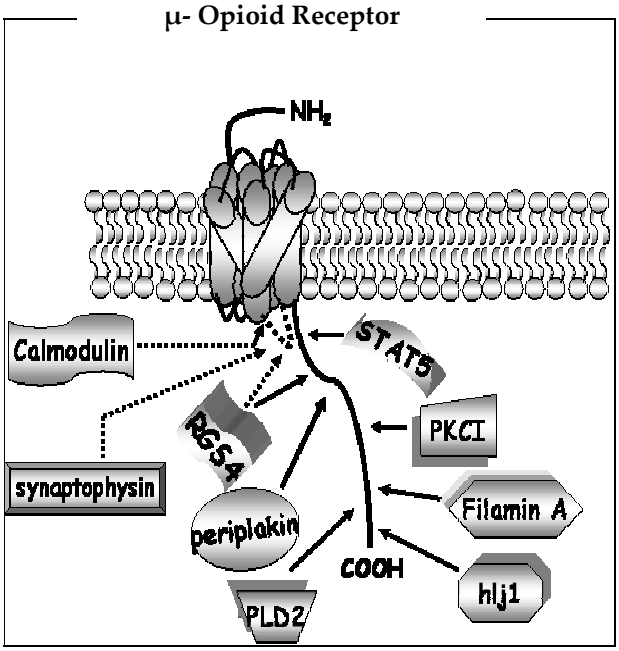
# *AGED-RELATED DISEASES*

**Research Group:** Cellular Signalling and Molecular Pharmacology

**Research Staff**
**Iro Georgoussi, Senior Researcher**
Adamantia Agalou, Postodoctoral Fellow
Danai Fourla, Postodoctoral Fellow
Leonidas LeontiadisGraduate Student
Irene Georganta, Graduate Student
Maria Papakonstantinou, Graduate Student
Michalis Sarris, Undergraduate Student

**Research Interests**

Our research interests are focused on the elucidation of the molecular signaling mechanisms mediated upon activation of the G protein coupled receptors (GPCRs) using as a model system the opioid receptors. In this respect we are interested in:




a) the identification of novel interactive proteins that participate in the GPCR signaling and can lead to new pharmacological targets (Figure 1).

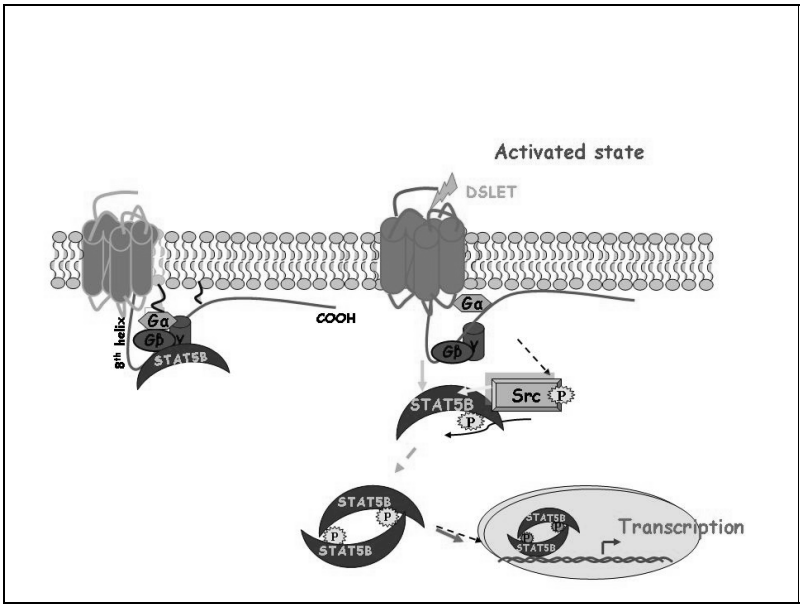
b) the identification of transcriptional factors and genes whose expression and function is affected by activation of opioid receptors upon morphine, or other opioid drug administration and finally

c) in the development of "smart drugs", novel analgesics, using either cell-based high throughput screening systems or chimeric-chameleon-peptides encompassing an opioid and other GPCR-like structure.

**2007 Findings**

**Identification of proteins that interact with μ- and δ- opioid receptors**

Previous results from our laboratory have shown for the first time that the RGS4 protein, which is implicated in the activation of G proteins, directly interacts with the ◎- and ◎- opioid receptors. Based on these observations we constructed a truncated version of the RGS4 protein and demonstrated the importance of the N-terminal domain for its interaction with the opioid receptors. In addition, using flow cytometry analysis we indicated that over-expression of RGS4 in HEK 293 cells leads to increased rate of◎opioid receptor internalization; while confocal microscopy studies suggested that activation of the ◎- opioid receptor leads to RGS4 translocation from the cytoplasm to the cell membrane (manuscript submitted).



**Identification of transcription factors and functional analysis of genes that are implicated in phenomena related to drug tolerance and dependence.** In an attempt to elucidate the molecular mechanisms that are involved in drug tolerance and dependence, we demonstrated that the transcription factor STAT5B binds to the C-terminal tail of the δ-opioid receptor in the resting state of the receptor and can form a multi-protein complex comprised by the receptor and the Gβγ subunits of the G proteins. STAT5B dissociation from the receptor occurs when STAT5B is phosphorylated by a Src kinase (Figure 2). These results reveal a novel signaling pathway through which δ-opioid receptor can probably regulate gene transcription and alter synaptosomal plasticity (manuscript in preparation).

In parallel, studies using RNA microarrays from SHSY-5Y cells, endogenously expressing the μ-opioid receptor and treated for various times with morphine, indicated a number of genes that are down- and/or up-regulated. The classification of these genes according to their functional role and correlation to STAT transcription factors is under further investigation (co-operation with Prof. K. Iatrou, laboratory of insect Molecular Biology and Biotechnology, Institute of Biology, and Dr Mayi Arcellana-Panlilio, University of Calgary, Canada).

**Identification of new opioid analogues with analgesic effect.** We participate in the EU network "Normolife" (LSHC-CT2006-037733) which consists of eleven partners from six European countries. Our goal in this European consortium is to develop new therapeutic substances for treatment of pain in patients with advanced stages of cancer. In this regard, we investigate the effect of novel synthetic chimeric-peptides on the activation of μ- and ◎ opioid receptor signaling mechanism in cellular systems that express the opioid receptors at high levels. Our results have shown that chimeric peptides targeting more than one GPCR, together with the opioid receptors, can reduce the cAMP levels, alter opioid receptor signaling and can be valuable targets with analgesic properties.

**Signaling mechanisms mediated by the olfactory receptors of the mosquito *Anopheles gambiae*.** In colaboration with the group of Insect Molecular Genetics and Biotechnology of the Institute of Biology headed by Prof. K. Iatrou, we aim to define the signaling mechanisms regulating the activity of olfactory receptors of mosquito *Anopheles gambiae.* In this respect, we identified that OR1 and OR2 receptors heterodimerize with the OR7 «helper» receptor and couple to specific G proteins when expressed in Bm5 Lepidopteran insect cells.

**Review Articles**

Georgoussi Z. (2007) In **"**Molecular aspects of G protein-coupled receptors: Interacting proteins and function" on "Novel interactive partners regulating opioid receptor signalling beyond the G protein paradigm" (Invited review) Nova Science publishers (eds F. Ciruela and R. Lujan) Chapter 6, 169-206

**2007 Presentations at International Scientific Conferences**

E. Georganta, Z. Georgoussi (2007). Functional complexes between δ-opioid receptor, Gβγ and STAT5B are implicated in STAT5B phosphorylation. INRC Annual Meeting, International Narcotic Research Conference, July 8-13, 2007, Berlin, Germany. (Poster Presentation)

L. Leontiadis, M.-P. Papakonstantinou, Z. Georgoussi (2007). RGS4 interacts directly with μ- and δ-opioid receptors to regulate their signaling. INRC Annual Meeting, International Narcotic Research Conference, July 8-13, 2007, Berlin, Germany. (Poster Presentation)

Z. Georgoussi (2007) Novel Signaling pathways mediated by the opioid receptors. 19th Polish Peptide Symposium p. 28. Pultusk, Poland 23-27 September. (**Invited Speaker**)

D., Dimitratos, S., Walter, M. and Biessmann, H. Anopheline mosquito olfaction and malaria control: OBPs, ORs and in vitro assays for ligand identification. Third International Meeting on "Molecular and Population Biology of Mosquitoes and Other Disease Vectors", 13 - 20 July 2007, Kolymbari, Crete, Greece.

Swevers, L., Andronopoulou, E., Labropoulou, V., Douris, V., Tsikou, D., Efrose, R., Kotzia, G., Stefanou, D., Morou, E., Georgoussi, Z., and Iatrou, K. (2007). Genetically transformed insect cell lines

as screening tools for improved insecticides and pharmaceutics. International Congress of Insect Biotechnology and Industry, Daegu, Republic of Korea, 19-24 August 2007 (**Invited lecture**)

**Citations 2007** (without self- citations): 16

**Total Citations 2005-2007** (without self- citations): 49

**h-factor**: 10

**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
Abraam El Hamitie, 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).

**2007 Findings**

Finally, it would be helpful to outline below my past productivity (until July of 2004 when I transferred my lab to Greece):

Impact factor and number of citations of the PI:

- Total Impact Factor for the work published after 1998 (19 papers): 139,012
- Average Impact Factor (for each of 19 papers from 1998 to present): 7,31
- Total number of citations (for 24 papers, self-references not included): 762
- Average number of citations (for each of 24 papers): 31,75

**Impact Factors**: 0

**Citations 2007** (without self- citations): 99

**Total Citations 2005-2007** (without self- citations): -

**h-factor**: 14

## Research Group: Mechanisms of Cell Proliferation and Ageing

**Research Staff**
**Dimitris Kletsas, Researche Director**
Dimitrios Stathakos, Emeritus Scientist
Haris Pratsinis, Postdoctoral Fellow
Antonia Spiropoulou, Postdoctoral Fellow
Panagiotis Handris, Graduate Student
Eleni Mavrogonatou, Graduate student
Vassilki Gioni, Collaborating Graduate Student (MSc)
Adamantia Papadopoulou, Collaborating Graduate Student (MSc)
Stafania Chrissouli, Collaborating Graduate Student (MSc)
Anastassios Malakassis, Collaborating Graduate Student (MSc)
Spiros Econopopoulos, Collaborating Graduate Student (MSc)

### Research Interests

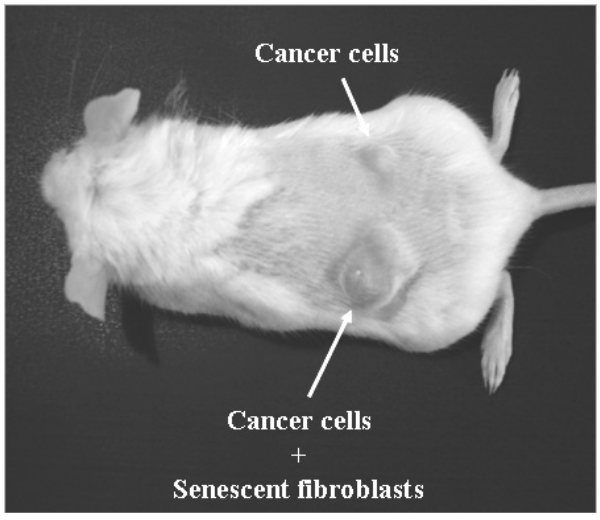
We are focusing on the role of growth factors, and especially of TGF-β, in tissue homeostasis during development and ageing. Their action on cell proliferation and extracellular matrix production, as well as the responsible signaling pathways are investigated. Alternative mechanisms of cell proliferation and differentiation, such as autocrine regulation, cell-matrix interactions, exogenous stress and the effect of mechanical forces, are also studied.

Main focus 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 stress - in comparison to that of the young or the cancer cell are investigated. Especially, we are interested on 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 the intervertebral disc, the degeneration of which provokes severe dysfunctions during ageing. Finally, the characteristics of centenarians, as an example of successful ageing, are also studied.

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

**2007 Findings**

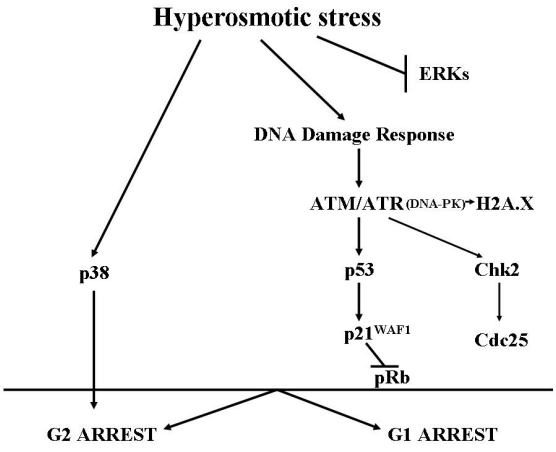
We have continued our studies on the role of growth factors in tissue repair. Having in mind the different repair strategies between fetuses and adults we have shown that Transforming Growth Factor-β (TGF-β) regulates the proliferation of human lung fibroblasts according to the developmental stage of the donor: it stimulates the proliferation of adult cells while it inhibits embryonic fibroblasts. TGF-β stimulates human fetal fibroblasts by induicing the synthesis and release of autocrine growth factors. In adult lung fibroblasts TGF-β inhibits proliferation via the activation of PKA and the subsequent up-regulation of the cyclin-dependent kinase inhibitor $p21^{WAF1}$. These data are the opposite of our previous findings in human skin fibroblasts.



In the same context, we are studying the role of the amniotic fluid, i.e. the physiological environment of embryonic cells, on various parameters of tissue repair. We have found that amniotic fluid stimulates cell prolioferation, migration, contraction and collagen synthesis. The mechanisms underlying these phenomena are currently under investigation.

Main goal of our laboratory is the investigation of the structural and functional feature of the senescent cell and its role in the development of age-related diseases, including cancer. Tumor stroma is an important factor in cancer development. We have shown that repeated therapeutic doses of ionizing radiation provoke premarure senescence in stroma fibroblasts and that senescent cells promote the growth of adjacent cancer cells in vitro and in immunocompromised mice (SCID) in vivo (Figure 1). Stem cells are currently studied in several biomedical applications. We, as part of a large EU-funded network aiming at the use of stem cells in autologous cell replacement therapies, are studying the characteristics of in vitro senescent human stem cells and the consequences of their use in vivo.

One of the tissues that is severely affected by ageing is the intervertebral disc. We have studied intervrtebral disc cells' proliferation, based on the hypothesis that proliferation in the disc is inhibited under normal conditions (due to the adverse nutritional environment) and it is stimulated during disc degeneration (aiming at tissue repair). We have shown that one of the stresses to which disc cells are subjected during daily activities, i.e. hyperosmotic stress, inhibits cell proliferation by activating the G2 and G1 cell cycle checkpoints. p38 MAPK was found to participate in G2 arrest under these conditions, since inhibition of its activity released the cells from G2 phase into mitosis. High osmolality resulted also in the ATM-mediated phosphorylation of p53 on Ser15, the up-regulation of $p21^{WAF1}$ and the hypophosphorylation of the retinoblastoma protein, in accordance to the observed G1 arrest. Furthermore, comet assay revealed the presence of DNA damage after hyperosmotic treatment, possibly attributed to the abrupt alterations in chromatin configuration observed early after exposure of the cells to this stress (Figure 2). In contrast with previous studies in renal cells, we have shown that disc cells exert a DNA damage response similar to the one expressed after ionizing radiation. Even more, disc cells residing within a hyperosmotic environment retained their ability to respond to newly introduced DNA damage, and possess an increased DNA repair efficiency. On the other hand, we have shown that various autocrine growth factors secreted during intervertebral disc damage, such as, PDGF, bFGF and IGF-I, stimulate cell proliferation via the activation of the MEK/ERK and the PI3K/Akt pathways.




In parallel, we have investigated the effect of anticancer agents on the homeostasis of stroma cells. In particular, we have shown that the known anticancer drug imatinib mesylate (STI571 or Glivec) has an antifibrotic effect on human breast fibroblasts, by inhibiting their proliferation and the synthesis of collagen I and III. These findings indicate that this compound may be suitable for the inhibition of desmoplasia, a crucial parameter of breast tumour development. Finally, we have continued our studies on the cytostatic/cytotoxic, anti-ageing and the would healing activity of natural products and new synthetic compounds.

