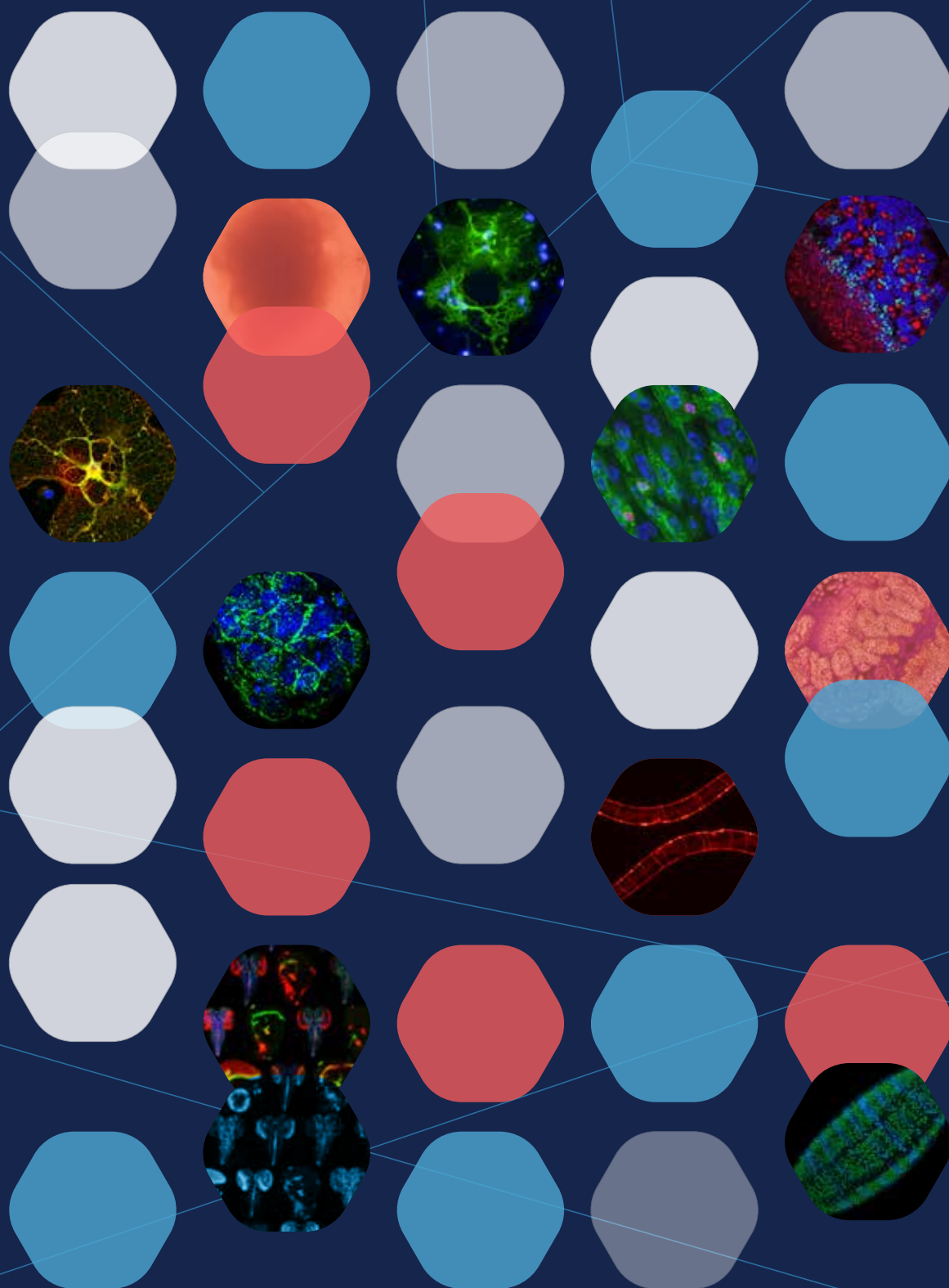




INSTITUTE OF
MOLECULAR
BIOLOGY AND
BIOTECHNOLOGY



BIENNIAL REPORT

2019-2020



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FACILITIES

IOANNINA

DIRECTOR'S SUMMARY



In the long history of our Institute, the years of 2019 and 2020 will be remembered as the period of exit from a decade-long economic crisis in Greece, with new hopes for increased government funding and opportunity for substantial improvements, which however were hindered by the sudden expansion of Covid-19 pandemic.

EXCEPTIONAL PERFORMANCE UNDER CHALLENGING CONDITIONS

Despite the high expectations, key government policies, which posed considerable restraints on the optimal development of Greek research institutions during the economic crisis, were not radically changed in 2019-2020. Greek scientists continued to suffer from low central funding, low salaries and a painfully high Bureaucracy in essentially all operational activities.

In late-2018 we had an MHV infection in our mouse facility, which affected the work of the majority of research groups until the fall of 2019.

The emergence of Covid-19 pandemic during the following year generated a worldwide situation that justified the use of dramatic superlatives in a literal sense: it was often described by many, as “Annus horribilis”. The recurring local and nationwide “lockdowns” and other regulations for controlling the pandemic in Crete and Greece, imposed unprecedented restrictions in our working routines and everyday life.

The combination of the above, tested us beyond measure in several ways. It is reasonable to say, that the past two years probably represent the most difficult period in IMBB history, under which pursuing our mission was a challenging task.

Intriguingly however, one could observe remarkable achievements, regarding IMBB's scientific output, financial growth and the development of novel collaboration schemes. Many IMBB researchers earned high-level distinctions and prizes and we could record significant successes in our innovation and outreach activities.

ACHIEVEMENTS AND PEOPLE

After a quick cataloguing of the major achievements of the period, it seems that, at least for IMBB, it may be more appropriate to call 2019-2020 “Anni mirabiles” instead of its complementary form.

Economy:

The data in the “Facts and Figures” section show that the Annual Central Budget from the government has been marginally (by 17%) increased in 2019. Unfortunately, this increase not only disappeared in 2020 budget but we had further reduction to about 88% of the amount received in 2018. In contrast, the total budget of the Institute has steadily increased in 2019 and 2020, respectively. The difference was due to the spectacular increase of funding from competitive sources: 16% increase in 2019 and 22% in 2020, compared to 2018 figures.

While the contribution of government funding to IMBB's total budget was generally around 25% for the past decades, the 2020 figure showing government support falling below 18% of the total, is alarming.

Science:

The publication statistics data show a promising increase in the total number of publications. A good percentage of the papers appeared in top 10% or top 5% scientific journals (using combined Scopus-Web of Science metrics). While the evaluation of such statistics is meaningful only in a longer time-frame, the numbers clearly indicate a developing trend during the period.