**2007 Publications**

Metwally K, Pratsinis H, Kletsas D. Pyrimido[4,5-c]quinolin-1(2H)-ones as a novel class of antimitotic agents: Synthesis and in vitro cytotoxic activity. Eur J Med Chem. 42 (2007) 344-50.

Papadimitriou K, Pratsinis H, Nebe-von-Caron G, Kletsas D, Tsakalidou E. "Acid Tolerance of Streptococcus macedonicus as Assessed by Flow Cytometry and Single-Cell Sorting". Appl Environ Microbiol. 73 (2007) 465-76.

Kletsas D, Pratsinis H, Gioni V, Pilichos K, Yiacoumettis AM, Tsagarakis S "Prior chronic in vivo glucocorticoid excess leads to an anabolic phenotype and an extension of cellular lifespan of skin fibroblasts in vitro." Ann. N.Y. Acad. Sci. 1100 (2007) 449-54.

Eliades T, Gioni V, Kletsas D, Athanasiou A, Eliades G. "Oestrogenicity of orthodontic adhesive resins" Eur J Orthod. 29 (2007) 404-407.

Tsimaratou K, Kletsas D, Kastrinakis N, Tsantoulis P, Evangelou K, Sideridou M, Liontos M, Poulias I, Venere M, Salmas M, Kittas C, Halazonetis T, Gorgoulis V. Evaluation of claspin as a proliferation marker in human cancer and normal tissues. J Pathol. 211 (2007) 331-339.

Pratsinis H, Kletsas D. "PDGF, bFGF and IGF-I stimulate the proliferation of intervertebral disc cells in vitro via the activation of the ERK and Akt signaling pathways". Eur. Spine J. 16 (2007) 1858-1866.

Stahtea XN, Roussidis AE, Kanakis I, Tzanakakis GN, Chalkiadakis G, Mavroudis D, Kletsas D, Karamanos NK. "Imatinib inhibits colorectal cancer cell growth and suppresses stromal-induced growth stimulation, MT1-MMP expression and pro-MMP2 activation". Int J Cancer. 2007 121, 2808-2814.

Liontos M, Koutsami M., Sideridou M., Evangelou K., Kletsas D., Levy B., Kotsinas A., Nahum O., Zoumpourlis V., Kouloukousa M., Lygerou Z., Taraviras S., Kittas C., Bartkova J., Papavassiliou A.G., Bartek J., Halazonetis T.D., Gorgoulis V.G. "Deregulated overexpression of the replication licensing factors, hCdt1 and hCdc6, occurs early in human carcinogenesis promoting malignant behavior" Cancer Res. 2007 67, 10899-909.

**2007 Presentations at International Scientific Conferences**

Papadopoulou and D. Kletsas "Ionizing radiation provokes premature senescence in human lung fibroblasts that enhance the growth of malignant lung epithelial cells in vitro and in vivo" SENECA European Conference on Cancer and Ageing. Warsaw, Poland 4-6 October 2007 (invited speaker).

D. Kletsas "Mechanisms of cell senescence and effects in age-related homeostasis" 2007 LINK-AGE Meeting and IBMC Symposium. Porto, Portugal, 28 November-1 December 2007.(invited speaker)

**Impact Factors** (for 8 publications):28,202

**Citations 2007** (without self- citations): 342

**Total Citations 2005-2007** (without self- citations): 712

**h-factor**: 17

## Research Group: The Role of Nuclear proteins and Chromatin Function

**Research Staff**
**Thomais Sourlingas, Researcher**
Kalliopi Sekeri, Emeritus Scientist
Marios Xydous, Graduate Student
Paraskevi Salpea, Graduate Student
Giannis Ninios, Collaborating Graduate Student
Kalliopi Kalokyri-Stylianidi, Research Technician

**Research Interests**

Studies of the expression of histone subtypes and more specifically, linker histone subtypes, as well as epigenetic histone modifications and their role in chromatin conformational changes during cellular ageing and apoptosis in the in vitro cell systems of human fibroblasts, human peripheral blood lymphocytes and human cancer cell lines. The central focus of these studies is the investigation of the potential involvement of the somatic H1 linker histones and of the H1.0 linker histone and their phosphorylation states, as well as the role of acetylation-deacetylation and methylation of nucleosomal histones in heterochromatic regions of chromatin and/or in the reorganization of euchromatic/heterochromatin regions of chromatin during ageing and apoptosis.

Concomitant to the above, the effect of histone deacetylase inhibitors in the acetylation of histones and non histone target molecules is also being studied. The aim of these studies is to find molecules and/or factors which may have a functionally active involvement during the course of apoptosis.

We are also studying the role of epigenetic histone modifications, such as acetylation and methylation, in changes in gene expression levels of age-related genes in peripheral blood leucocytes.

The acetylation of promoters of genes of the biological clock (circadian rhythm) of mammalian cell systems and how the products of these circadian genes affect cell cycle-related gene expression and carcinogenesis is also being studied.

**2007 Findings**

We studied the phosphorylation levels of the linker DNA H1 histone somatic subtypes in human peripheral blood lymphocytes as a function of donor age and found a significant reduction of the mono-phosphorylated forms of the H1.4 and H1.5 subtypes in the elderly donors. This result was found to be related with the observed increase in the heterochromatin protein, HP1$\alpha$, from lymphocyte lysates of the same elderly donors. These results associate for the first time the dephosphorylation of two histone H1 subtypes with an increase in a heterochromatin protein and the possible role that this specific epigenetic change may have in the reorganization or increase in heterochromatic regions of chromatin during ageing.

The activation of the DNA fragmentation factor (DFF) was studied after the induction of apoptosis by histone deacetylase inhibitors in six leukemic cell lines. Activation of the heterodimeric complex DFF which consists of DFF40/DFF45 is initiated by the proteolytic degradation of DFF45 by the activated caspase 3. Our results showed that the apoptotic levels as well as the degree of DFF45 proteolysis (1) are time-dependent, (2) differ in the different cell lines studied and (3) that this proteolytic degradation is proportional to the degree of induced apoptosis. Two cell lines were selected which had the greatest response to the induction of apoptosis by the histone deacetylase inhibitor, trichostatin A, for co-immunoprecipitation experiments. The goal of these experiments was to ascertain the contribution of histone H1 in DFF40 activation. Previous work of others, using recombinant DFF in an *in vitro* cell-free system, showed that the presence of histone H1 is necessary for maximum DFF40 activation. From our work, we found that histone H1 co-immunoprecipitates with DFF40 in human cell lines. These results indicate that histone H1 is associated with DFF40 *in vivo* and may be a contributing factor to DFF40 activation under apoptotic conditions in human cells.

The apoptotic levels after trichostatin A treatment were compared in leukemic cell lines and human peripheral blood lymphocytes. Results from this line of work showed that lymphocytes had lower apoptotic levels as compared to leukemic cells and moreover that the levels of induced, by trichostatin A, apoptosis was not donor age-dependent. Also, whereas DFF40 and DFF45 are immuno-detected in lymphocytes, trichostatin A-induced apoptosis does not lead to DFF45 proteolysis, in contrast to leukemic cells.

Comparative studies amongst leukemic cells and normal lymphocytes are also in progress so as to investigate the acetylation of cytoplasmic proteins in apoptotic signal transduction pathways. The results showed that tubulin is acetylated after trichostatin A treatment. Tubulin of normal lymphocytes have stable levels of acetylation which do not change as a function of donor age. On the other hand, the six leukemic cell lines studied (MOLT-4, U937, NB-4, K562, HL60, Jurkat) showed a differential response to the inhibitor with respect to tubulin acetylation levels. Kinetic studies also showed that acetylation occurs from the first hour of trichostatin A treatment for both tubulin and the nucleosomal histone H4. However after treatment with the more general histone deacetylase inhibitor, sodium butyrate, that though does not inhibit the only deacetylase that specifically deacetylates tubulin, HDAC6, the levels of apoptosis remain unchanged. This result indicates that tubulin acetylation, in contrast with nucleosomal histone acetylation, may perhaps not be involved in the apoptotic signaling process.

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 and cell cycle genes that may be regulated by clock genes. Fundamental molecular elements of the biological clock are the genes, *per1, per2, cry1, cry2, clock, bmal1, rev-erbα*. It is known that the acetylation levels of histones H3 and H4 in the promoter regions of the genes, *per1, per2,* and *cry* show circadian rhythm which follows the rhythmic changes of their mRNA levels. This strongly indicates that there is a direct relationship amongst histone acetylation and the regulation of the biological clock. Moreover, it has also been shown that the expression levels of certain cell cycle/proliferation genes are influenced by clock genes. Therefore the focus of this study is to investigate whether histone deacetylase and acetyltransferase inhibitors can change the expression levels of clock genes and whether these changes can also affect the expression levels of cell cycle/proliferation genes at specific circadian times. The general aims of this study are being carried out within the framework of a research collaboration with the laboratory of Dr. Anastasia Prombona of the Institute of Biology NCSR "D".

The cellular system used in our lab's investigation is mouse NIH3T3 cell cultures whose circadian clock rhythm has been synchronized for 48 hours. The agents that we used to change histone acetylation levels at specific circadian times are trichostatin A, a histone deacetylase inhibitor and curcumin, an inhibitor of the histone acetyltransferase, p300. With RT-PCR, we found that the expression levels of the clock genes *per1, per2,* and *cry1* as well as the genes *c-myc* and *wee1* change differentially with respect to the specific agent used. Moreover, we found that these changes are also influenced by the specific circadian time that the cell cultures were treated with these inhibitors.

**2007 Publications**

Sekeri-Pataryas, K.E., Sourlingas, T.G. (2007). The differentiation-associated linker histone, H1.0, during the in vitro aging and senescence of human diploid fibroblasts. Ann. N Y Acad. Sci. 1100, 361-367.

Sourlingas, T.G., Kypreou, K.P., Topakas, G.N. Karchilaki, I.N., Stavropoulou-Giokas, C., Sekeri-Pataryas, K.E. (2007). Effect of the histone deacetylase inhibitor trichostatin A in human peripheral blood lymphocytes as a function of donor age. Ann. NY Acad. Sci. 1119, 64-71.

**2007 Presentations at International Scientific Conferences**

T.G. Sourlingas, G.N. Topakas, I.N. Karchilaki, C. Stavropoulos-Giokas, K.E. Sekeri-Pataryas. (2007). Effect of the histone deacetylase inhibitor, trichostatin A, in peripheral blood lymphocytes as a function

of donor age. 12th International Congress of Biomedical Gerontology (IABG), "Molecular Mechanisms and Models of Ageing", May, 2007, Spetses, Greece,

Y.P. Ninios, K.E. Sekeri-Pataryas, T.G. Sourlingas. (2007). Trichostatin A alters $\alpha$-tubulin and histone acetylation levels and differentially induces apoptosis in four leukemic cell lines. 12th International Congress of Biomedical Gerontology (IABG), "Molecular Mechanisms and Models of Ageing", May, 2007, Spetses, Greece.

P. Salpea, V.R. Russanova, K.E. Sekeri-Pataryas, B.H. Howard, T.G. Sourlingas.(2007). Epigenetic changes of the *dfna5* gene region during differentiation and ageing. 12th International Congress of Biomedical Gerontology (IABG), "Molecular Mechanisms and Models of Ageing", May, 2007, Spetses, Greece.

A. Repouskou, K.E. Sekeri-Pataryas, T.G. Sourlingas, A. Prombona. (2007). Changing the acetylation status at specific circadian times has differential effects on the expression levels of cell cycle genes in N2a cells. EMBO Workshop, "Molecular Mechanisms of Cell Cycle Control in Normal and Malignant Cells", Oct., 2007, Spetses, Greece.

M. Xidous, K.E. Sekeri-Pataryas, A. Prombona, T.G. Sourlingas. (2007). Histone acetylation and circadian clock gene regulation: effects on the expression of cell cycle genes. EMBO Workshop, "Molecular Mechanisms of Cell Cycle Control in Normal and Malignant Cells", Oct. 2007, Spetses, Greece.

**Impact Factors** (for 2 publications): 6,950

**Citations 2007** (without self- citations): 8

**Total Citations 2005-2007** (without self- citations): 36

**h-factor:** 6

## Research Group: Cell & Matrix Biochemistry/Pathobiology

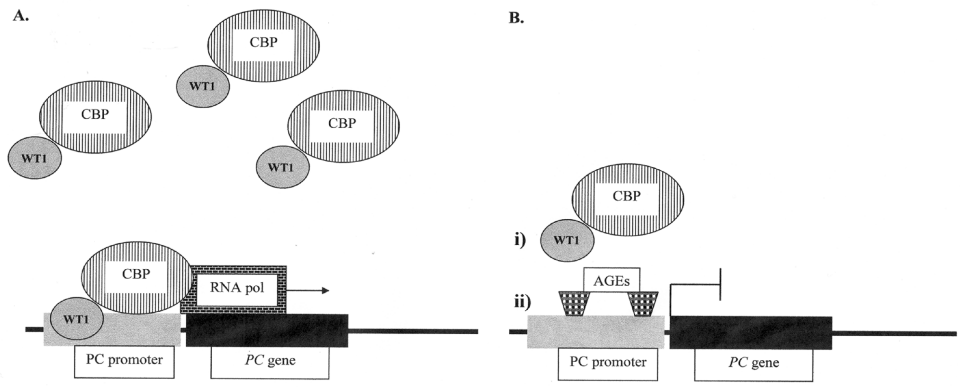
**Research Staff**
**Fotini-Effie Tsilibary, Research Director**
**Athina Tzinia, Researcher**
**Paraskevi Kitsiou, Researcher**
**Angelika Chroni, researcher**
Apostolia Fragouli, Postdoctoral Fellow
Garyfallia Drossopoulou, Postdoctoral Fellow
Vassiliki Skamnaki, Postdoctoral Fellow
Argyris Talamagas, Graduate Student
Panayotis Venieratos, Graduate Student
Ioanna Tsagaraki, Graduate Student
Nikos Tsotakos, Graduate STudent
Maria Manta, Graduate student
Nefeli Lagopati, Collaborating Graduate Student
Myrto Kostomiri, Collaborating Graduate Student
Georgios Mihas, Graduate Student *(MSc)*
Georgios Daniel, Graduate Student *(MSc)*
John Daphnis, Graduate Student *(MSc)*
Eleni Kotsopoulou, Research Technician

**Research Interests**

- Regulation of gene expression of the cell surface-associated sialoprotein podocalyxin, in renal glomerular epithelial cells, during renal development, and in normal or diabetic conditions
- Biological effects of innovative nanomaterials for diagnostic and therapeutic use
- Glucose-induced apoptosis of pancreatic β-cells through insulin-mediated signal transduction
- Examination of the role of the enzyme MMP-9 in neurodegenerative conditions of the CNS/Alzheimer
- Examination of the role of lipids and proteins derived from lipid metabolism in cells or blood circulation, in normal and pathological conditions such as atherosclerosis
- Examination of the role of lipids and lipoproteins in neuro-degenerative conditions such as Alzheimer's disease. Unraveling of the correlations between apo4 and $A_\beta$ metabolism in the brain
- Regulation of glutamate transporters in normal and neurodegenerative conditions
- Mechanisms of osteoblastic cell survival and apoptosis in conditions of inflammation

**2007 Findings**

***Regulation of gene expression of the cell surface-associated sialoprotein podocalyxin, in renal glomerular epithelial cells, during renal development, and in normal or diabetic conditions***: Previous lab data had demonstrated that podocalyxin (PCLP) expression was suppressed in high glucose. Transcription factor CBP was observed to form a functional complex with WT1 in the presence of both normal and high glucose concentration; however this association was decreased by 40% in the presence of high glucose. To continue, chromatin immunoprecipitation (ChIP) assays were performed, which indicated that the binding of the transcriptional complex WT1-CBP on the promoter of PCLP gene was substantially decreased in high glucose (Fig.1). At the cellular level we observed that high glucose can induce changes/damage via increased formation of advanced glycation end products (AGEs). Therefore we examined the effect of aminoguanidine (AG), an inhibitor of AGEs and observed that AG prevented glucose-induced suppression of podocalyxin expression. We conclude that prolonged administration of AG in our *in vitro* cell model prevents AGE-induced toxicity and apparently reinforces the binding of the WT1-CBP transcriptional complex on the PCLP promoter.

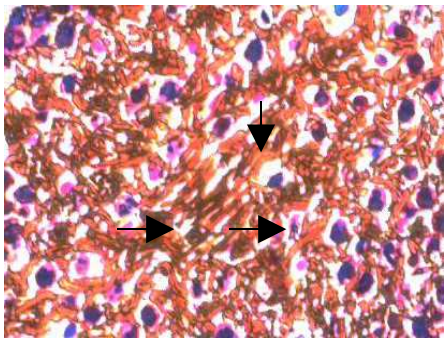



**Διάγραμμα ρύθμισης της έκφρασης της ποδοκαλυκίνης (PC) σε φυσιολογική (A) και αυξημένη (B) συγκέντρωση γλυκόζης. (A) Ο μεταγραφικός παράγοντας WT1 συνδέεται με τον παράγοντα CBP και το σύμπλοκο συνδέεται με τον υποκινητή της PC εκκινώντας τη διαδικασία μεταγραφής. (B) Σε συνθήκες αυξημένης συγκέντρωσης γλυκόζης (i) η διασύνδεση του WT1 με τον CBP είναι ελαττωματική, και (ii) γλυκο-επαγώμενα AGE στον υποκινητή παρεμποδίζουν τη διασύνδεση με το σύμπλοκο WT1- CBP, αναστέλλοντας την έναρξη μεταγραφής**

In parallel, in collaboration with Dr. V. Pachnis (Division of Molecular Neurobiology, MRC National Institute for Medical Research, London, UK), we started examining the role of PCLP during nephrogenesis and differentiation of glomerular podocytes. The model used for these studies is the RET51/51 transgenic mouse which was developed by Dr. Pachnis and has renal malformations during development. We observed that in embryonic kidneys of RET51/51 mice, WT1 transcription factor was substantially decreased compared to the control.

***Biological effects of innovative nanomaterials for diagnostic and therapeutic use:***

This project is part of collaboration with the Laboratory for Cell Regeneration and Cell senescence (DR. Kletsas in charge), as well as with the Institutes of Material Science and Physical Chemistry (collaboration funded by PEP ATT_28), aiming at the development of novel bioactive nanomaterials (magnetic, Fig.2) & Ti02 nanoparticles, for the diagnosis and treatment of pathological conditions. This collaboration has already yielded one publication (I. Rabias et. al. Biomicrofluidica, 2007), and one oral presentation at the meeting: *International Conference for Nanomedicine* (Halkidiki, 2007)

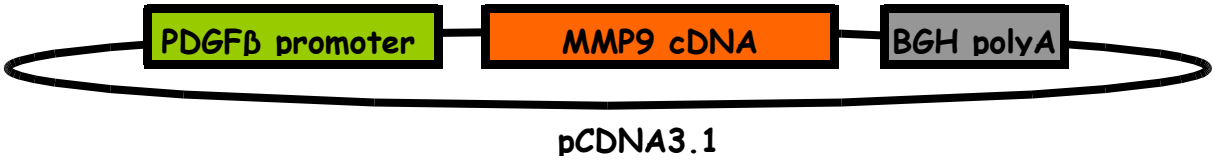


Local infusion of magnetic nanoparticles (arrows) in rat glioma tumor induced apoptosis of tumor cells (arrowheads).

***Glucose-induced apoptosis of pancreatic β-cells through insulin-mediated signal transduction:*** Type 2 diabetes is characterized by dysfunction and eventual apoptosis of pancreatic β-cells. Unperturbed insulin signaling is pivotal for survival of this cell type, the only cell which produced and secretes insulin. We examined the effect of high glucose on the insulin signaling pathway in cultured, mouse pancreatic β-cells (βTC-6). The observed data indicated that prolonged exposure of βTC-6 cells to high glucose lead to substantial down-regulation of the activation of the insulin signalling pathway (Insulin receptor/IRS-2/PI3-kinase/Akt/FoxO).This resulted to the activation of pro-apoptotic caspases and increased cell apoptosis. A study of the mechanisms involved in the apoptotic process yielded data documenting the expression of IL1β interleukin from pancreatic βTC-6 cells, which was also accompanied by increased expression of the cytoplasmic protein SOCS-1 whereas the levels of SOCS-3 remained unaltered. SOCS or "suppressor of cytokine signaling" proteins are induced by cytokines and down-regulate cytokine and insulin-mediated signaling. SOCS-1 inhibits the IRS-2-mediated signaling, whereas SOCS-3 inhibits both IRS-1 and IRS-2 –mediated insulin signaling. Our data demonstrated that prolonged exposure of βTC-6 cells resulted in suppressed activation of the IRS-2 substrate only (IRS-1 remained unaffected), in accordance with the observed increased expression of SOCS-1 specifically. We conclude that high glucose suppressed insulin-mediated signal transduction in βTC-6 cells, probably as a result of increased SOCS-1 and IL1β expression, leading to cell apoptosis.

***Examination of the role of the enzyme MMP-9 in neurodegenerative conditions of the CNS/Alzheimer:***

Our experimental data showed that MMP-9 eliminates Aβ peptide by processing APP towards α-secretase cleavage. To study the effect of MMP-9 on reducing amyloid load in an Alzheimer's disease mouse model, we generated constructs containing the cDNA coding for wild type, auto activated or mutant forms of MMP-9 (Fig.3), which will be used to generate double transgenic (APP/MMP-9) mice.




General structure of the construct (~ 3.7kb) which will be used for the development of transgenic mice over expressing MMP-9 in the CNS

***Examination of the role of lipids and proteins derived from lipid metabolism in cells or blood circulation, in normal and pathological conditions such as atherosclerosis:***

***Structure-function relationship of apoA-I.***

**a)** Following adenovirus-mediated gene transfer in apoA-$I^{-/-}$ mice we found that mice expressing the apoA-I[Δ(185-243)] or the apoA-I[Δ(220-243)] (that diminish the ABCA1-mediated cholesterol efflux *in vitro*) didn't form HDL, while mice expressing the WT apoA-I, the apoA-I[Δ(232-243)] or the apoA-I[E191A/H193A/K195A] (that promote normal ABCA1-mediated cholesterol efflux) formed HDL normally. The findings indicate that a) the C-terminal region 220-231 of apoA-I is important for the formation of HDL *in vivo* and b) mutations in apoA-I that diminish its functional interactions with ABCA1 diminish the formation of HDL *in vivo*.

*b*) The capacity of four natural occurring apoA-I mutants (apoA-I[Leu141Arg]$_{Pisa}$, apoA-I[Leu159Arg]$_{FIN}$, apoA-I[R151C]$_{Paris}$, apoA-I[R160L]$_{Oslo}$) and one bioengineered mutant (apoA-I[R149A]) to activate the cholesterol esterifying enzyme LCAT in vitro was greatly reduced. Gene transfer studies showed that mice expressing any of the five apoA-I mutants didn't form spherical HDL. Simultaneous treatment of mice with adenovirus expressing any of the five mutants and human LCAT led to formation of spherical HDL particles, suggesting a potential therapeutic intervention for HDL abnormalities that result from specific mutations in apoA-I.

***Analyses of HDL composition and functions in Greek patients who survived myocardial infraction before the age of 36 years.*** HDL-cholesterol and apoA-I levels as well as LCAT and CETP (cholesteryl ester transfer protein) activities were lower in patients compared to controls. There was no significant difference on the anti-oxidative/anti-inflammatory activities of HDL between the two groups. The lower HDL-LCAT and CETP activities in patients affect the composition and possibly the functions of HDL in humans.

***Examination of the role of lipids and lipoproteins in neuro-degenerative conditions such as Alzheimer's disease. Unraveling of the correlations between apo4 and $A_\beta$ metabolism in the brain: Insights on the relationship between apoE4 and $A_\beta$ metabolism in brain.*** We examined the effects of carboxy-terminal truncated apoE4 forms (apoE4[Δ(186-299)] and apoE4[Δ(166-299)]), which are present in the brain of Alzheimer' disease patients on the processing of amyloid precursor protein (APP) and on $A_\beta$ production in HEK and human neuroblastoma SK-N-SH cells transiently transfected with human APP. Lipid-free apoE4-165 decreased $A_\beta$ production in both cells and showed a much greater ability to stimulate the clearance of $A_\beta$ compared to WT apoE4. The levels of APP and APPα were not affected apoE4-165 as compared to WT apoE4. ApoE4-185 had similar effects on APP processing as WT apoE4.

***Regulation of glutamate transporters in normal and neurodegenerative conditions:*** In order to study possible association between CKIδ and the glutamate transporter EAAT3, HEK-293 cells were co-transfected with the cDNAs encoding for both CKIδ and EAAT3. No association between the two molecules was observed by Immunoprecipitation experiments.

***Mechanisms of osteoblastic cell survival and apoptosis in conditions of inflammation:*** We investigated the effect of TNF-$\alpha$ in apoptosis of an osteosarcoma cell line MG63. Cells were found to be resistant to TNF-$\alpha$-induced apoptosis via the activation of cell survival kinase Akt/PKB pathway. Treatment of MG63 cells with TNF-$\alpha$ resulted in the increase of TIMP-1, a molecule recently shown to have antiapoptotic properties. Co-treatment of MG63 cells with TNF-$\alpha$ and CHX facilitated cell death, as it was determined morphologically with nuclei DAPI staining and activation of caspase-3 and its substrate PARP, whereas the expression levels of TIMP-1, $\beta_1$ and $\alpha_v\beta_3$ integrins were reduced. Moreover, silencing TIMP-1 expression by siRNA treatment with TNF-$\alpha$ resulted in cell apoptosis.

**2007 Publications**


Moutzouris, D., Kitsiou, P., Talamagas, A., Drossopoulou, G., Kassimatis, Patrakos (2007). Chronic exposure of human glomerular epithelial cells to high glucose concentration results in modulation of high-affinity glucose transporters expression. *Renal Failure* Volume 29, Issue 3, 353-358

AA. Talamagas, S Efthimiopoulos, EC. Tsilibary, ME. Figueiredo-Pereira and AK. Tzinia, (2007) Abeta(1–40)-induced secretion of matrix metalloproteinase-9 results in sAPPα release by association with cell surface APP. *Neurobiology of Disease* **28** 304–315

Chroni, A.*, Koukos, G., Duka, A. and Zannis, V.I. (2007) The carboxy-terminal region of apoA-I is required for the ABCA1-dependent formation of α-HDl but not preβ-HDL particles in vivo. *Biochemistry*, 46, 5697-5708. *: corresponding author

Koukos, G, Chroni, A., Duka, A., Kardassis, D. and Zannis, V.I. (2007) Naturally occurring and bioengineered apoA-I mutations that inhibit the conversion of discoidal to spherical HDL: the abnormal HDL phenotypes can be corrected with treatment with LCAT. Biochem. J., 406, 167-174.

Koukos, G, Chroni, A., Duka, A., Kardassis, D. and Zannis, V.I. (2007) LCAT can rescue the abnormal phenotype produced by the natural apoA-I mutations $(Leu141Arg)_{Pisa}$ and $(Leu159Arg)_{FIN}$. *Biochemistry*, 10713-10721.

I. Rabias, H. Pratsinis, G. Drossopoulou, M. Fardis, N. Boukos, N. Tsotakos, D. Kletsas, E. Tsilibary and G. Papavassiliou (2007)[#] In vitro studies on ultra small superparamagnetic iron oxide nanoparticles coated with gummic acid for MRI contrast agent *Biomicrofluids** 1,(044101. 1-12 (* new journal)

[#] Selected for the December 1, 2007 issue of the Virtual Journal of Biological Physics Research, published by the American Physical Society (APS) and the American Institute of Physics ( http://www.vjbio.org )

**2007 Presentations at International Scientific Conferences**

G. Drossopoulou, N. Tsotakos, E. Kotsopoulou, E.C. Tsilibary: Chronis exposure of human glomerular epithelia cells to high glucose permamnent;y suppresses PCLP expression: Inhibition of AGE formation partially restores PCLP expression. 19 Meeting of the European Renal Cell Study Group, March 22-25, Abbaye des Vaulx de Cernay, France, 2007

G. Drossopoulou, N. Tsotakos, E. Kotsopoulou, E.C. Tsilibary. "Transcriptional regulation of podocalyxin in cultured immortalized human glomerular epithelial cells". XLIV European Renal Association-European Dialysis and Transplant Association (ERA-EDTA), June 21-24 Barcelona, Spain 2007

Koukos G, Chroni A, Duka A, Kardassis D, Zannis VI**.** (2007) Lecithin:Cholesterol Acyl Transferase Can Rescue the Abnormal Phenotype Produced by the Natural Apolipoprotein A-I Mutations (Leu141Arg)Pisa and (Leu159Arg)FIN. 8th Annual Conference on *Arteriosclerosis, Thrombosis and Vascular Biology*, April 19-21, 2007, Chicago, IL, USA.

**Impact Factors** : E. Tsilibary /A. Tzinia, P. Kitsiou (for 4 publications): 6,275
A. Chroni (for 3 publications): 11,8. Total : 18,075

**Citations 2007** (without self- citations): 136
(Tsilibary EC, Tsilibary E, Tsilibary PC): 59
(Tzinia A, Tzinia AK): 9
(Kitsiou P): 7
(A. Chroni): 60

**Total Citations 2005-2007** (without self- citations): 393
(Tsilibary, EC. Tsilibary E, Tsilibary PC): 201
(Tzinia A, Tzinia AK): 36
(Kitsiou P): 26
(A. Chroni): 130

**h-factor:** E. Tsilibary:29 , A. Tzinia:7, P. Kitsiou:4, A. Chroni:8

## Research Group: Environmental Mutagenesis -Carcinogenesis

**Research Staff**

**Gerassimos Voutsinas, Researcher**
Vassilis Nikas, Graduate Student (until 6/2007)
Panagiotis Karkoulis, Graduate Student
Dimitra Anastasiou, Collaborating Graduate Student
Stefanos Kachrilas, Collaborating Graduate Student
Antonis Lampidonis, Collaborating Graduate Student
Eleni Litsiou, Collaborating Graduate Student
Evmorphia Konstantatou, Collaborating Graduate Student
Sofia Melachrinou, Collaborating Graduate Student (*MSc*)
Katerina Vestaki, Collaborating Graduate Student (*MSc*)
Sokratis Avgeris, Research Technician

**Research Interests**

1. Identification and validation of drug targets for cancer therapy
2. Development and evaluation of biomarkers for diagnosis and prognosis of human diseases
3. Development of protocols for molecular diagnosis of human genetic diseases

**2007 Findings**

**Targeting $BRAF_{V600E}$ in thyroid carcinoma: therapeutic implications**

B-Raf is an important mediator of cell proliferation and survival signals transduced via the Ras-Raf-MEK-ERK cascade. BRAF mutations have been detected in several tumors, including papillary thyroid carcinoma, but the precise role of B-Raf as a therapeutic target for thyroid carcinoma is still under investigation. We analyzed a panel of 93 specimens and 14 thyroid carcinoma cell lines for the presence of BRAF mutations and activation of the mitogen-activated protein/ERK kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway. We also compared the effect of a B-Raf small inhibitory RNA construct and the B-Raf kinase inhibitor AAL881 on both B-Raf wild-type and mutant thyroid carcinoma cell lines. We found a high prevalence of the T1799A (V600E) mutation in papillary and anaplastic carcinoma specimens and cell lines. There was no difference in patient age, B-Raf expression, Ki67 immunostaining, or clinical stage at presentation between wild-type and BRAFV600E specimens. Immunodetection of phosphorylated and total forms of MEK and ERK revealed no difference in their phosphorylation between wild-type and BRAFV600E patient specimens or cell lines. Furthermore, a small inhibitory RNA construct targeting the expression of both wild-type B-Raf and B-RafV600E induced a comparable reduction of viability in both wild-type and BRAFV600E mutant cancer cells. Interestingly, AAL881 inhibited MEK and ERK phosphorylation and induced apoptosis preferentially in BRAFV600E-harboring cells than wild-type ones, possibly because of better inhibitory activity against B-RafV600E. We conclude that B-Raf is important for the pathophysiology of thyroid carcinomas irrespective of mutational status. Small molecule inhibitors that selectively target B-RafV600E may provide clinical benefit for patients with thyroid cancer. [Mol Cancer Ther 2007;6(3):1070–8]

**Glutathione-S-transferase T1 and M1 gene polymorphisms in Greek patients with multiple sclerosis: a pilot study**

Oxidative stress has been implicated in the pathogenesis of multiple sclerosis (MS). Glutathione-S-transferases (GSTs) are detoxification enzymes, evolved to protect cells against reactive oxygen metabolites. Both GSTT1 and GSTM1 genes exhibit a homozygous deletion polymorphism (null genotype) leading to abolished enzyme activity. We studied the impact of the GSTT1 and GSTM1 polymorphisms on MS susceptibility in a case–control study of 47 Greek patients and 165 controls. Correlations between genotype, gender and disability status were also investigated. The incidence of both GSTT1 and GSTM1 genotypes did not differ significantly between controls and patients. A

significantly increased frequency of GSTM1 null genotype was found amongst female patients (65.5%) as compared with males (33.3%, P Ό 0.04). The results suggest that GSTT1 and GSTM1 have no major pathogenetic role on the MS occurrence, nor any strong modifying effect on the disability status. The higher incidence of GSTM1 null genotype observed in female patients, suggests a possible role of the GSTM1 detoxification pathway in a gender-dependent manner.