Having the eye on quality, IMBB Researchers have made several key discoveries with high scientific impact. Some of them are summarized in the ‘Scientific Highlights’ section.

Among the many high impact papers produced by IMBB Researchers and collaborators during 2019-2020, I proudly mention here the study on a new class of calcium mediated dendritic action potentials effecting neuronal output,

which was published in Science Vol 367 pp. 83-87 and was named Breakthrough of the Year by the Quanta Magazine.

People:

The scientific excellence of our faculty was recognized through international prizes and distinctions, such as the Friedrich Wilhelm Bessel Award to George Garinis who was also elected EMBO member, the Albert Einstein Fellowship award to Panagiota Poirazi and the election of Nektarios Tavernarakis as member of the Academy of Athens and the German Academy of Sciences Leopoldina. In addition, Giorgos Chamilos received an ERC Consolidator Grant, increasing the number of IMBB's current and former ERC grantees to 8.

We were also pleased by the election of Nektarios Tavernarakis as Vice President of ERC Scientific Council, which reinforces the influential role of IMBB Researchers in the highest-level European science decision-making bodies.

We are happy to welcome 5 new Group Leaders, Anastasios Pavlopoulos, Emmanouil Froudarakis, Panos Verginis and George Gouridis, Christos Gkogkas, who started their new labs at IMBB during 2019-2020.

For the first time IMBB has appointed Adjunct Researchers, an affiliation designed for high profile scientists who have their main activities abroad, but have developed long-term and close collaborations with IMBB Researchers. With great excitement we recruited Dr Jean-Paul Latge (Professor Emeritus from Pasteur Institute), Dr Alexander Dömling (Professor at Univ. Groningen), Dr Triantafyllos Chavakis and Dr Ioannis Mitroulis (TU Dresden), as part-time Adjunct Researchers. Their experience will substantially enhance our competitiveness. We look forward to all of the new researchers making significant impacts over the years to come.

We were sorry to say Goodbye to our Researchers John Strouboulis and Vasiliki Nikolettou, who left us to join the Kings College in London and the University of Lausanne. Our farewell wishes are tied with the confidence of keeping close ties with them through future collaborations.

We express our appreciation and gratitude to Theodore Fotsis, the former director of the Ioannina Branch, on the occasion of his retirement in September 2019. Dr Fotsis was a founding member of IMBB-BR, which under his leadership has developed to one of the key biomedical research centers in Greece. We wish the very best for his successor, Savvas Christoforidis.

The year of 2020 had also some tragic events: We and the entire Greek scientific community was shocked by the passing away of IMBB Researcher and former Deputy Director, Dimitris Kafetzopoulos. We learned with great sadness about the premature loss of IMBB Alumni Alekos Athanasiadis, a group leader at the Gulbenkian Institute in Portugal. Both of our colleagues were at the peak of their active career. Their passing away, constitutes a great loss for the Greek and the International academic community.

Another tragic event was the senseless murder of our esteemed and dear colleague, Suzanne Eaton, on Cretan soil in the summer of 2019. It has left the entire scientific community and particularly those of us on the island of Crete baffled and hurt.

Innovation and Science-Business interactions:

A significant contribution from the Biosensors Lab related to Real-time digital DNA assays, provided the basis of the development of a point-of-care-device and the launching of the spin-off company BIOPIX-T.

Another start-up company EnzyQuest was developed by former post-docs of the Minotech laboratory of IMBB, which focuses on developing and commercializing molecular biology enzyme products.

Both BIOPIX-T and EnzyQuest has recently succeeded in obtaining substantial funds from venture capital investors, which provides high promise for further development and long-term growth.

Finally, IMBB-FORTH further extended its strategic research collaboration agreements with the global enterprise Bayer, towards the identification and characterization of potential molecular insecticide targets and the discovery of novel pest control solutions.

ASSOCIATION WITH EU-LIFE, A RECOGNITION OF IMBB'S DECADES-LONG EFFORTS TO REACH THE EXCELLENCE STANDARDS OF LEADING EUROPEAN CENTERS

EU-LIFE is an alliance of 15 leading European research institutes in the life sciences with a mission to promote excellence in research by implementing and disseminating best practices in the organization and operation of scientific establishments.

Throughout its history IMBB pursued principles similar to those of EU-LIFE Institutes, with key characteristics of adherence to merit-based recruitment strategy, rigorous evaluation by international Scientific Advisory Committees, organizational and management structures focusing on creating optimal environment for performing top quality research and by putting the scientific quality standards high, to the level of the top-performing international centers.

IMBB has multiple benefits from joining the distinguished group of EU-LIFE institutions, including new opportunities for scientific collaborations and sharing experience in science resources, organization and use of research facilities, funding opportunities, postgraduate training, bioethics, gender equality and technology transfer issues.

IMBB shares the view of the EU-LIFE community that scientific excellence in life sciences can only be achieved through strong adherence to principles of quality, scientific integrity, ethical responsibility, societal accountability, ecological sustainability, gender equality and cultural diversity while promoting a strong dialogue with society. Needless to say we are very excited to be part of the EU-Life alliance and look forward to contribute to its mission of promoting scientific excellence across Europe.