**Drug-Mediated Targeted Disruption of Multiple Protein Activities Through Functional Inhibition of the Hsp90 Chaperone Complex**

Hsp90 is an evolutionarily conserved and ubiquitously expressed molecular chaperone that mainly modulates, along with a group of co-chaperones, the general platform of protein folding and prevents the nonspecific aggregation of misfolded or unfolded proteins. In this review, after a description of the above phenomena and a report on the numerous clients of Hsp90, among which a variety of important regulatory proteins of the cell is included, we mainly dealt with the involvement of Hsp90 in the carcinogenic process and its molecular targeting in human cancer therapy. We discuss all up to date categories of Hsp90 inhibitors and we provide data for the ongoing relative clinical trials.

**2007 Publications**

Mitsiades, C.S., Negri, J., McMullan, C., McMillin, D.W., Sozopoulos, E., Fanourakis, G., Voutsinas, G., Tseleni-Balafouta, S., Poulaki, V., Batt, D., Mitsiades, N. (2007). Targeting BRAFV600E in thyroid carcinoma: therapeutic implications. Mol. Cancer Ther. 6, 1070-1078.

Stavropoulou, C., Korakaki, D., Rigana, H., Voutsinas, G., Polyzoi, M., Georgakakos, V., Manola, K., Karageorgiou, C. and Sambani, C. (2007). Glutathione S-transferase T1 and M1 gene polymorphisms in Greek patients with multiple sclerosis: a pilot study. Eur. J. of Neurol. 14, 572-574.

Stravopodis, D.J., Margaritis, L.H. and Voutsinas, G.E. (2007). Drug-mediated targeted disruption of multiple protein activities through functional inhibition of the hsp90 chaperone complex. Curr. Med. Chem. 14, 3122-3138.

**Impact Factors** (for 3 publications): 12,622

**Citations 2007** (without self- citations): 40

**Total Citations 2005-2007** (without self- citations): 76

**h-factor**: 6

# *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 Lampropoulou, Researcher**
Lydia Ignatiadou, Emeritus Scientist
Rodica Efrose, Postodoctoral Fellow
Georgia Kotzia , Postdoctoral Fellow
Theodoros Georgomanolis, Graduate Student
Konstantinos Ioannides, Graduate Student
Christiana Magrioti, Graduate Student
Dimitra Stefanou, Technical Specialist
Dimitrios Kopanelis, Research Technician

### Research Interests

**1. Regulatory mechanisms controlling insect physiological functions:** (a) Oogenesis in lepidopteran insects: a model for long-term differentiation programs induced by ecdysteroid hormones (b) Mechanisms of immunosuppression in lepidopteran insects following parasitization by hymenopteran endoparasitoids: the role of the interactions between proteins produced by hymenopteran endosymbiotic polydna viruses and hemocyte proteins of the lepidopteran hosts (c) Mechanisms controlling olfactory function in the malaria mosquito vector *Anopheles gambiae*.

**2. Molecular Biology and genetic manipulation of insect nuclear polyhedrosis viruses:** (a) Viruses expressing proteins harmful to the insect hosts (b) Incapacitated viruses as vectors for insect genetic transformation and continuous high-level heterologous protein production (c) Modified viruses as vectors for human gene therapy applications.

**3. Functional genomics:** (a) Systems for production of proteins of economic importance in lepidopteran insect cell lines (b) High throughput screening systems for bioactive substances (activators and inhibitors of pharmacological targets) in chemical libraries and collections of natural products (plants and microorganisms).

**2007 Findings**

**Regulatory control of oogenesis in lepidopteran insects.**
Expression analysis and functional characterization was carried out for the regulatory factor BmSH3, a putative modulator of the function of the nuclear receptor BmE75. After the detection of three protein isoforms, which are expressed in different compartments of ovarian follicles, 5'RACE (Rapid Amplification of cDNA ends) experiments were carried out to isolate new isoforms of BmSH3 that differ in their N-terminal regions.

**Molecular mechanisms of endoparasitoidism in lepidopteran insects**
We continued the study of the functions of proteins of the endosymbiotic virus CcBV of the endoparasitoid wasp Cotesia congregate, which is injected in the lepidopteran host Manduca sexta during its parasitism by Cotesia. Our studies were pursued in two directions. The first one concerned the interaction of the viral proteins with those of the host and the elucidation of the role of the interactions in the inhibition of the immune response that the host is capable of mounting. The second direction focussed on the function of a family of viral proteins, Vank, which contain extensive similarities with the ankyrin repeats of the IκBα inhibitor of the mammalian transcription factor NF-κB that is involved in the regulation of genes of the immune response.
During the previous year, our studies concentrated on the second part of this project. To investigate the role of Vank proteins in the NFκB/Rel signalling pathway, a series of experiments were carried out using mammalian cell lines that are induced with the factor TNFα. From these experiments it was evident that some members of the family of Vank proteins of CcBV act as inhibitors of the NFκB/Rel transcription factor. For other members of the Ank family, further tests are required. Because the action

of Ank proteins also needs to be tested in insect cells, studies of the functional properties of reporter plasmids for BmRelA/B and BmRelish were carried out and their involvement in the induction of antmicrobial peptide genes was investigated in insect cell lines such as Bm5 (*Bombyx mori*), HighFive (*Trichoplusia ni*), S2 (*Drosophila melanogaster*) and Sf21 (*Spodoptera frugiperda)*. Our results indicate that the transcription factor BmRelB is specifically induced by lipopolysaccharides in the Sf21 cell line, which was therefore selected as the most suitable insect cell line for the characterization of the function of Ank proteins.

**Regulatory control of mosquito olfaction**

We continued our efforts for the development of a high-throughput screening system for ligands of odorant receptors of the mosquito *Anopheles gambiae*, to which we referred in our previous annual report. The screening system is expected to lead to the identification of new environmentally friendly methods for a reduction on the size of *Anopheles* populations and the reduction in transmission frequency of the malaria parasite. In the framework of this effort we had already generated transformed insect cell lines that expressed recombinant mammalian or insect Gα proteins (Gα16, Gαq και Gαs) that are involved in the function of G protein coupled receptors (GPCRs). During the previous year we generated multiple 'second generation' lepidopteran cell lines that express in addition the mosquito odorant receptors OR1 and OR2 in the presence or absence of OR7, the heterodimerization partner of all mosquito odorant receptors.

Detailed characterization of transformed cell lines (documentation of expression of exogenous genes and assessment of functionality of receptors after addition of known ligands to the cell cultures) is in progress. Furthermore, based on the observation that only female individuals of *Anopheles* are carriers of the malaria parasite and the consequent understanding that the technological platform under development must be based on the employment of odorant receptors that are mainly expressed in the antennae (the major olfactory organs) of female mosquitoes, we completed a study that determined the differential expression of all 80 genes, which encode odorant receptors in the antennae of male and female mosquitoes. On the basis of these results we selected a small subset of odorant receptors, which will be used subsequently as targets for the development of high-throughput screening systems for ligand mimetics for odorant receptors specifically expressed in female *A. gambiae* mosquitoes.

**Molecular biology and genetic engineering of insect nuclear polyhedrosis viruses**

After the encouraging results obtained by the collaborating group of Dr. R. Matsas (Hellenic Pasteur Institute) in *in vitro* neural trauma assays (scratch assays) using primary Schwann cells that were genetically transduced with recombinant BmNPV baculovirus of the silkworm that express the candidate therapeutic gene L1, further functional tests were conducted. Thus, recombinant BmNPV-transduced Schwann cells were 'transplanted' to organotypic cultures of brain tissue from rats. The results showed that Schwann cells transduced with recombinant baculovirus expressing the L1 protein show increased migration activity in brain tissue compared with cells that are transduced with baculovirus expressing green fluorescent protein (GFP).

Furthermore, we constructed transfer vectors that allow the generation of BmNPV baculoviruses containing (1) the gene of the PiggyBac transposase and (2) PiggyBac recognition sequences at the ends of cloned genes that need to be over-expressed. It is expected that baculoviruses equipped with this transposition system will direct the incorporation of the expression cassette that is flanked by the transposase recognition sequences into the chromosomes of the transduced cells with concomitant destruction of the baculovirus vector, leading to permanent transgene expression in infected cells.

Finally, we generated a series of stably transformed lepidopteran cell lines that express constitutively the baculoviral transcription factor IE-1. The cell lines will be used for the production of IE-1 knockout baculoviruses created by the laboratory of Dr. D. Theilmann (Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada), which are unable multiply in infected wild-type lepidopteran cells. The use of defective baculoviruses for mammalian gene transfer in gene therapy protocols is expected to have significant advantages with respect to the use of baculovirus transfer vectors with functional *ie-1* gene, because the defective baculoviruses are expected to be less likely to trigger cellular immune responses in the transduced cells.

**Functional genomics**

*Drosophila*-derived S2 cells were used to screen a dibenzoyl hydrazine library for the presence of ecdysone activity (Greece-Japan collaboration program). When compared with earlier screening results, much lower activity of dibenzoyl hydrazines was observed in dipteran S2 relative to lepidopteran Bm5 cells. The data obtained are used to construct a quantitative structure activity (QSAR) model for the ecdysone activity specific to dipteran insects.
In addition, a new cell-based screening system was established for ecdysone activity that is specific to the lepidopteran insect pest *Spodoptera littoralis* (Greece-Spain collaboration program). Using this system, the activity of potential ligands from collections of dibenzoylhydrazine, diaminoacylketone and tetrahydroquinoline compounds was measured and compared with the Bm5-based screening system that is specific to the lepidopteran beneficial insect *B. mori*.

**2007 Publications**

Machado, E., Swevers, L., Sdralia, N., Medeiros, M.N., Fernando G. Mello, F.G. and Iatrou, K. (2007). Prostaglandin signaling and ovarian follicle development in the silkmoth, *Bombyx mori*. Insect Biochem. Mol. Biol. 37, 876-885.

Iatrou, K. and Biessmann, H. (2007). Sex-biased expression of odorant receptors in antennae and palps of the African malaria vector Anopheles gambiae. Insect Biochem. Mol. Biol. doi:10.1016/j.ibmb.2007.11.008. [Epub ahead of print Nov 24, 2007]

Soin, T., Swevers, L., Mosallanejad, H., Efrose, R., Labropoulou, V., Iatrou, K., Smagghe, G. (2007). Juvenile hormone analogs do not affect directly the activity of the ecdysteroid receptor complex in insect culture cell lines. J. Insect Phys. doi:10.1016/j.jinsphys.2007.11.001. [Epub ahead of print Nov 24, 2007]

Ignatiades, L., Gotsis-Skretas, O., Metaxatos, A., (2007). Field and culture studies on the ecophysiology of the toxic dinoflagellate Alexandrium minutum (Halim) present in Greek coastal waters, Harmful Algae 6, 153-165

**2007 Presentations at International Scientific Conferences**

Iatrou, K., Andronopoulou, E., Labropoulou, V., Swevers, L., Georgoussi, Z., Woods, D., Dimitratos, S., Walter, M. and Biessmann, H. Anopheline mosquito olfaction and malaria control: OBPs, ORs and in vitro assays for ligand identification. Third International Meeting on "Molecular and Population Biology of Mosquitoes and Other Disease Vectors", 13 - 20 July 2007, Kolymbari, Crete, Greece.

Smagghe, G., Soin, T., Mosallanejad, H., Decombel, L., Van de Velde, S., Vandenborre, G., Ryckaert, J., Van Damme, E., Goodman, C., Caputo, G., and Swevers, L. (2007). Insect cell lines as tools for developing rational insecticides. International Congress of Insect Biotechnology and Industry, Daegu, Republic of Korea, August 19-24, 2007. Entomological Research 37, Supplement 1, A57.

Swevers, L., Andronopoulou, E., Labropoulou, V., Douris, V., Tsikou, D., Efrose, R., Kotzia, G., Stefanou, D., Morou, E., Georgoussi, Z., and Iatrou, K. (2007). Genetically transformed insect cell lines as screening tools for improved insecticides and pharmaceutics. International Congress of Insect Biotechnology and Industry, Daegu, Republic of Korea, August 19-24, 2007. Entomological Research 37, Supplement 1, A86.

Soin, T., Swevers, L., Efrose, R., Labropoulou, V., Mosallanejad, H., Iatrou, K., and Smagghe, G. (2007). The ecdysteroid receptor complex in insect cell lines is not directly affected by juvenile hormone agonists. Vth International Conference on Arthropods: Chemical, Physiological and Environmental Aspects. Bialka Tatrzanska, Poland. September 16-21, 2007.

Labropoulou V, Douris V, Stefanou D, Magkrioti C, Andronopoulou E, Swevers L, Iatrou K. The interaction of the Cotesia congregata bracovirus CcV1 protein with Manduca sexta hemolin. J. Insect Sci. 7: 2007 Meeting Abstract

Gotsis-Skretas O. & Ignatiades L. 2007. Patterns of phytoplankton community structure and related environmental parameters in Greek coastal waters. (Oral presentation), International Congress for the Exploration of the Mediterranean Sea, 9-13 April, 2007, Istanbul, Turkey. Abstracts Vol. 38, p. 70.

**Impact Factors (for 4 publications):9,901**

**Citations 2007 (without self- citations): 144**

Iatrou K. (Swevers′ publications are included): 61
Swevers L.: 6
Lampropoulou V.: 34
Ignatiades L.: 43

**Total Citations 2005-2007** (without self- citations): 426
Iatrou K. (Swevers′ publications are included): 165
Swevers L.: 14
Lampropoulou V.: 107
Ignatiades L.: 140

**h-factor:**
22 (K. Iatrou)
11 (L. Swevers)
5 (V. Lampropoulou)
14 (L. Ignatiadou)

## Research Group: Transcriptional Regulation by the Biological Clock

**Research Staff**
**Anastassia Prombona, Senior Researcher**
Anastasia Repouskou, Graduate Student
Marianna Kapi, Undergraduate Student
Dafni Antoniou, Undergraduate Student
Irene Nomikou, Undergraduate Student
Aggeliki Galeou, Training Student
Sokrates Avgeris, Research Technician

**Research Interests**

- *Investigation of the biological function in plants*

Study of the biological clock function in *Phaseolus vulgaris*. Study of genes involved in the synchronization of the biological clock by input light signals and photoperiodism. Regulation of gene expression by the clock and the white light. Role of rhythmically expressed genes in the central oscillator function. Interaction of clock proteins.

- *Investigation of the biological clock influence on $H_2$ production in the alga Chlamydomonas reinhardtii*

Study of the biological clock function under $H_2$ producing conditions. Study of the role of photoperiodism and light wavelength on the $H_2$ production levels.

- *Investigation of the involvement of the biological clock function in carcinogenesis.*

Regulation of cell cycle and cell proliferation by components of the biological clock in mouse fibroblasts and cancer cell lines. Modulation of the histones' acetylation levels and study of its effects on the biological clock function and the cell cycle. Our goal is to study the effects of drugs that control the proliferation of cancer cells in dependence of the circadian time to achieve improved therapy (chronotherapy).

**2007 Findings**

***Investigation of the biological clock function in plants***

In synchronization experiments with 2-min white light pulses and photoperiods, the expression pattern of the genes *PvELF4*, *PvTOC1* and *PvLHY* were studied. *PvELF4* and *PvTOC1* were found to be down regulated by light, while both are under photoperiod evening expressed genes in *Phaseolus vulgaris*. New experiments will investigate the role of *PvELF4* and *PvTOC1* in the light induced expression of *PvLHY.*

***Investigation of the biological clock influence on $H_2$ production in the alga Chlamydomonas reinhardtii***

Initial experiments with *C. reinhardtii* revealed an influence of growing conditions for $H_2$ production on *Lhcbm1* expression levels. The exact influence of the biological clock on *Lhcbm1* expression levels will be studied by using different light sources and duration. This project is executed in collaboration with Dr. K. Stamatakis (Membrane biophysics and biotechnology group).

***Investigation of the involvement of the biological clock function in carcinogenesis***

Study of the expression levels of the cell cycle genes *wee1, c-myc, cyclinD1* in circadian clock synchronized N2A cells in culture, showed no changes after trichostatin A (TSA, an HDAC inhibitor) application at the circadian time 6. Contrary to that, TSA application at the circadian time 18, negatively affected the expression levels of all three genes *wee1, c-myc, cyclinD1.* Curcumin, on the other hand, an inhibitor of HAT p300, caused no significant change of the expression levels of the above mentioned cell cycle genes, independently of the time of application. This project is executed in collaboration with Dr. T. Sourlingas (Histone Biochemistry group).

**2007 Presentations at Inrernational Scientific Conferences**

A. Repouskou, K.E. Sekeri-Pataryas, T.G. Sourlingas, A. Prombona (2007) Changing the acetylation status at specific circadian times has differential effects on the expression levels of cell cycle genes in N2A cells. EMBO workshop 2007, Molecular mechanisms of cell cycle control in normal and malignant cells, October 5-8, Spetses, Greece

M. Xidous, K.E. Sekeri-Pataryas, A. Prombona T.G. Sourlingas, (2007) Histone acetylation and circadian clock gene regulation: effects on the expression of cell cycle genes. EMBO workshop 2007, Molecular mechanisms of cell cycle control in normal and malignant cells, October 5-8, Spetses, Greece

**Citations 2007** (without self- citations): 9

**Total Citations 2005-2007** (without self- citations): 27

**h-factor**: 5

## Research Group: Microbial Molecular Genetics

**Research Staff**
**Vassiliki Sophianopoulou, Researcher Director**
Eleftherios Sideris, Emeritus Scientist
Dimosthenis Kizis, Postdoctoral Fellow
Dimitra Bouzarelou, Graduate Student
Ioannis Vaggelatos, Graduate Student
Katerina Roumelioti, Collaborating Graduate Student
Anna Bombori, Collaborating Graduate Student (MSc)
Alexandros Pittis, Undergraduate Student
Marina Laspa, Undergraduate Student
Ioannis Tampakis, Training Student
Areti Tsolomiti-Gourgou, Research Technician

## Research Interests

- Research on the molecular mechanisms of recognition and transport of amino acids and nucleobases through cellular membranes via specific transmembrane transporters.
  Transporters of medical, pharmacological and agricultural importance: amino acid and nucleobase transporters
  a) identification and regulation of the expression of genes encoding amino acid and purine transporters b) studies on structure-function relationships of amino acid transporters c) identification of *trans*-acting molecular determinants involved in expression and activity of amino acid transport systems

- Basic research on mechanisms involved in cell wall expansion and phytopathogenicity in fungi
  Study of non-plant Expansin-like proteins:
  a) Identification and regulation of the expression of genes encoding expansin-like protein(s) in *Aspergillus nidulans* b) physiological / functional characterization and cellular localization of the encoded proteins

- Functional genomics: Use of *Aspergillus nidulans* as a novel microbial model system for functional expression and biochemical characterization of members of the Nucleobase Ascorbate Transporter (NAT) family from higher organisms.

- Molecular mechanisms of intracellular homeostasis of $Na^+$ and $H^+$ ions in microbial cells. Identification and study of $Na^+/H^+$ antiporters in cyanobacterial cells.

**2007 Findings**

**In the context of the project: "Research on the molecular mechanisms of recognition and transport of amino acids and nucleobases through cellular membranes via specific transmembrane transporter: Transporters of medical, pharmacological and agricultural importance"**

**a)** We identified $\alpha$ gene encoding the specific di-carboxylic amino acid transporter AgtA of *Aspergillus nidulans*, we carried out a preliminary kinetic characterisation of this transporter and we studied its transcriptional and post-translational regulation in response to the nitrogen status of the cell. The identification and characterisation of this transporter provides a tool to study amino acid transporter topogenesis and the interplay of the different regulatory mechanisms involved in nitrogen source utilisation. Moreover, it provides a new target protein to analyse novel signalling processes.

**b)** We examined the functional replacement of the three native Cys residues (Cys54, Cys352 and Cys530) of the major proline transporter PrnB in *Aspergillus nidulans* and the properties of an engineered Cys-less PrnB protein, as background for employing a Cys-scanning mutagenesis approach. We show that simultaneous replacement of Cys54 with Ala, Cys352 with Ala and Cys530 with Ser results in a functional Cys-less PrnB transporter. We also introduce the use of a biotin-

acceptor domain tag to quantitate protein levels of engineered PrnB mutants by Western blot analysis. Finally, we use the background of the Cys-less PrnB transporter to evaluate the functional importance of amino acids Q219, K245 and F248 of PrnB in the mechanism of PrnB-mediated proline uptake.

**c)** We presented data showing that a single copy gene encoding a putative thio-redoxin complements the mutant phenotype of an *aau* (**a**mino **a**cid **u**ptake) strain in the transport of many amino acids used by the lower eukaryote *Asprgillus nidulans* as sole nitrogen sources. Furthermore, we presented data showing that in this mutant strain chimeric AgtA-GFP molecules do not go to the plasma membrane as in an isogenic wild-type strain, implying that the product of the cloned gene is involved in appropriate folding of the AgtA transporter possibly by catalysing disulfide bond formation.

**In the context of the project: "Basic research on mechanisms involved in cell wall expansion and phytopathogenicity in fungi Study of non-plant Expansin-like proteins"**

We identified and characterized an expansin-like gene (*eglD*) in *A. nidulans*, the product of which shows strong similarity with bacterial and fungal endo-β1,4-glucanases. The *eglD* gene is constitutively expressed in all developmental stages and compartments of *A. nidulans* asexual life and its expression is regulated at a post-transcriptional level. The temporal expression pattern and cellular localization of the EglD protein and its role in morphogenesis, growth rate and germination rate of conidia were investigated. Our results showed that EglD is a protein with putative endoglucanase activity involved in conidial cell wall integrity.

**In the context of the co-operative project: "Molecular mechanisms of intracellular homeostasis of $Na^+$ and $H^+$ ions in microbial cells. Identification and study of $Na^+/H^+$ antiporters in cyanobacterial cells"**

*In silico* analyses of the genome sequence of *Synechococcus* sp. PCC 7942 cyanobacterium revealed the presence of seven genes (*synnhA1-7*) encoding putative $Na^+/H^+$ antiporters. All seven genes were functionally characterized by their heterologous expression in *E. coli*. Five of them (*synnhA1*, *synnhA2*, *synhA3*, *synnhA4* and *synnhA7*) were inactivated in order to examine participation of the encoding $Na^+/H^+$ antiporters in salt and pH homeostasis of the freshwater *Synechococcus* sp. PCC 7942 cells. Our results showed differential responses of the corresponding mutant strains to distinct environmental NaCl concentrations and pHs. Additionally, the expression of *sycnhA* genes was monitored in terms of acute response, acclimation and functional redundancy under salt and alkaline stress conditions using real-time RT-PCR. To our knowledge, this is the first report of $Na^+/H^+$ antiporters in a freshwater cyanobacterium, providing new insights in understanding the presence and function of specific homologues among cyanobacteria in respect to their habitat preferences.

**2007 Publications**

P. Kafasla, D. Bouzarelou, S. Frillingos and V. Sophianopoulou, 2007. The proline permease of *Aspergillus nidulans*: functional replacement of the native cysteine residues and properties of a Cysteine-less-transporter. Fungal Genet. Biol. 44 (7): 615-26

N. Mourtzis, K. Eliadou, C. Aggelidou, V. Sophianopoulou, I. M. Mavridis and K. Yannakopoulou, 2007. Per (6-guanidino-6-deoxy) cyclodextrins: Synthesis, characterisation and binding behaviour toward selected small molecules and DNA. Org. Biomol. Chem. 5 (1): 125-131

Amillis S Hamari Z, Roumelioti K, Scazzocchio C, and Diallinas G, 2007. Regulation of expression and kinetic modeling of substrate interactions of a uracil transporter in *Aspergillus nidulans.* Mol. Memb. Biol.24(3): 206-14.

**2007 Presentations at International Scientific Conferences**

M. Billini, K. Stamatakis and V. Sophianopoulou, 2007. A network of $Na^+/H^+$ antiporters in the freshwater cyanobacterium *Synechococcus sp*. PCC 7942. Proceedings of the 32nd Febs Congress on Molecular Machines (Vienna). Febs Journal Volume 274, Supplement 1, BS4-37, page 131

**Impact Factors** (for 3 publications): 9,5

**Citations 2007** (without self- citations): 24
**Total Citations 2005-2007** (without self- citations): 75

**h-factor**: 10

## Research Group: Biophysics and Biotechnology of Membranes

**Research Staff**
**Kostas Stamatakis, Senior Researcher**
George Papageorgiou, Emeritus Scientist
Meropi Tsimilli – Michael, Collaboratig Scientist
Maria Billini, Graduate Student
Tilemachos Voreas, Undergraduate Student
Eleni Zagari, Training Student

**Research Interests**

Membrane and cytosolic defense mechanisms mobilized by photosynthetic organisms when provoked by water deficit and salinity. Permeability of plasma membranes to water, ions, and neutral molecules. Critical role of turgor for adaptation to salinity and cell division. Studies on $N\alpha^+/H^+$ antiporters. Studies on Chlorophyll fluorescence induction curves. Studies on the photosynthetic Hydrogen production.

**2007 Findings**

***A. Study of sodium proton antiporters in the cyanobacterium Synechococcus sp. PCC 7942***

We conducted gene inactivation of the five (*synnhA1*, *synnhA2*, *synhA3*, *synnhA4* and *synnhA7*) out of seven genes with gene disruption via a kanamycin resistance cartridge. Mutant strains derived from the above procedure were further subjected in growth rate measurements under various growth conditions in order to be phenotypically characterized. Our results showed that Δ*synnhA2* strain is sensitive in growing under low concentrations of $Na^+$ especially in increased pH values (9.0). Moreover, Δ*synnhA7* strain cannot grow in media with low pH values (6.2) when high concentrations of NaCl are present.

***B. Study of Fluorescence induction curves in higher plants and cyanobacteria***

We have investigated the slow chlorophyll (Chl) a fluorescence induction (FI) transient of cyanobacterium Synechococcus sp PCC 7942. At room temperature, the transient comprises rise (S → M) and decay (M → T) phases in the s-to-min time window and is a sequel to the fast transient. In cyanobacteria, light-harvesting phycobilisomes (PBS) donate electronic excitation to Chl holochromes of photosystems (PS) II and I in a regulated manner. Here, we examined the phenomenology of the SMT transient at conditions at which the regulation of the PBS → PS I excitation transfer was eliminated. When the PBS → PS I electronic excitation transfer is not regulated, the FI pattern of Synechococcus becomes plant-like.

***C. Study of photosynthetic Hydrogen Production***

$C_1$. Preliminary studies shows that the transcription levels of *Lhcbm1* are affected by the growth conditions for the production of hydrogen. (In collaboration with Dr. A. Prombona)

$C_2$. We study the photochemical efficiency (Fv/Fm) of photosystem II (PS II) during the transition from photosynthetic $O_2$ to photosynthetic $H_2$ evolution. Isolation and characterization of thylakoid membranes.

**2007 Publications**

Stamatakis, K., Tsimilli-Michael, M., Papageorgiou, G. C. (2007). Fluorescence induction in the phycobilisome-containing cyanobacterium *Synechococcus* sp PCC 7942: II.-Analysis of the slow fluorescence transient. Biochim. Biophys. Acta (Bioenergetics) 1767, 766–772

Papageorgiou, G. C., Tsimilli-Michael, M., Stamatakis K, (2007). The fast and slow kinetics of chlorophyll *a* fluorescence induction in plants, algae and cyanobacteria: a viewpoint. Photosynth Res 94, 275–290.

**2007 Presentations at International Scientific Conferences**

Stamatakis, K., Tsimilli-Michael, M., Papageorgiou, G. C. (2007). Chlorophyll a fluorescence induction in the cyanobacterium Synechococcus sp PCC 7942: Analysis of the slow induction phase. State Transitions, 28-32 July, 2008 – Queen Mary U of London; JF Allen & CW Mullineaux, organizers)

Tsimilli-Michael M., Papageorgiou, G. C. Stamatakis, K. (2007). Investigation of the Kautsky curve OJIPSM(T) in Synechococcus sp PCC 7942 ($10^{-5}$-$10^{3}$ s) and a method to determine the (Chl a)PSII fluorescence component. State Transitions, 28-32 July, 2008 – Queen Mary U of London; JF Allen & CW Mullineaux, organizers)

**Impact Factors** (for 2 publications): 36,495

**Citations 2007** (without self- citations): 5

**Total Citations 2005-2007** (without self- citations): 12

**h-factor**: 6

## Research Group: Pending

**Research Staff**
**Maria Konstantopoulou, Researcher**
Vassilios Mazomenos, Emeritus Scientist
Kelly Martinou, Postdoctoral Fellow
Anatsassia Pantazi – Mazomenou, Research Technician

**Research Interests**

- Chemical ecology: isolation and identification of biologically active substances, relating to the chemical communication of insects and to plant – insect interaction (pheromones, volatile compounds of plant origin etc.) that may be used in integrated pest management programs.
- 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 of semiochemicals.
- Endosymbiotic bacteria of insects: Isolation and studies on their mutualistic relations with the 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.

**2007 Findings**

The toxicity studies of the secondary metabolites produced by the entomotoxic strain (SMU-21) of *Mucor hiemalis* were continued. The specific strain has been isolated from naturally occurring insect populations and is being evaluated against dipteran insects of high economic importance. Toxicity levels of the secondary metabolites, mode of action and the most efficient isolation techniques were evaluated. The chemical characterization of the toxins is under way using up-to-date organology (HPLC, NMR, FAB-MS, FTIR).

Research for semiochemicals with kairomonal action on parasitoids of the genus *Trichogramma* was continued, under the scope that they will aid substantially in the effectiveness of utilizing such egg parasitoids in integrated pest management programs. Under this scope the occurrence of plant originating semiochemicals influencing the parasitoids' behavior has been investigated. Furthermore the "learning" ability of the parasitoids when exposed to certain semiochemicals as well as how this specific property may be utilized to enhance their parasitization under natural conditions has been studied. To this approach different concentrations of its host, the olive moth *Prays oleae* pheromone have been tested along with varying polarity body extracts of both larvae and adults.Volatiles naturally occurring in olive leaves, flowers and fruits were also tested.

The approach of ways dealing with the pine dying-out phenomenon was continued. The aim is to control *Marchalina hellenica* populations in the framework of a pest management program that will respect both environment and the ecosystem. Consequently, factors that were studied are: a) *M. hellenica*'s bio-ecology (life cycle, reproductive potential, phenology), b) detection of natural enemies (parasitoids, predators) that influence its population fluctuation.

**2007 Publications**

E.M. Hegazi, M. Konstantopoulou, P. Milonas, A. Herz, W.E. Khafagi, A. Zaitun, S. Mostafa, I. Helal and S. El-Kemny, B.E. Mazomenos, 2007. Mating disruption of the jasmine moth *Palpita unionalis* (Lepidoptera: Pyralidae) using the two pheromone components blend: A case study over three consecutive olive growing seasons in Egypt. *Crop Protection* 26(6): 837-844.

E.P. Siskos, M.A. Konstantopoulou, B.E. Mazomenos and M. Jervis, 2007. Insecticidal activity of *Citrus aurantium* fruit, leaf and shoot extracts against adult olive fruit flies (Diptera: Tephritidae). *Journal of Economic Entomology* 100(4): 1215-1220.

C.G. Athanassiou, N.G. Kavallieratos, S.F. Gakis, L.A. Kyrtsa, B. Mazomenos and F.T. Gravanis, 2007. Influence of trap type, trap colour, and trapping location on the capture of the pine moth *Thaumetopoea pityocampa*. *Entomologia Experimentalis et Applicata* 122(2): 117-123.

A.P. Mihou, A. Michaelakis, F.D. Krokos, B.E., Mazomenos, B.E. and E.A. Couladouros, 2007. Prolonged slow release of (Z)-11-hexadecenyl acetate employing polyurea microcapsules. *Journal of Applied Entomology* 131(2): 128-133.