OUTREACH TO PUBLIC AND SOCIETY

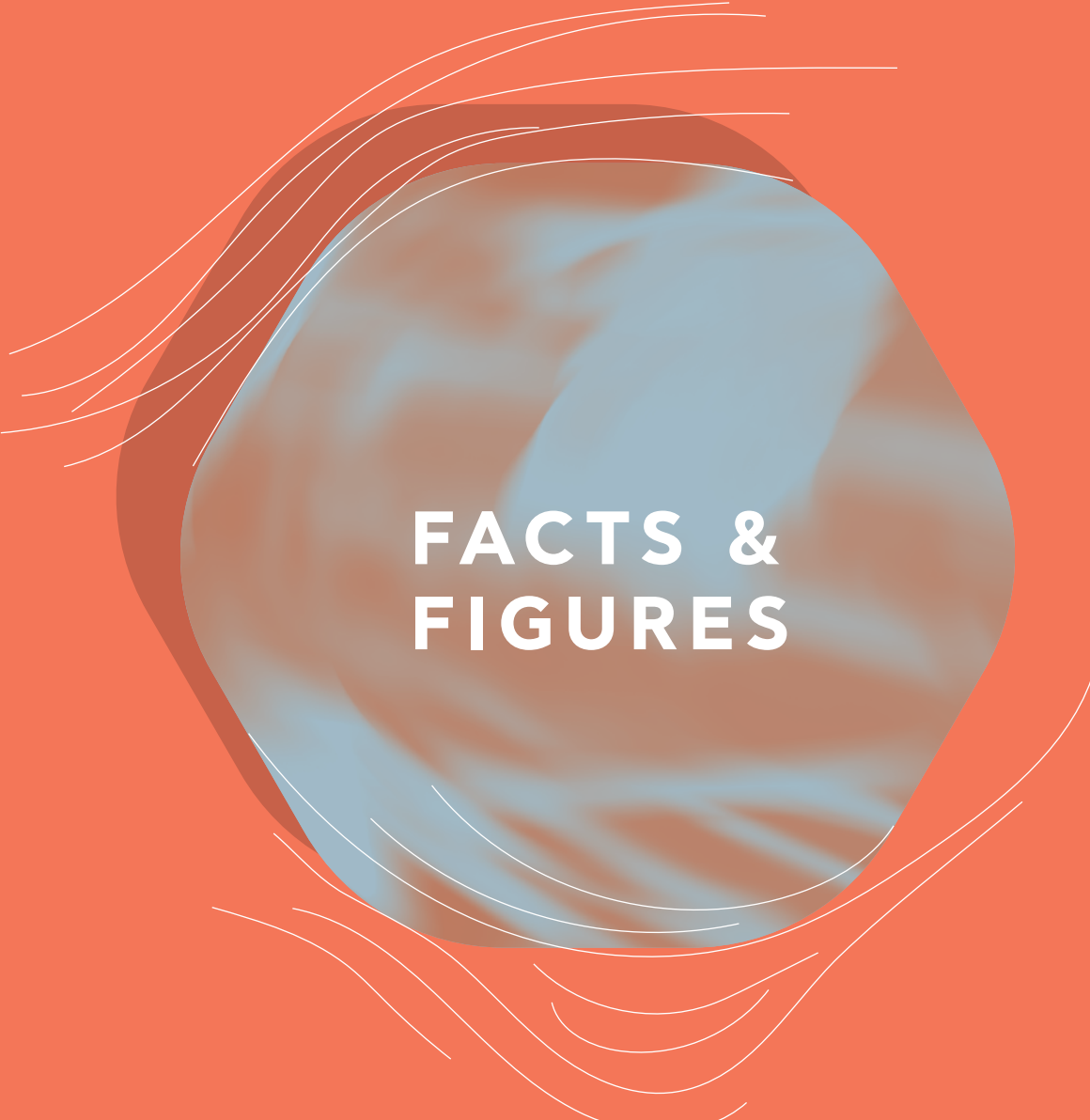
Following the tradition of many years, IMBB members have actively participated in the organization of 2019 and 2020 Researcher's Night in Heraklion, one of the largest celebration of science and research in European cities. We had many presentations, experiments, events specifically designed for students and teachers, and a variety of original events, where the public can experience research at first hand and have direct contact with scientists. In 2020 all of the activities were conducted online.

Our outreach activities aiming at raising public awareness also included several presentations in Athens Science Festival in 2019 and numerous articles and interviews in popular press, in local and national TV stations about the role of science and especially basic research in advancement of the society.

Throughout 2020 IMBB Researchers had actively participated in the national campaign to inform the public about Covid-19 disease pathogenesis, spreading, about pharmaceutical treatment strategies and the necessity of vaccination. Our Researchers had >100 appearances in electronic media and newspaper articles and participated in a number of discussion forums that have attracted very large audiences.

In 2019 IMBB has relaunched the online database of pesticide resistance "Galanthus", an invaluable informatics tool supporting Greek farmers for the timely and evidence-based actions to fight seasonal and region-specific agricultural pests.

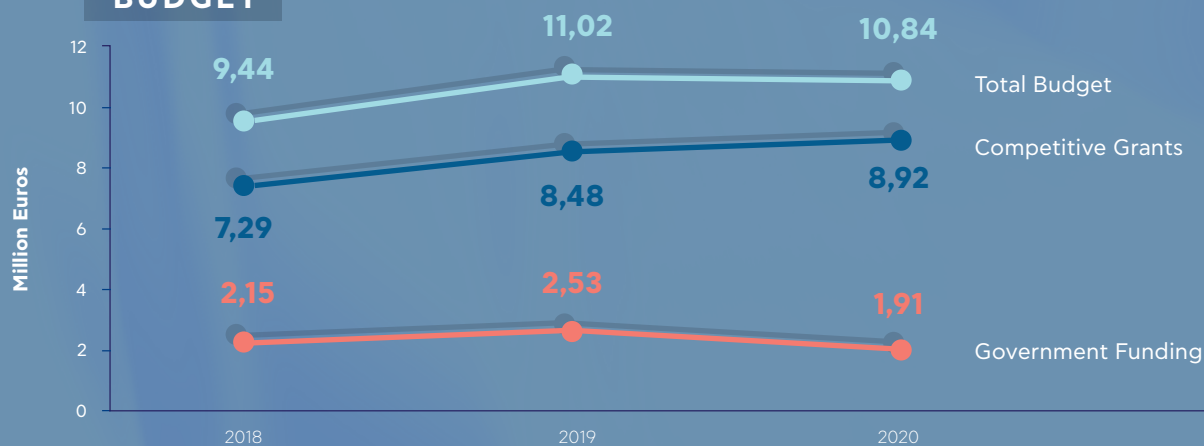
Finally, in the framework of a research project, IMBB in collaboration with the UoC Medical School has organized a screening program for the regular testing of FORTH employees, where 26 IMBB members, including students, PIs, technicians and post-docs worked in a voluntary basis.

An abstract graphic featuring a large, irregular blue circle with a white outline, set against a solid orange background. Several thin, white, curved lines radiate from the top and bottom of the circle, creating a sense of motion or flow. The text "FACTS & FIGURES" is centered within the blue circle in a bold, white, sans-serif font.

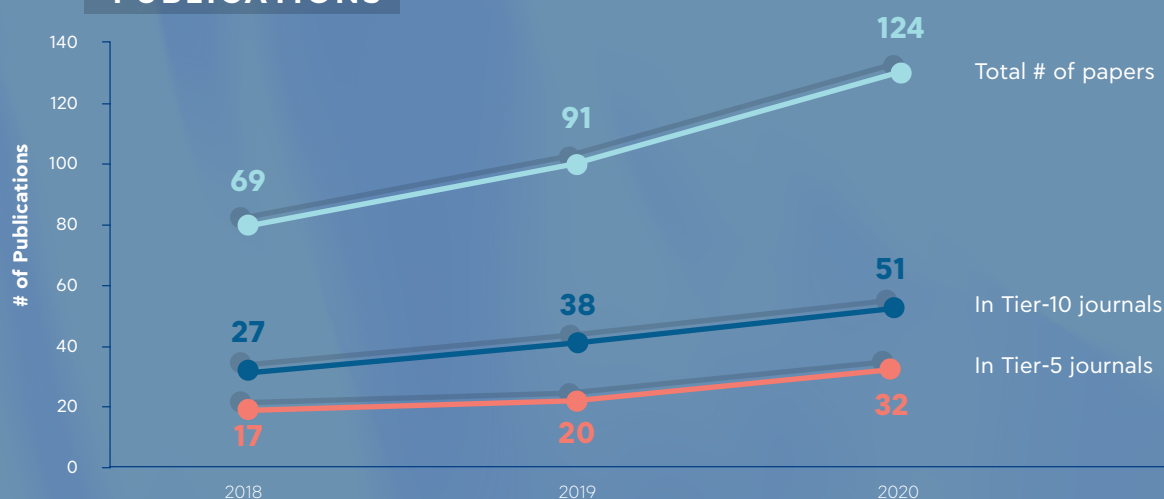
FACTS & FIGURES

FACTS & FIGURES

BUDGET

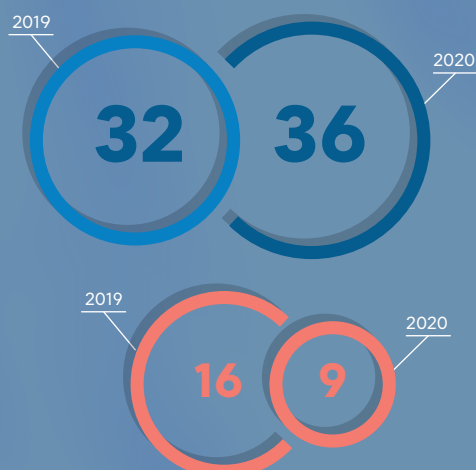


PUBLICATIONS

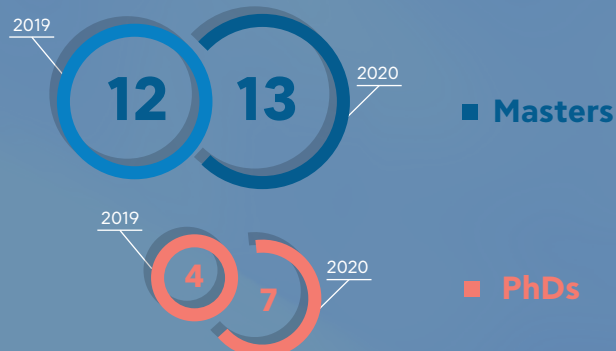


MASTERS AND PHD DEGREES AWARDED

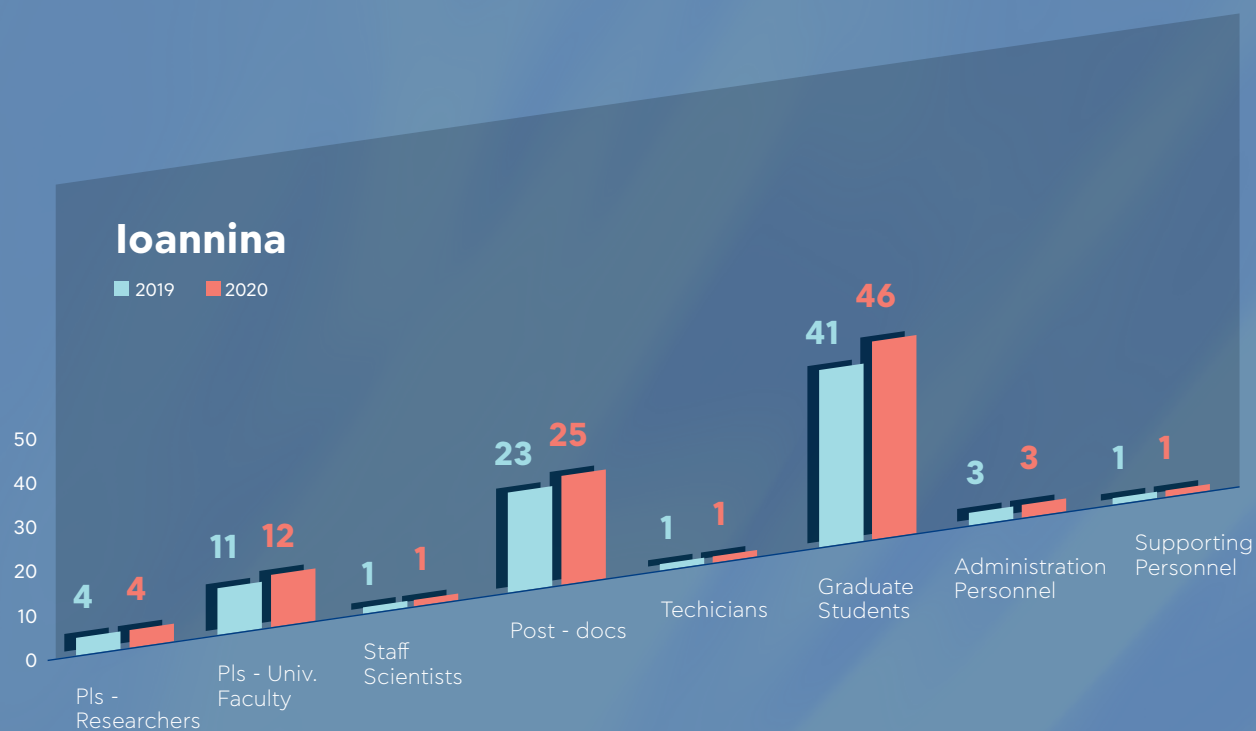
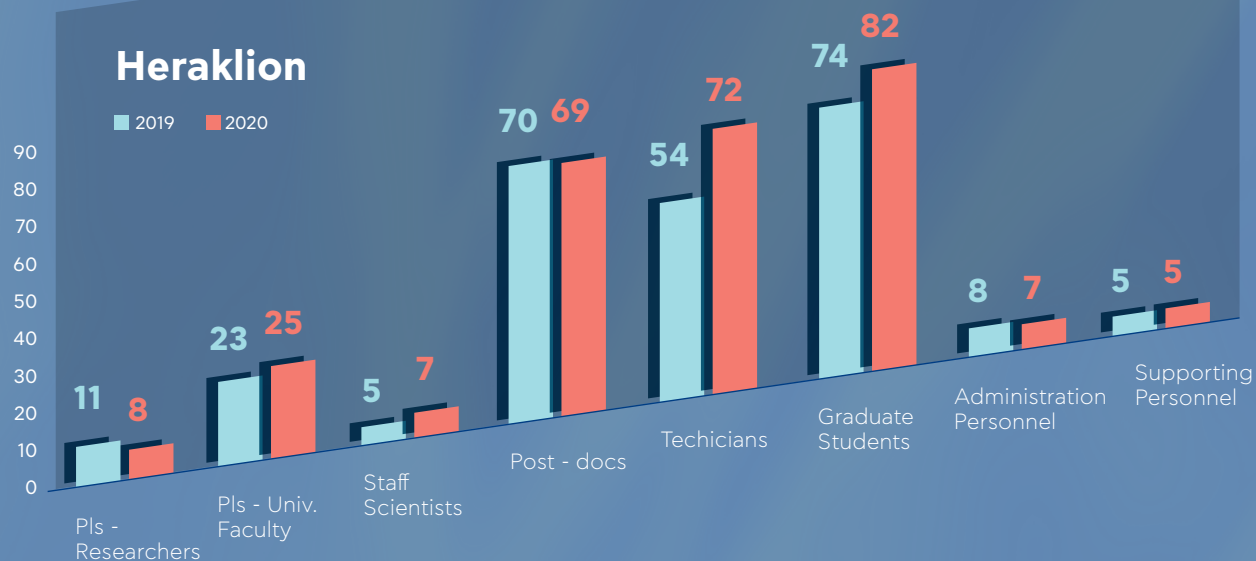
IMBB Heraklion