**Impact Factors** (for 4 publications): 4,706

**Citations 2007** (without self- citations): 48
M. Konstantopoulou: 16
V. Mazomenos: 32

**Total Citations 2005-2007** (without self- citations): 102

**h-factor**:
M. Konstantopoulou: 5
V. Mazomenos: 12

# *PROGRAMME C:*

# *STRUCTURAL AND COMPUTATIONAL BIOLOGY*

## Research Group: Theoritical Biology and Computational Genomics

**Research Staff**
**Yannis Almirantis, Research Director**
Spyros Papageorgiou, Emeritus Scientist
Alexandros Klimopoulos, Graduate Research Associate

**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 (tenths of nucleotides), 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 other than Zipf's laws in the genome: redundancy – multiple coding – asymmetry etc.
- "Conservation laws" at the genome structure. The case of "Chargaff's 2$^{nd}$ 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.

**2007 Findings**

Spatial distribution and clustering of repetitive elements are extensively studied during the last years, as well as their co-localization with other genomic components. We have investigated the large-scale features of Alu and LINE1 spatial arrangement in the human genome, by studying the size distribution of inter-repeat distances. In most cases, we have found power law size distributions, extending in several orders of magnitude. We have also studied the correlations of the extent of the power law (linear region in double logarithmic scale) and of the corresponding exponent (slope) with other genomic properties. A model has been formulated to explain the formation of the observed power laws. According to the model, two kinds of events occur repetitively in evolutionary time: random insertion of several types of intruding sequences and occasional loss of repeats belonging to the initial population due to "elimination" events. This simple mechanism is shown to reproduce the observed power law size distributions and is compatible with our present knowledge on the dynamics of repeat proliferation in the genome.

**2007 Publications**

Sellis, D., Provata, A. & Almirantis, Y. Alu and LINE1 distributions in the human chromosomes. Evidence of a global genomic organization expressed in the form of power laws. *Molecular Biology and Evolution* (2007) 24, 2385-2399.

**Impact Factors** (for 1 publication): 6,73

**Citations 2007** (without self- citations): 20

**Total Citations 2005-2007** (without self- citations): 52

**h-factor:** 9

## Research Group: NMR Studies of Biomolecules and Parmaceuticals

**Research Staff**
**Maria Pelekanou, Researche Director**
**Marina Sagnou, Lecturer**
Dimitra Benaki, Collaborating Scientist
Aggeliki Panagiotopoulou, Technical Specialist
Stamatia Tzanopoulou, Graduate Student

**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
- Labelled compounds (e.g. complexes of technetium and rhenium) designed as potential radiopharmaceuticals

The areas of application of our work is mainly Alzheimer's disease and cancer, but also other diseases of the central nervous system, bacterial infections, etc.
We use NMR spectroscopy and circular polarimetry (CD) for the structural studies in combination with methodologies of organic synthesis, radiolabelling, and in vitro and in vivo biological assessment.

**2007 Findings**

In the area of NMR structural investigations of peptide/proteins of biological interest, the study of colivelin (CL), a potent derivative of the neuroprotective peptide humanin (HN), was accomplished by circular dichroism and NMR spectroscopy. Compared to the parent peptide, CL is more prone to self-association through the formation of $\beta$-sheet structure, a finding that is in accordance with literature data on the mode of action of the HN family of peptides. The *in vitro* and *in vivo* evaluation of the iodinated form of CL ($^{124}$I-CL) in cell lines, tissue homogenates, and experimental animals is in progress. Furthermore, the interactions of the amyloid peptide $\beta$-AP(1-28) with a variety of small molecules was investigated with NMR. The study revealed that the hormone melatonin, the natural antioxidant oleuropein, and the histological dye thioflavin T have the ability to interact with the soluble form of $\beta$-AP(1-28) and deserve further investigation as potential inhibitors of its aggregation (submitted to *Biopolymers*).

In the field of development of rhenium and technetium radiopharmaceuticals, the design and synthesis of a complex of the tricarbonyl $Re(CO)_3^+$ core with a derivative of thioflavin T was accomplished. This complex exhibits marked selectivity *in vitro* for the amyloid plaques of Alzheimer's disease, while biodistribution studies of its $^{99m}$Tc analogue in mice show an adequate crossing of 0.2% of the blood-brain barrier. Expanding our activities to PET imaging of amyloid plaques, the design of a derivative of thioflavin T suitable for labeling with PET isotopes was completed and initial synthetic steps towards its synthesis were undertaken. Furthermore, aiming at the development of tumor-targeted radiodiagnostic agents the uptake of complexes of the tricarbonyl metal core $M(CO)_3^+$ (M = Re, $^{99m}$Tc) with derivatives of the anticancer agent 2-(4-aminophenyl)benzothiazole is currently being evaluated in breast and ovary cancer cell lines.

In 2007 the chemistry of the coordination sphere of the tricarbonyl core $M(CO)_3^+$ (M= Re, $^{99m}$Tc) was further investigated through the synthesis of a new type of $M(CO)_3$(NNO) complex suitable for the efficient attachment of pharmacophore groups through the formation of amide bonds (*Inorg. Chem.* 2008, in press). Other studies with the $M(CO)_3^+$ core in progress include synthesis, characterization, and

preliminary evaluation of complexes for the distinction of inflammation from infection, imaging of the myocardium and imaging of hypoxic tissue (*Eur. J. Med. Chem*. 2008, in press).

**2007 Publications**

D. Papagiannopoulou, I. C. Pirmettis, M. Pelecanou, D. Komiotis, M. Sagnou, D. Benaki, C. P. Raptopoulou, A. Terzis, M. Papadopoulos (2007). Synthesis and structural characterization of neutral "3 + 2" oxorhenium and oxotechnetium complexes of the 2-mercaptoethyl-N-glycine (SNO)/2,2′-bipyridine (NN) mixed ligand system. Inorg. Chim. Acta 360, 3597-3602

Stamopoulos, D., Benaki, D., Bouziotis, P., Zirogiannis, P.N. (2007). In vitro utilization of ferromagnetic nanoparticles in hemodialysis therapy. Nanotechnology 18 495102

**2007 Presentations at International Scientific Conferences**

A. Papadopoulos, C. Tsoukalas, A. Panagiotopoulou, M. Pelecanou, M. Papadopoulos, I. Pirmettis (2007). Development of $^{99m}$ Tc-Labelled biomarkers for heart metabolism using the technetium tricarbonyl core. labelling of small biomolecules using novel technetium-99m cores. Technical Report Series No. 459 IAEA, 2007, Vienna, Austria.

N. Margaritis, N. Bourkoula, M. Paravatou, E. Livaniou, A. Papadopoulos, A. Panagiotopoulou, C. Tsoukalas, M. Pelecanou, M. Papadopoulos, I. Pirmettis (2007). Development of $^{99m}$ Tc-Labelled Biomarkers for EGFR-TK Using the Technetium Tricarbonyl Core. Labelling of Small Biomolecules Using Novel Technetium-99m Cores. Technical Report Series No. 459 IAEA, 2007, Vienna, Austria.

D. Benaki, C. Zikos, A. Evangelou, M. Vlassi, J. Slaninova, E. Livaniou, E. Mikros, M. Pelecanou (2007). Structural studies of humanin and its derivatives in the investigation of their neuroprotective role against Alzheimer's disease. Biologically Active Peptides. X. Czech and Slovak National Peptide Conference. Prague, 2007, Czech Republic.

P. Kyprianidou, A. Chiotellis, D. Papagiannopoulou, I. Iakovou, C. Tsoukalas, A. Panagiotopoulou, M. Pelecanou, N. Karatzas, M. Papadopoulos, I. Pirmettis (2007). Novel fac-[$^{99m}$Tc-$CO_3$] labeled fluoroquinolones as potential infection imaging agents. European Nuclear Medicine Congress, Copenhagen, Eur. J. Nucl. Med., 34, S329, 2007.

**Impact Factors** (for 2 publications): 4,711

**Citations 2007** (without self- citations): 22

**Total Citations 2005-2007** (without self- citations): 69

**h-factor:**12

## Research Group: Protein Structure by Crystallography and Theoretical Modeling

**Research Staff**
**Metaxia Vlassi, Senior Researcher**
Dimitris Vlachakis, Postodoctoral Fellow
Giorgos Nikolopoulos, Postodoctoral Fellow
Giorgos Villias, Collaborating Graduate Student (MSc)
Stamatia Vatsi, Collaborating Graduate Student (MSc)
Konstantina Dragoumani, Undergraduate Student

**Research Interests**

Our current research activities focus on structural studies of 1) protein interactions with emphasis on sequence repeat containing protein-protein interaction modules and 2) enzymes and peptides of medical interest with the aim to elucidate structure/stability/function relationships towards a structure-based drug design. The approach we follow includes a combination of bioinformatics techniques (*in silico* 3D-Modelling, docking, Molecular Dynamics simulations) with biochemical and biophysical methods (Circular dichroism (CD), x-ray Crystallography).

**2007 Findings**

- ✓ Towards the elucidation of the TPR-mediated interaction mechanism of the Ssn6 and Tup1 proteins (see previous annual reports) and using a combination of limited proteolysis, molecular dynamics simulations, disorder predictions and CD experiments we showed that this particular protein interaction takes place via the "folding coupled to binding" mechanism. More specifically, the TPR1 of Ssn6 is partially unfolded in the absence of Tup1, whilst conformational changes (disorder to helix) accompany the complex formation. We propose that the flexibility of TPR1 is essential for Tup1 recognition by Ssn6 through hydrophobic interactions. Moreover our experimental data suggest that the binding of Tup1 involves the convex surface formed by the TPRs of Ssn6 in contrast to modes of interactions observed so far in other TPR-mediated interactions. This work was accepted for publication in *Proteins* (Palaiomylitou/Tartas *et al*, 2008).
- ✓ A new protein, potential target for rational de novo design of anti-cancer drugs, was overexpressed in *E. Coli* cells and purified biochemically towards structural studies. In a previous work we predicted that this protein contains sequence repeats/patterns of protein interaction (see previous annual reports). This work is funded by GSRT ( 05Non-EU-356)
- ✓ With the aim of elucidating the structure/stability/function relationships of BRCT-mediated protein interactions we studied the thermal stability of various BRCA1 (see previous annual reports) variants (in collaboration with G. Nounesis group, IRRP). This work was published in *BBA* (Nikolopoulos et al, 2007)
- ✓ A 3D-model of the interacting proteins AtoC-AtoS, which act as a two component system (see previous annual reports) was constructed and showed that Asp55 rather that His73 of AtoC is in the proximity of His398 of AtoS suggesting that Asp55 is involved in the phosphotrasfering. This prediction was confirmed by biochemical data by the collaborating lab of prof. Kyriakidis (AUTh). This work was published in *BBA* (Grigoroudis et al, 2007).

**2007 Publications**

Nikolopoulos, G., Pyrpassopoulos, S., Thanassoulas, A., Klimentzou, P.,Zikos,C., Vlassi, M., Vorgias, C, Yannoukakos, D., Nounesis, G. (2007) Thermal Unfolding of Human BRCA1 BRCT-Domain Variants. *Biochim Biophys Acta - Proteins & Proteomics* 1774(6):772-80 .

Grigoroudis, AI, Panagiotidis, CA, Lioliou, EE, Vlassi, M.*, Kyriakidis, D. (2007) Molecular modeling and functional analysis of the AtoS-AtoC two-component signal transduction system of Escherichia

coli. *Biochim Biophys Acta-General Subjects* 1770(8):1248-58. **Corresponding Author*

**2007 Presentations at International Scientific Conferences**

Vlachakis, D., A. Tartas and M. Vlassi (2007) Using molecular dynamics simulations to investigate the structural stability of a repeat containing protein 32nd FEBS Congress, Molecular Machines, Vienna, Austria, July 7-12, 2007.

**Impact Factors** (for 2 publications): 5,40

**Citations 2007** (without self- citations): 25

**Total Citations 2005-2007** (without self- citations): 65

**h-factor:** 10

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

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

# HUMAN TISSUE BANK

**Research Staff**
**Helen Vavouraki, Reseracher**
Konstantinos Makris (MD), Graduate Research Assosciate
Stilianos Kakkos, Research Technician

**Description**

Our permanent task is the continuous search of human tissues from suitable donnors, the effort for the optimization of the production processes, the introduction of new techniques and methods, the application of new quality controls according to the latest national and international standards and legislation for this type of products.

**2007 Findings**

a) Accreditation of the Bank operation according the ISO 9001/2000.

b) Completion of our products preparation to be launched in the market, under the program AKMON. A range of appropriate printed matter was edited as well as an up to date computerizing system to support the whole task of the Bank.

c) In parallel, the production of grafts was amounted to 195 of various type bone grafts, mainly.

**Other Acticities at the IB**

Responsible of Quality Assurance Project of the Bank according the ISO 9001/2000 stds,

Application of the ISO stds and market specifications to the major Bank activities, like new labeling format, technical equipment supply and functioning, data recording e.t.c.

**Other Scientific Activities**

Representative of Greece to the European Regulatory Experts Committee for the Directives 23/2004 17/2006 and 86/2006 concerning the tissues and cells of human origin.

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

Collaboration with the National Transplant Organisation, and the Ministry of Health and Social Solidarity for the adaptation of the above Directives into the National Law.

# EXPERIMENTAL ANIMAL COLONY

**Research Staff**
**Dimitris Kletsas, Senior Researcher**
Ioannis Zafiropoulos, Research Technician
George Doulgeridis, Research Technician

**Description**

The animal facility maintains and reproduces inbred strains of experimental animals. The following strains are currently available:

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

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 labs, hospitals, pharmaceutical companies, etc.

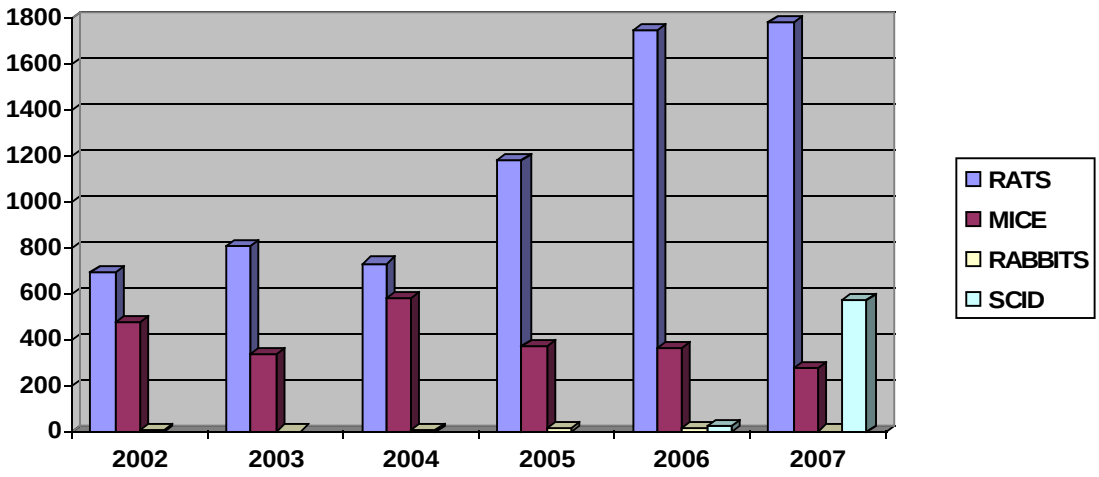
During 2007, the Animal Facility provided the following animals:

| *Users* | *Rats* | *Mice* | *Rabbits* | *SCID* |
|---|---|---|---|---|
| Institute of Biology | 30 | 2 | | 33 |
| Institute of Radioisotopes & Radiodiagnostics | 52 | 273 | 4 | 542 |
| External Users | 1698 | - | - | - |
| **Total of animals provided** | **1780** | **275** | **4** | **575** |

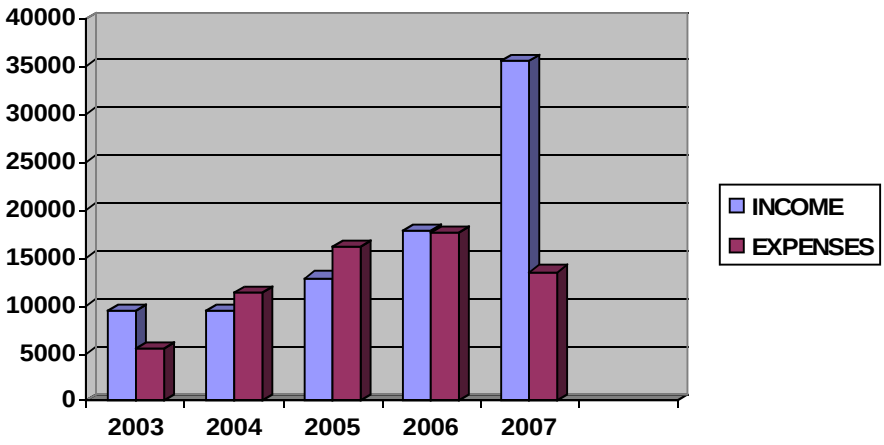
The total income of the Unit for 2007 was 35.601,13 € and total expenses were 13.572,18 €.