IMBB Ioannina



IMBB PERSONNEL IN 2019 & 2020





SCIENTIFIC HIGHLIGHTS



THE ACHILLES' HEEL OF MALARIA VECTORS: NOVEL INSECTICIDE RESISTANCE MECHANISMS EXPRESSED IN THEIR LEGS

Malaria has halved since 2000 (approximately 500,000 lives saved every year), with 80% of the reduction attributable to the use of insecticides. However, insecticide resistance is at a critical tipping point in public health, with some mosquito populations showing resistance to all insecticides and the strength and impact of this resistance is escalating every year. As a result, for the first time after many years, malaria cases were increased in several places after 2015, despite the far more intense use of insecticides.

The Molecular Entomology group of IMBB led by John Vontas, in close collaboration with researchers at the Liverpool School of Tropical Medicine revealed novel insecticide resistance mechanisms expressed in mosquito legs, the most relevant insect tissue for contact insecticide uptake.

Modification of epicuticular hydrocarbons and remodeling of leg cuticles, as well as the overexpression of leg-enriched sensory appendage proteins (SAP2) confers pyrethroid resistance in *Anopheles gambiae*. SAP2 expression is elevated in insecticide-resistant populations and is further induced upon mosquito contact with pyrethroids. SAP2 silencing fully restores mosquito mortality, whilst its overexpression results in increased resistance, likely due to the high-affinity SAP2 binding to pyrethroid insecticides. Mining of genome sequence data reveals a selective sweep near the SAP2 locus in three West African countries, with the observed increase in haplotype associated SNPs mirroring increasing resistance reported in Burkina Faso.

These studies identify new insecticide resistance mechanisms that are highly relevant to malaria control efforts.

Balabanidou V. et al. (2019) **Proceedings of the Royal Society B** 286:20191091

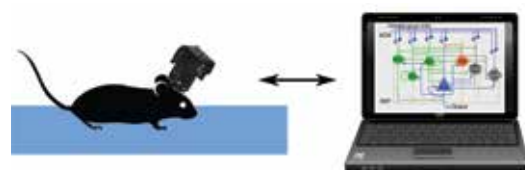
Ingham VA et al. (2020) **Nature** 577:376-380

NEW INSIGHTS INTO THE PATHOGENESIS OF EPILEPSY

It is widely known that the hippocampus – a brain area that is well preserved across species – is involved in the encoding of spatial information, namely information regarding an animal's location in space. This information is used to enable animals to navigate effectively in different environments. The hippocampus is divided into distinct subareas that process such spatial information. For this study, the researchers focused on two areas: the Dentate Gyrus (DG) and the area Cornu Ammonis 1 (CA1), which serves as the output of the hippocampus. While the neuronal mechanisms of spatial coding by CA1 neurons are well characterized under healthy conditions, the effects of epilepsy remain unknown.

In a collaborative effort, the laboratories of Drs. Peyman Golshani at UCLA, Tristan Shuman at Mount Sinai Hospital and Panayiota Poirazi at IMBB, used a multitude of techniques to investigate the network mechanisms that underlie spatial coding deficits in epileptic mice. They found increased interneuronal death in area CA1 of epileptic animals, along with a significant desynchronization between CA1 and Dentate Gyrus interneurons. To pin down which of these alterations has a causal effect on spatial coding, the authors combined molecular analyses, *in vivo* cellular imaging, *in vivo* electrophysiology and computational modeling.

Using a novel, wire-free two-photon miniscope, the teams of Drs. Golshani and Shuman monitored the activity of CA1 pyramidal neurons as mice ran on treadmills laden with various sights and sounds, some familiar and others new. This allowed the examination of how the animals' brains responded, as they freely explored their surroundings. They found that the number of cells encoding the animal's location in CA1 – so called place cells – was reduced in epileptic animals, while those that



remained functional were unstable and less informative compared to the cells of healthy mice. Complementary data from silicon probes demonstrated that interneuronal populations, between the DG and CA1 areas, were out of sync in epileptic but not in healthy mice.

Computational modeling undertaken by the team of Dr. Poirazi at IMBB incorporated both of these findings in computational models to dissect their relative contributions. Specifically, PhD student Ioanna Pandi and postdoctoral fellow Spiros Chavlis developed a detailed CA1 circuit model that simulated spatial coding in both healthy and epileptic virtual mice, while navigating respective virtual environments. Modelling revealed that temporally precise intrahippocampal communication is critical for the encoding of spatial information by CA1 place cells. Epilepsy-induced desynchronization of these inputs significantly impairs the animals' ability to encode spatial information while the respective interneuronal loss has a minor effect.

Shuman et al. (2020) *Nature Neuroscience* 23: 229-238

NOVEL ROLE OF MITOPHAGY IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE

Alzheimer's disease is the most common form of dementia affecting millions of patients worldwide. Although intense research efforts have been focused on the development of novel therapeutic strategies against Alzheimer's disease, at the moment there is no effective treatment. Mitochondrial impairment is a major hallmark of Alzheimer's disease pathophysiology. Mitochondria are indispensable and highly dynamic, energy-generating organelles in all eukaryotic cells that also play pivotal roles in fundamental cellular processes. Alterations in mitochondrial homeostasis heavily impact cellular metabolism, and critically influence organismal physiology. Neuronal cells are critically dependent, perhaps more than any other cell type, on proper mitochondrial function. Thus, maintenance of neuronal homeostasis



necessitates a tight regulation of mitochondrial quality control. A wide range of complex and highly specialized molecular and cellular pathways have evolved to preserve mitochondrial homeostasis. Mitophagy is a selective type of autophagy mediating the elimination of dysfunctional mitochondria, and is the major mechanism, by which cells adjust their mitochondrial content in response to stress of metabolic state.