The certification of the Unit according to ISO, as well as the elaboration of the ΑΚΜΩΝ project funded by GSRT and in collaboration with ELPEN Pharma Company, are under process. Collaborations with the University of Ioannina and EKEBE "Al. Fleming" have been realized.

## DISPOSAL OF LABORATORY ANIMALS 2002-2007




## INCOME-EXPENSES 2003-2007


40000
35000
30000
25000
20000
15000
10000
5000
0
2003
2004
2005
2006
2007
INCOME
EXPENSES

# 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, face-contrast, Nomarsky etc techniques on both fixed and living cells.

**2007 Findings**

During the year 2007, 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, Senior Researcher**
**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.

**2007 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 Programmme, which has been successfully carried out for the past 30 years. This Programme includes:

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

During the year 2007, 13 scientists were trained at the postdoctoral level at our Institute. Furthermore, 29 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 2007, 1 of our graduate students finished their thesis work and became PhDs.

Moreover, 8 students from the University are carrying out their pre-graduate project thesis work at the Institute and 4 students did practical job training.

In the framework of Graduate Programme, during the year 2007 the Biology Institute organized one course in which had as participants graduate students of the IB and of other Institutes of N.C.S.R. "Demokritos". The following courses were given by scientists of the Biology Institute:

- *Structural Biology and Theoritical Approaches* [course lecturer: M. Pelecanou, course coordinator: M. Vlassi].
- *Chromatin Structure and Regulation of gene Expression* [course lecturers: Th. Sourlingas, L. Swevers, course coordinator: V. Sophianopoulou ].

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:

- *Teaching in the framework of the postgraduate course: "Biochemistry"* (**Dr. Iro Georgoussi,** Department of Biology, University of Athens)
- *Lecture with title "Heptahelical receptors coupled to G proteins" in the framework of the postgraduate course "Biochemistry" "* (**Dr. Iro Georgoussi,** Department of Biology, 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 and Dr. H. Pratsinis**, Department of Biology, University of Athens).
- *Teaching in the framework of the postgraduate course: "Applications of Biology to Medicine", the course "Cell Cycle: Checkpoints and Consequences for Physiological Cell Function"* (**Dr. Th. Sourlingas,** Department of Biology, University of Athens).
- *Lecture with title "Molecular mechanisms and therapeutic approaches for diabetes mellitus in the framework of the postgraduate course "Pathobiochemistry"* (**Dr. E. Tsilibary**, Department of Biology, University of Athens)
- *Lecture with title "Molecular mechanisms and theurapeutical interventions in diabetes – mellitus" in the framework of the course"Pathobiochemistry"* (**Dr. E. Tsilibary**, Department of Biology, University of Athens)

- *Lecture with title "Technologies for the protein structural analysis" in the farework of of the interdepartemental programme of graduate studies "Protein Biotechnology"* (**Dr. M. Pelecanou,** Department of Biology, University of Crete)
- *Lecture with title "Functional Expression and Study of Transmebrane Transporters of Higher Organisms" in the framework of the course "Model Systems of Molecular Microbiology" of the postgraduate programme 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 courses: "Clinical Biochemistry & Molecular Diagnostics" (***Dr. M. Vlassi**, Department of Biology, University of Athens)
- *Teaching in the framework of the postgraduate courses: "Introduction to Research Methods" (***Dr. M. Vlassi**, Department of Biology, University of Athens)

During July 2007, 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 (2007) are presented analytically in the following pages.

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

## COMPLETION/AWARD OF DOCTORAL THESES IN 2007

| GRADUATE STUDENT | TITLE OF DOCTORAL THESIS | ADVISOR (in Institute of Biology) | UNIVERSITY |
|---|---|---|---|
| **Athanassia Aggeli** | *Interactions between isoform collagen IV chains and renal cell types* | Effie Tsilibary | Medical School, University of Athens |

**LECTURE CONTRIBUTIONS TO**
**THE 2007 SUMMER SCHOOL**
**OF THE NCSR "DEMOKRITOS"**
*(July 2007)*

| DATE | SPEAKER | TITLE |
|---|---|---|
| 9/7/07 | **Dr. A. Prombona**<br>Institute of Biology, NCSR<br>"Demokritos" | The biological clock and its role as tumor depressor |
| 9/7/07 | **Dr. D. Kletsas**<br>Institute of Biology, NCSR<br>"Demokritos" | Cellular senescence and tissue homeostasis |
| 9/7/07 | **Dr. L. Swevers**<br>Institute of Biology, NCSR<br>"Demokritos" | Regulatory mechanisms of oogenesis in Lepidotera: characterization and study of molecules with a pivotal role in the successive stages of oogenesis of *Bombyx mori* |
| 12/7/07 | **Dr. Th. Sourlingas**<br>Institute of Biology, NCSR<br>"Demokritos" | Histone subtypes and post translational modifications: fundamental factors for chromatin remodelling during ageing and apoptosis |
| 12/7/07 | **Dr. I. Almyrantis**<br>Institute of Biology, NCSR<br>"Demokritos" | Introduction to the study of the genome by means of statistical and probabilistic methods. Is a linguistic approach to the description of "genomic text" possible? |
| 18/7/07 | **Dr. I. Georgoussi**<br>Institute of Biology, NCSR<br>"Demokritos" | Heptahelical receptors and G proteins: health, pathogenesis and development ogf new drugs |
| 19/7/07 | **Dr. E. Tsilibary**<br>Institute of Biology, NCSR<br>"Demokritos" | Basic research and clinical applications for interfering with human disease: from bench to bedside |
| 20/7/07 | **Dr. I. Almyrantis**<br>Institute of Biology, NCSR<br>"Demokritos" | Organisms, genomes and evolution: The understanding of the origin of life and of biological function through the interaction of biology with other sciences |

**SEMINAR PROGRAMME 2007**
**INSTITUTE OF BIOLOGY**

| DATE | SPEAKER | TITLE |
|---|---|---|
| 12/1/07 | **Dr. D. Vassilakopoulou**<br>Dpt of Biology, Univ of Athens | New data for the expression and regulation of human L-Dopa decarboxylase |
| 26/1/07 | **Dr. V. Panoutsakopoulou**<br>Academy of Athens, IIBEAA | Osteopontin involvement in allergic asthma: regulation of dendritic cell subsets |
| 9/2/07 | **Assoc. Prof. D. Alexandraki**<br>Dpt of Biology, Univ of Creta | New metal –regulated transcriptional complexes in the monocellular organism saccharomyces |
| 23/2/07 | **Prof. G. Kapetanaki**<br>Academy of Athens, IIBE | Cellular and Molecular Mechanisms of Cardioprotection by Desmin Cytoskeleton |
| 9/3/07 | **Assoc. Prof. E. Frilingos**<br>Medical School. Univ. of Ioannina | Oxidize purine transporters in enterobacteria: Structure –function relationship xanthine transporter YgfO |
| 23/3/07 | **Assoc. Prof. D. Kardassis**<br>Medical School. Univ. of Creta | Mechanisms of TGF-b mediated transcriptional regulation |
| 29/3/07 | **I. Georganta**<br>Institute of Biology, NCSR "Demokritos" | Multi-protein complexes of the δ – opoiod receptor |
| 12/4/07 | **Ch. Magrioti**<br>Institute of Biology, NCSR "Demokritos" | Hymenopteram parasitism on lepidopteram hosts with the use of polydna viruses |
| 19/4/07 | **N. Tsotakos**<br>Institute of Biology, NCSR "Demokritos" | Mechanisms that control the suppression of the anti-adhesive protein podocalyxin in human podocytes exposed to diabetic conditions |
| 20/4/07 | **Prof. Th. Fotsis**<br>Medical School. Univ. of Ioannina | Signal transduction in endothelial cells |
| 26/4/07 | **G. Ninios**<br>Institute of Biology, NCSR "Demokritos" | Study of the activation of DFF –(DNA fragmentation factor) after induction of apoptosis by HDACIs (histone deacetylase inhibitors) in cancer cells |
| 10/5/07 | **M. Xedous**<br>Institute of Biology, NCSR "Demokritos" | The Effect of Histone Acetylation Levels in the Regulation of the Biological Clock: Consequences for Cellular Function |
| 24/5/07 | **A. Repouskou**<br>Institute of Biology, NCSR "Demokritos" | Circadian clock and histone acetylation: interaction with the cell cycle in *in vitro* murine cell systems |
| 1/6/07 | **Prof. M. Kokkinides**<br>Dpt of Biology, Univ of Creta | Structural studies of proteins from the type III secretion system, a bacterial device for the close combat with euraryotic host cells |
| 14/6/07 | **E. Mavrogonatou**<br>Institute of Biology, NCSR "Demokritos" | The effect of osmotic stress on the proliferation of nuclens pulposus intervertebral disc cells |
| 18/6/07 | **Dr. I. Almyrantis**<br>Institute of Biology, NCSR "Demokritos" "Demokritos" | Alu and LINE1 distributions in the human chromosomes: Evidence of global genomic organization expressed in the form of power laws |
| 21/6/07 | **P. Venieratos**<br>Institute of Biology, NCSR "Demokritos" "Demokritos" | Glucose-induced alterations in signal transduction mechanisms and fuctions of pancreatic β-cells |
| 21/6/07 | **D. Anastassiou**<br>Institute of Biology, NCSR | The study of molecular signaling pathways in urinary bladder cancer |

| | | |
|---|---|---|
| | "Demokritos" | |
| 5/7/07 | **M. Papakonstantinou**<br>Institute of Biology, NCSR<br>"Demokritos" | Interactions of the seven transmembrane receptors with different proteins. Characterisation of new signaling pathways |
| 5/7/07 | **S. Alimperti**<br>Institute of Biology, NCSR<br>"Demokritos" | The role of molecular chaperones in the regulation of mammalian kinases |
| **Prof. J. C.** 19/9/07 | **States**<br>Dpt. of Pharmacology & Toxicology<br>University of Louisville, School of Medicine | In utero arsenic exposure-induced alterations in liver gene expression associated with accelerated atherogenesis |
| 4/10/07 | **N. Tsotakos**<br>Institute of Biology, NCSR<br>"Demokritos" | Symoylation inhibis Cleavage of Sp1 N-terminal Negative Regulatory Domain and inhibits Sp-1-dependant Transcription |
| 18/10/07 | **K. Ioannides**<br>Institute of Biology, NCSR<br>"Demokritos" | An improved zinc-finger nuclease architecture for highly specific genome editing |
| 25/10/07 | **K. Roumelioti**<br>Institute of Biology, NCSR<br>"Demokritos" | Nitrogen catabolite repression and pathogenicity of candida albicans require GATA-type transcription factor which encoded by GAT1-gene |
| 25/10/07 | **Dr. J. Slaninova**<br>Institute of Organic Chemistry and Biochemistry (IOCB), Czech Academy of Sciences | Behavioral testing of the potentially neuroprotective factor humanin and its analogues |
| 1/11/07 | **E. Salpea**<br>Institute of Biology, NCSR<br>"Demokritos" | In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state |
| 8/11/07 | **M. Papakonstantinou**<br>Institute of Biology, NCSR<br>"Demokritos" | Regulator of G-Protein Signaling (RGS) Proteins Differentially Control Chondrocyte Differentiation |
| 15/11/07 | **A. Repouskou**<br>Institute of Biology, NCSR<br>"Demokritos" | The Circadian Gene Per1 Plays an Important Role in Cell Growth and DNA Damage Control in Human Cancer Cells |
| 22/11/07 | **I. Georganta**<br>Institute of Biology, NCSR<br>"Demokritos" | A nuclear function of β- Arrestin 1 in GPCR signaling: Regulation of histone acetylation and gene transcription |
| 6/12/07 | **S. Aliberti**<br>Institute of Biology, NCSR<br>"Demokritos" | Analysis of the CK2-dependent phosphorylation of serine 13 in Cdc37 using a phospho-specific antibody and phospho-affinity gel electrophoresis |
| 13/12/07 | **Ch. Magrioti**<br>Institute of Biology, NCSR<br>"Demokritos" | Targeted inhibition of the maturation of miRNA molecules with the use of morpholinos reveals the role of miR-375 on the development of pancreatic islets |
| 13/12/07 | **G. Ninios**<br>Institute of Biology, NCSR<br>"Demokritos" | Sequence elements in both subunits of the DNA fragmentation factor are essential for its nuclear transport |

# *COLLECTIVE DATA*

# FINANCIAL REPORT 2007

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

| | PROGRAMMES | | | | | | |
|---|---|---|---|---|---|---|---|
| ***INCOME*** | **464 IB** | **1240 EPAN** | **1269 EXCELLENCE** | **1333 AKMON** | **1334 AKMON** | **1397 PEP** | **1334 Experimental Animal Colony** |
| CARRIED OVER FROM 2006 | 1.185 | 430.425 | 46.892 | 4.400 | -18.348 | 50.333 | |
| FUNDING FROM NCSR "D" | 50.000 | | | | | | |
| MATCHING FUNDS | 20.263 | 333.880 | | 9.984 | | | |
| INCOME FROM SERVICES | | | | | 6.393 | | 35.601,13 |
| DONATIONS FROM COMPANIES | | | | | | | |
| TRANSFER FROM OTHER SOURCES | 24.764 | 7.683 | 428 | 22.500 | 104 | | |
| ***TOTAL INCOME*** | **96.212** | **771.988** | **47.320** | **36.884** | **-11.851** | **50.333** | **35.601,13** |

***EXPENSES***

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| EQUIPMENT | 4.737 | 140.613 | 13.717 | 207 | 2.096 | 9.710 | 1990,51 |
| SUPPLIES | 3.187 | | | 2.849 | 747 | 13.388 | 3.321,85 |
| SALARIES | 17.608 | | | 22.962 | | 24.000 | |
| TRAVELS | 1.185 | | 5.760 | | | | |
| OTHER EXPENSES | 5.869 | | 74.101 | | 4.165 | | 315,90 |
| COMMITTED | 2.184 | | 11.882 | 12.488 | 5.123 | | |
| TRANSFER FROM OTHER SOURCES | 26.724 | | | 5.000 | 1.358 | | |
| ***TOTAL EXPENSES*** | **61.496** | **140.613** | **105.459** | **43.507** | **13.489** | **47.098** | **5.628,26** |

***2. GOVERNMENTAL FUNDING***

| | |
|---|---|
| ANIMAL CHOW | 7.944 |
| ***TOTAL GOVERNMENTAL FUNDING*** | ***7.944*** |

## 2. *EXTERNAL FUNDING FROM THE PROGRAMMES OF THE INSTITUTE*

| **SOURCE OF FUNDING (number of programmes)** | **FUNDING (in EUROS)** | | | |
|---|---|---|---|---|
| | **Programme A** | **Programme B** | **Programme C** | **INSTITUTE** |
| European Union (4) | 262.836 | - | - | 262.836 |
| General Secretariat for Research & Technology (19) | 342.063 | 53.875 | 36.500 | 432.438 |
| Ministry of health & Social Solidarity (2) | 14.635 | - | - | 14.635 |
| International Atomic Energy Ageny (IAEA) (1) | - | - | 500 | 500 |
| Aspis Bank (1) | - | - | 10.000 | 10.000 |
| AO Foundation (1) | 11.970 | - | - | 11.970 |
| Greek Society of Oncologists (1) | 6.000 | - | - | 6.000 |
| Bristol Myers Squibb (1) | 15.000 | - | - | 15.000 |
| **TOTAL** | **652.504** | **53.875** | **47.000** | **753.379** |