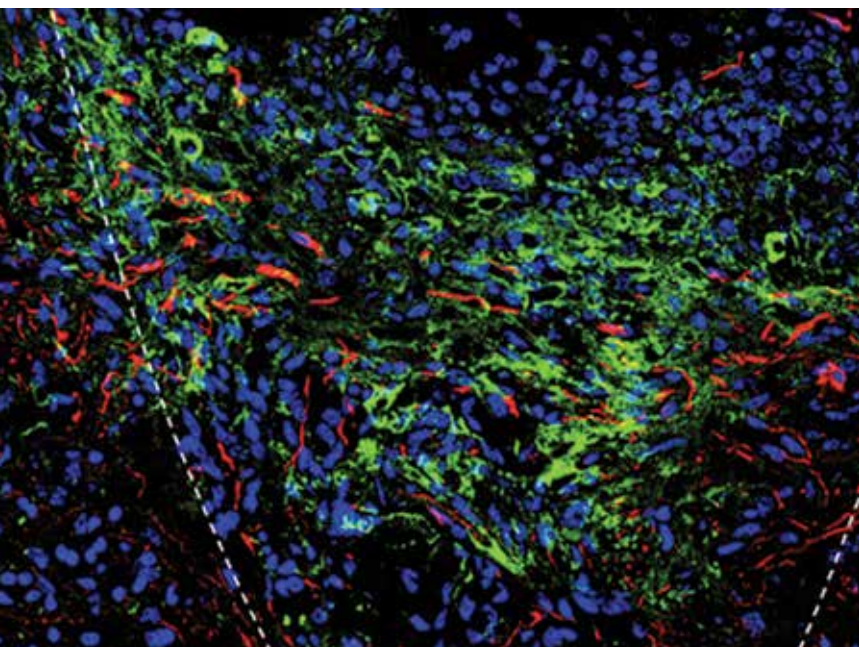
Using a cross-species multifaceted approach involving both human patient samples and cells, the mouse (*Mus musculus*) and the simple nematode *Caenorhabditis elegans* the research teams of Prof. Tavernarakis, Dr. Fang and Prof. Bohr now show that defective mitophagy has a pivotal role in the development and progression of Alzheimer's disease. Accumulation of dysfunctional mitochondria has been detected, post-mortem, in brains of Alzheimer's disease patients, as well as in mouse and nematode transgenic models of the disease. Interestingly, pharmacological upregulation of mitochondrial clearance ameliorates several pathological features of Alzheimer's disease. Importantly, mitophagy induction reverses cognitive impairment both in transgenic nematodes and mice. These findings identify defective removal of damaged mitochondria as a critical contributor to Alzheimer's disease pathogenesis and highlight modulation of mitophagy as a potential therapeutic intervention strategy. The evolutionary conservation of the regulatory factors involved in the elimination of defective mitochondria underscores the pivotal role of mitophagy across species. The identification of specific mitophagy modulators may lead to the development of effective therapeutic intervention strategies tackling mitochondrial-associated pathologies and provide critical insights with broad relevance to human health and quality of life.

Fang EF et al. (2019) *Nature Neuroscience* 22: 401-4

NEUROIMPLANTS TO TREAT SPINAL CORD INJURIES

A pioneering research study by researchers at IMBB-FORTH and the University of Crete suggests the development of neuroimplants for the regeneration of nerve tissue in cases of spinal cord injury. The materials and cells used are non-toxic for humans, and approved from FDA for their immediate translation in humans. This study demonstrates for the first time that neuroimplants based on well-characterized porous collagen scaffolds, similar to FDA-approved ones used in human regenerative medicine, can prepare for culture, transfer and protect embryonic neural stem cells, transplanted to spinal cord injury areas, leading to an almost complete recovery of movement and sensation in an experimental model of spinal cord injury in the mouse, so that the ability of the animals to move 12 weeks after their injury and transplantation is no different from the control healthy animals with no injuries.

Kourgiantaki et al. (2020) *Nature Regenerative Medicine* 5:12

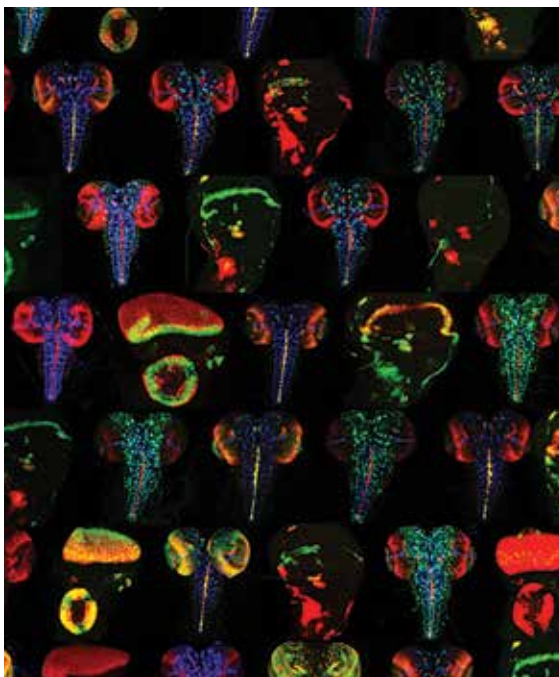


A NOVEL MECHANISM OF INTERCHROMOSOMAL GENE REGULATION

Precise activity of a gene requires its promoter to be matched with an appropriate enhancer. Enhancers are stretches of DNA near a gene, which recruit transcription factors, a cohort of regulatory proteins that instruct the gene to get transcribed (turn on) or remain silent. They do so by looping the chromosome thread to bring the enhancer near the promoter, another DNA stretch at the start of the gene's coding sequence, where the transcriptional machinery is recruited to initiate copying of the DNA to RNA. Although promoters and enhancers are intensely studied, another less prominent type of DNA element in this fundamental process of gene regulation are the so-called insulators. Insulators serve to limit inappropriate enhancer-promoter interactions and are important players in subdividing animal genomes into domains of independent gene activity.

Pawel Piwko and coworkers in Christos Delidakis group, revisited a phenomenon of inter-chromosome gene regulatory interaction. The two homologous chromosomes (one from each parent) are closely aligned in all cells of the fruitfly *Drosophila melanogaster*. This apparently enables enhancers from one homologue to activate promoters on the opposing homologue. This is an unusual mechanism of gene regulation, since normally enhancers, promoters and coding regions are in proximity of each other on the same DNA molecule. When it was first recognized by Nobel laureate Ed Lewis in the 1950's, it was dubbed transvection.

IMBB scientists used modern transgenesis techniques to insert artificially assembled genes in specific places (loci) in the fruitfly's genome. They then studied the activity of a large number of pairs of such transgenes, each member of the pair inserted in the same locus of two homologous chromosomes. They found that transvection requires the presence of an insulator element on both homologues. When the same insulator is placed next to a transgene in both homologues, an enhancer from the one transgene can robustly activate transcription from a promoter in the other. They showed that four different insulators can support transvection, and that this can happen at any of several loci in the genome, as



long as chromosome pairing is not disrupted. Though necessary, the presence of homotypic insulators is not sufficient for transvection; their position, number and orientation matters. The identity of enhancers and promoters in the vicinity of a trans-interacting insulator pair is also important, indicative of complex insulator-enhancer-promoter interactions. It therefore appears that transvection is simply an epiphenomenon of normal gene regulatory mechanisms (that are traditionally thought to take place within the same chromatin thread) and the ability of *Drosophila* homologous chromosomes to pair with each other. As such, it highlights a heretofore little appreciated positive role of insulators in transcription and proposes that insulators could generally function as powerful and versatile gene regulators.

Piwko et al. (2019) *Genetics* 212:489-508

DNA DAMAGE TRIGGERS METABOLIC REPROGRAMMING, LEADING TO AGING

Inborn defects in DNA repair mechanisms are associated with cancer, aging but also complex metabolic and endocrine disorders. Integrity of the genome is critical for normal cellular function but the DNA is continually challenged by intrinsic and extrinsic genotoxic factors. To counteract DNA damage, cells have evolved DNA repair mechanisms ensuring that the genome remains functionally intact and is faithfully transmitted to progeny. Nucleotide excision repair (NER) is a major DNA

repair mechanism that cells employ to remove a wide class of bulky, DNA-distorting lesions from the genome. The importance of NER defects in man is illustrated by rare syndromes that either show increased cancer predisposition or dramatic features of accelerated aging, including depletion of fat depots. However, with the exception of cancer and aging, the links between defects in NER and the rapid onset of developmental defects in humans are not well understood.

A new study from George Garinis lab provides evidence that persistent DNA damage triggers an exosome-based, metabolic reprogramming that leads to chronic inflammation and tissue pathology in DNA repair-deficient progeroid syndromes and likely also during aging. Using mice with an engineered DNA repair defect in tissue-infiltrating macrophages (cell type-specific ablation of a NER factor), the scientists revealed a fundamental mechanism by which irreparable DNA lesions in circulating macrophages secrete exosomes that trigger a metabolic switch leading to chronic inflammation.

Specifically, persistent DNA damage accumulation in tissue-infiltrating macrophages triggers robust cytoplasmic changes, autophagy and exosome biogenesis leading to the secretion of extracellular vesicles *in vivo* and *ex vivo*. The researchers discovered that the exosome cargo is taken up by recipient cells leading to enhanced cellular glucose uptake and to higher cellular oxygen consumption rate in mice. In turn, the IMBB researchers discovered that high glucose levels in recipient cells triggers pro-inflammatory stimuli. This, in turn, establishes chronic inflammation and tissue pathology in mice with important ramifications for DNA repair-deficient, progeroid syndromes and aging.

The findings provide a novel mechanism to explain how DNA damage is causally linked to metabolic abnormalities and inflammation in man.

Goulielmaki et al. (2020) *Nature Communications* 11:42



CHALLENGE OF THE DOGMA THAT WANTS INTERNEURONS TO BE "SIMPLE"

Interneurons are the main inhibitory class of cells in the brain of mammals. They come in many flavors, varying in shape, localization, connectivity and intrinsic properties. Despite this variability, they are mainly attributed a generic task: to maintain the excitation / inhibition balance necessary for normal brain functioning. Indeed, disruption of their proper functioning is associated with several neurodegenerative disorders such as Alzheimer's, Parkinson's, epilepsy etc.

For decades, interneurons were assumed to sum their inputs and, if strong enough, cause the cell to fire. This simple type of computation, also referred to as a "point neuron" computation, ignores any non-linear influences that could be generated in the dendrites of these cells. Recent experimental findings however, report nonlinear dendritic events in many interneuron subtypes, implying that interneurons may be much more than simple point devices.

To test this proposition, PhD student Alexandra Tzilivaki and postdoctoral fellow George Kastellakis in the Poirazi Lab at IMBB, developed a toolset of computational models covering two brain areas related to learning and memory functions, the hippocampus and the prefrontal cortex. This toolset consisted of a) full-blown morphologically and biophysically detailed models of Fast Spiking Basket Cells (FSBCs), b) mathematical reductions of these cells in the form of artificial neural networks and c) a network model with both excitatory and inhibitory cells, able to form new associative memories. They used these models to test how FSBCs integrate at the single neuron level and how their computational properties may affect learning and memory.

The first interesting finding was that in all model cells

tested, in both brain areas, FSBCs had non-linear dendrites. Depending on their morphology and biophysics, these dendrites could either boost incoming inputs (supra-linear dendrites) or suppress them (sub-linear dendrites). The presence of these non-linearities enabled individual FSBCs to produce a wide range of responses that could not be captured by a point neuron reduction. Instead, the researchers showed that the best way to capture what an FSBC is actually computing was to assume a 2-layer Artificial Neural Network that incorporates the two types of dendrites found in these cells: supra-linear and sub-linear. This finding raises this particular interneuron subtype to the same level of processing power as pyramidal neurons, the main excitatory neurons involved in most complex behaviors, including learning and memory.

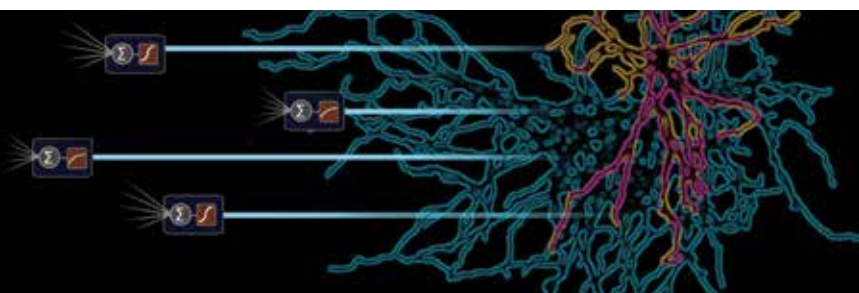
Taking it one step further, the researchers implemented a network model with both excitatory and inhibitory neurons, capable of forming associative memories. They showed that the nonlinear dendrites of FSBCs can significantly enhance learning and memory in this network model, compared to a respective network with only point FSBCs (implemented with linear dendrites). This prediction shows the wider implications of considering dendritic nonlinearities in interneurons, a deed that has been ignored for years.