# COLLECTIVE DATA ON PRODUCTIVITY OF SCIENTIFIC PROGRAMMES

| | PROGRAMME | | | |
|---|---|---|---|---|
| | **A** | **B** | **C** | **INSTITUTE** |
| Researchers | 9 | 7 | 4 | 21* |
| Technical Specialist | - | 1 | 1 | 2 |
| Emeritus & Collaborating Scientists | 2 | 5 | 2 | 9 |
| Postdoctoral Fellows | 7 | 4 | 2 | 13 |
| Graduate Students | 20 | 8 | 1 | 29 |
| Collaborating Graduate Students | 14 | 1 | 2 | 17 |
| Graduate Research Associates | - | - | 1 | 2[£] |
| Undergraduate Students | 1 | 9 | 1 | 11 |
| Research Technicians | 4 [#] | 3 | - | 9 [@] |
| Administrative Staff | - | - | - | 2 |
| **Total Personnel** | **57**[#] | **38** | **14** | **115** |
| **Publications in Peer-Reviewed Journals** | **20** | **13** | **5** | **38** |
| Publications (Average) in Peer-Reviewed Journals per Scientist | 2.22 | 1.85 | 0.8 | 1.9 |
| **Cumulative Impact Factor in Peer-Reviewed Journals (number of publications)** | **65.849 (20)** | **60.602 (13)** | **16.841 (5)** | **143.292 (38)** |
| Average Impact Factor in Peer-Reviewed Journals | 3.292 | 4.661 | 3.368 | 3.770 |
| Cumulative Impact factor per Scientist | 7.316 | 8.657 | 4.210 | 7.164 |
| **Proceedings to Conferences** | **16**[$] | **4**[$] | **5** | **24** |
| Proceedings (Average) per Scientist | 1.777 | 0.571 | 1.25 | 1.2 |
| **Total Publications** | **36**[$] | **17**[$] | **10** | **62** |
| Publications (Average) per Scientist | 4 | 2.428 | 2.5 | 3.1 |
| **Citations** | **542** | **230** | **67** | **839** |
| **International Patents** | - | - | - | - |
| **Greek Patents** | - | - | - | - |
| **Presentations to International Conferences** | **18**[+] | **14**[+] | **5** | **35** |
| Presentations (Average) per Scientist to International Conferences | 2 | 2 | 0.75 | 1.75 |
| **Presentations to Greek Conferences** | **50** | **6** | **8** | **64** |
| Presentations (Average) per Scientist to Greek Conferences | 5.555 | 0.857 | 2 | 3.2 |
| **Total Presentations to Conferences** | **68**[+] | **20**[+] | **13** | **99** |
| Presentations (Average) per Scientist to Conferences | 7.555 | 2.857 | 3.25 | 4.95 |

** 1 Scientist of Human Tissue Bank is included*

*# 1 Research Technician who is occupied in other programme also 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*

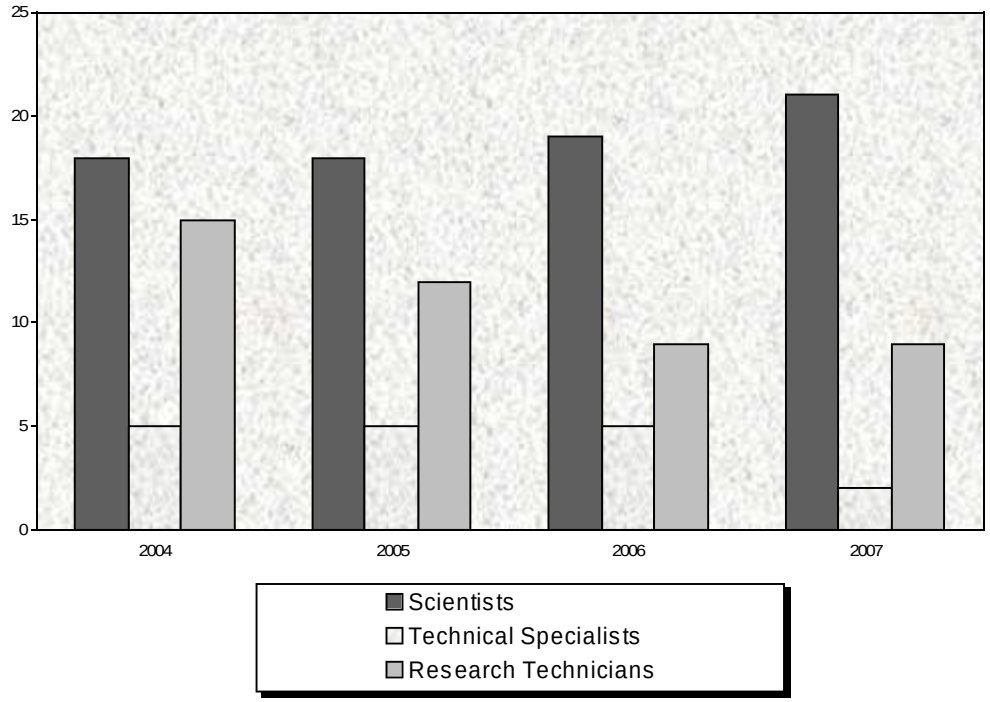
*$1 publication in proceedings of international conference common to A and B programme is included*

*+2 presentations to international conferences common to A and B programme are included*

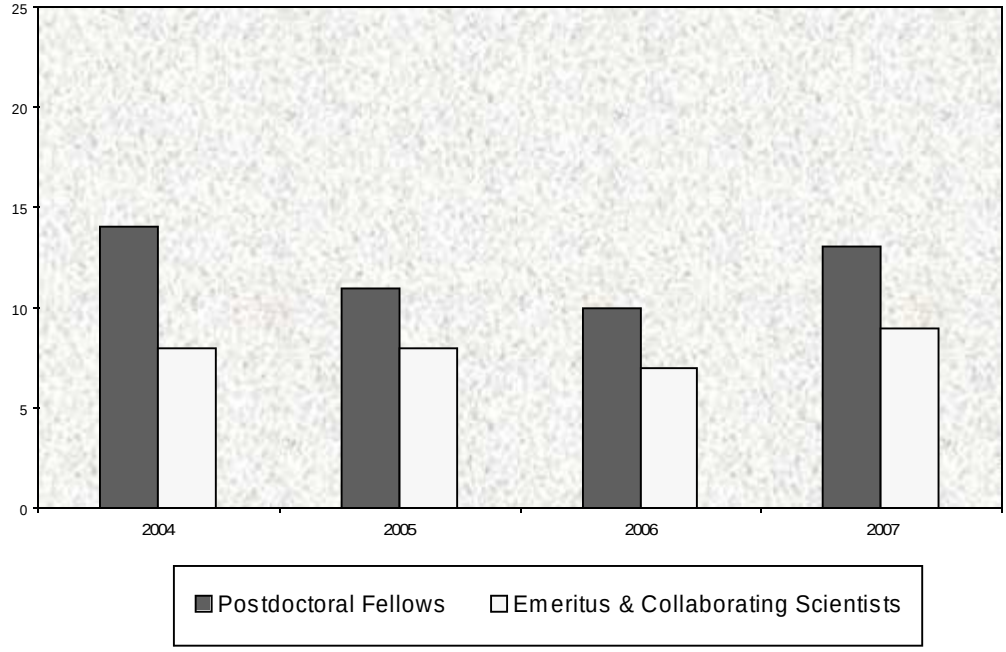
*£ 1 GraduateResearch Associate of Human Tissue Ban kis included*

# CHANGES OF IB STAFF
# DURING 2004-2007

**"TENURED EMPLOYEES"**

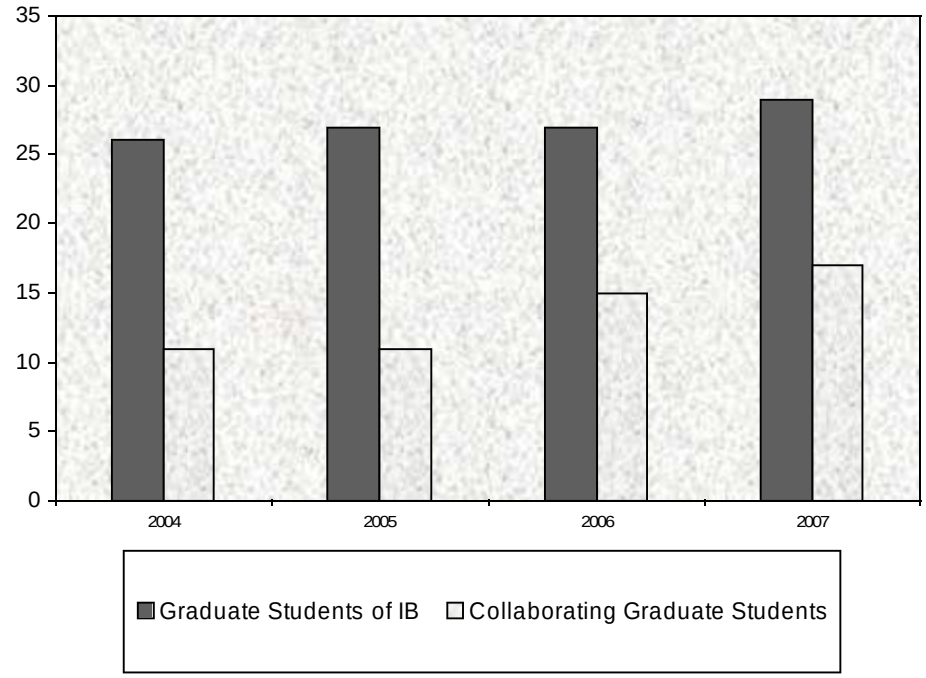



**"POSTDOCTORAL FELLOWS and EMERITUS & COLLABORATING SCIENTISTS"**

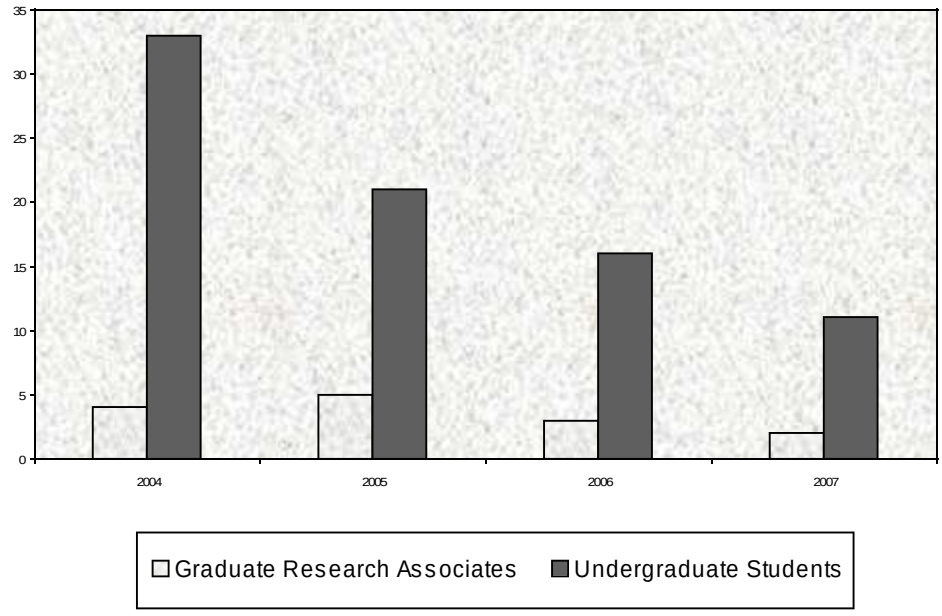


# CHANGES OF IB STAFF
# DURING 2004-2007

**"GRADUATE STUDENTS"**

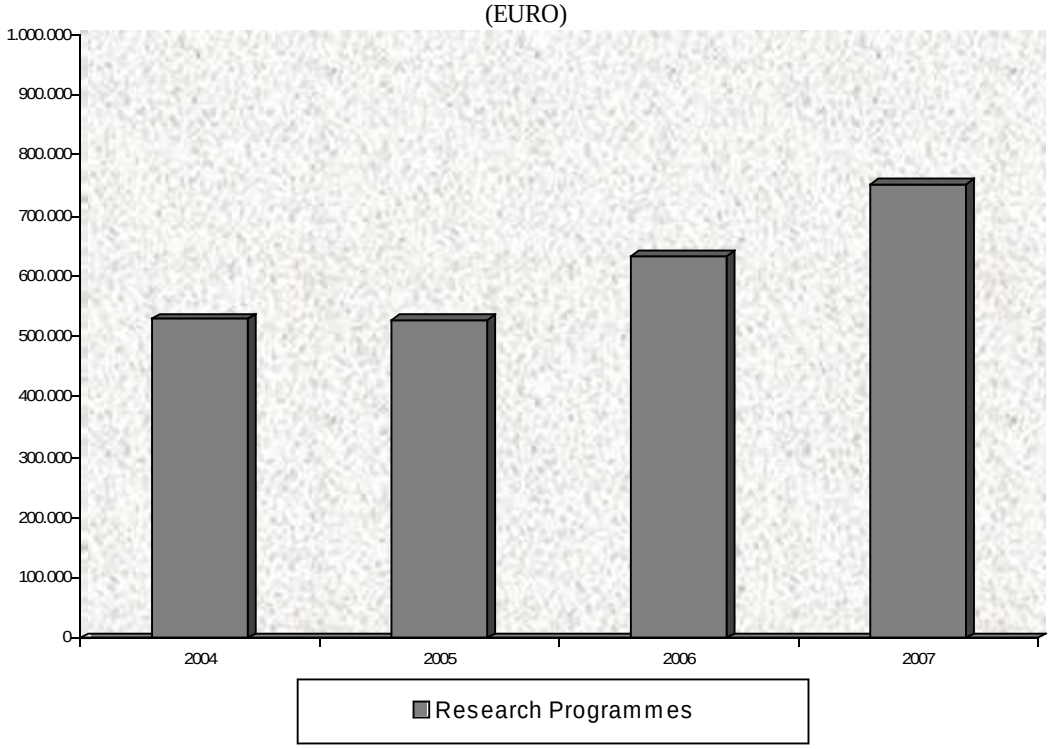



**"GRADUATE RESEARCH ASSOCIATES AND UNDERGRADUATE STUDENTS "**



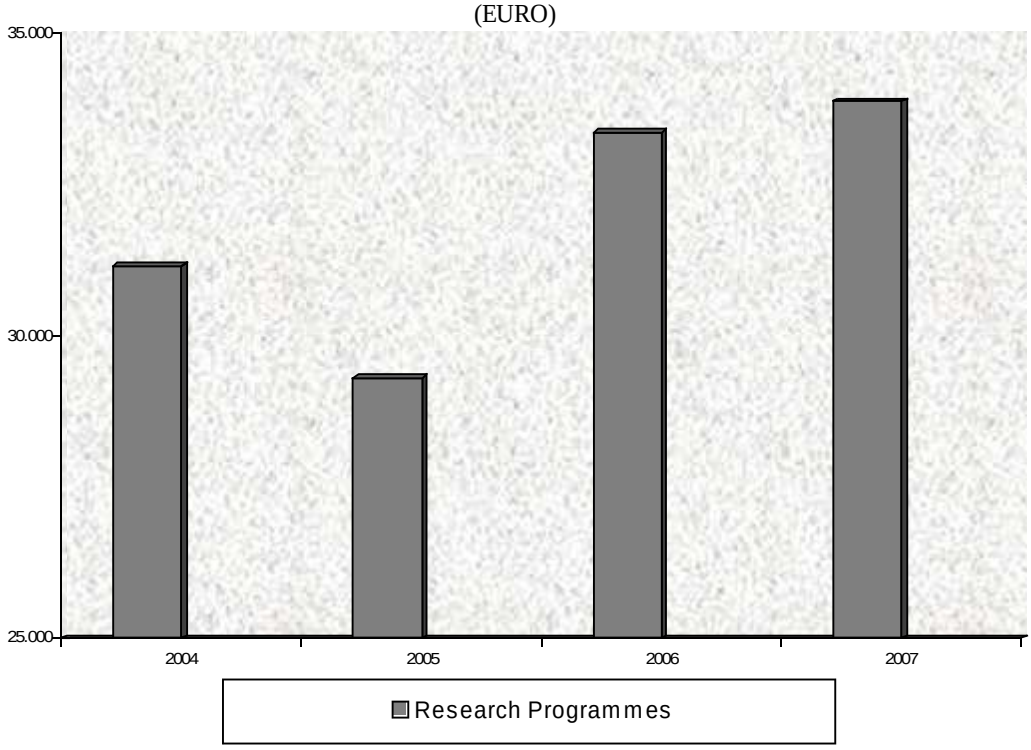
**CUMULATIVE EXTERNAL FUNDING OF THE INSTITUTE DURING 2004-2007**

(EURO)




**EXTERNAL FUNDING OF THE INSTITUTE PER SCIENTIST DURING 2004 - 2007**

(EURO)



**PUBLICATIONS IN PEER-REVIEWED JOURNALS**
**AND CUMULATIVE IMPACT FACTOR DURING 2004-2007**