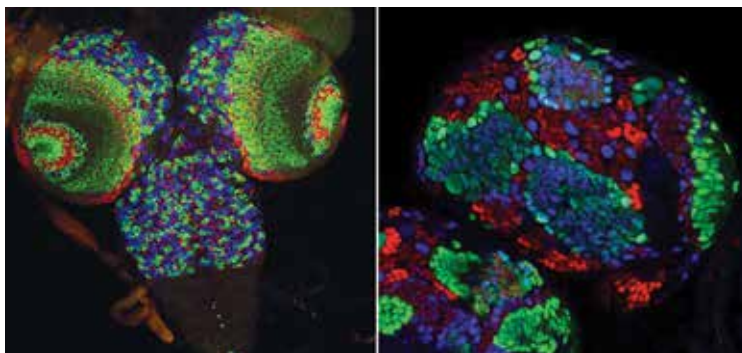
The work provides a novel view of dendritic integration in FS interneurons that extends in hippocampal and cortical areas. It also provides a more accurate reductionist description of interneuron processing that may have substantial influences in fields beyond Neuroscience, including machine learning and artificial intelligence.

Tzilivaki et al. (2019) *Nature Communication* 10:3664

A SINGLE GENETIC INSULT CAN INDUCE MALIGNANCY TO NEURAL STEM CELLS

Somatic stem cells are long-lived cells that populate many animal tissues and retain the ability to divide in order to generate new differentiated cells to expand the tissue and/or to replenish dying cells. Stem cell longevity coupled to their division competence makes them particularly prone to carcinogenesis. A simple fruitfly model for neural stem cell (NSC) tumours was described





over a decade ago by the lab of Cayetano Gonzalez, IRB Barcelona. They showed that disrupting a well-studied process, the asymmetric division of NSCs in juvenile fruitflies (*Drosophila* larvae) often leads to malignancy, manifested as uncontrolled proliferation, which can be perpetuated by transplanting the larval tissue to an adult host. NSCs in flies (and mammals) divide asymmetrically, giving rise to a new NSC and a more differentiated neuronal or glial progenitor. In flies this asymmetry has been studied in great molecular detail and involves asymmetric partitioning a small group of proteins and RNAs to one or the other daughter cell. These asymmetrically partitioned factors affect transcription, translation and Notch signaling, a cell-cell communication module that allows neighbouring cells (in this case the two NSC daughters) to influence each other's functions. New work from the group of Christos Delidakis has shown that disrupting only one of these processes, Notch signaling, is sufficient to generate NSC tumours.

In normal asymmetric divisions, only the new-NSC daughter receives Notch signaling. If we artificially impose this signal on both daughters, we often produce supernumerary aberrant stem cells. These can go on to overproliferate and can cause malignant tumours when transplanted to adult hosts. The researchers dissected the gene regulatory networks that underlie this malignant transition using genomic tools. They showed that a group of transcriptional repressors induced by Notch, the Hes proteins, occupy a number of sites in the stem cell chromatin and repress nearby genes. Many of the Hes target genes are promoters of differentiation. Two that were studied in more detail were able to suppress tumorigenesis when they were re-expressed in Notch-induced NSC tumours. This work has therefore opened a path for dissecting the complex gene regulatory circuits that lead to neoplastic transformation of stem cells.

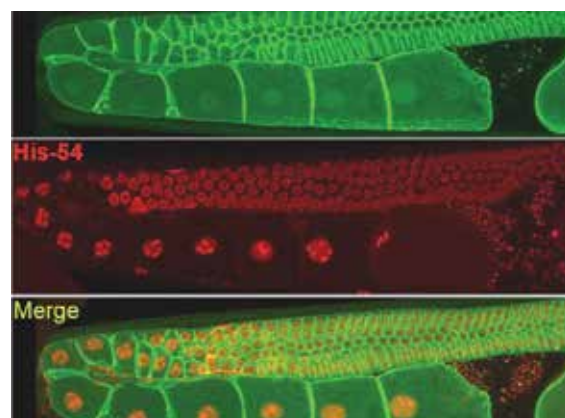
Magadi et al. (2020) *Development* 147, doi: 10.1242/dev.191544

A LINK BETWEEN MITOCHONDRIAL MATURATION AND GERM CELL DIFFERENTIATION

Unlike somatic cells, gametes are virtually immortal and fulfill the pivotal task of protecting and faithfully transferring genetic material across generations. Mitochondria are cellular organelles of prokaryotic origin which execute numerous functions in eukaryotic cells, with ATP production being the most prominent one. IMBB researchers have demonstrated that inhibition of mitochondrial genome (mtDNA) transcription leads to germline tumor formation in nematodes, due to impaired differentiation and oocyte production. Thorough monitoring of germline mitochondria highlighted that they transition from a globular to a tubular shape during differentiation. Elongated organelles, in contrast with globular mitochondria of undifferentiated germ cells, exhibit various signs of functional maturation, such as, increased ATP production and elevated membrane potential, as well as, enhanced production of reactive oxygen species (ROS). This mitochondrial maturation process is tightly controlled by sperm-originating (MSP) signals, which are known to promote oogenesis and ovulation.

These findings integrate mitochondria and their homeostasis in the developmental modules which shape the *C. elegans* germline. Moreover, the results establish enhanced mitochondrial maturation as a prerequisite for stem cell differentiation in diverse model organisms, and further implicate augmented mitochondrial activity in tumorigenesis.

Champilas N. and Tavernarakis N. (2020) *Cell Death and Differentiation* 27:601





TIMELINE

TIMELINE

20
19

Jean-Eric Piquet EU Director General of Research and Innovation visits FORTH Institutes and the Genomics Facility of IMBB

New Group Leaders Anastasios Pavlopoulos and Christos Gkogkas join IMBB

MARCH

MAY

IMBB's Proteomics Facility joins the European Proteomics Infrastructure Consortium (EPIC-XS), a network of Europe's most renowned proteomics laboratories.

IMBB Researchers co-organize the EMBO Workshop: Cell Biology of the Neuron: Polarity, Plasticity and Regeneration



OCTOBER



IMBB-FORTH hosts the 12th Hellenic Bioinformatics Society Meeting entitled "Precision Medicine, Biodiversity and Genome biology"

Triantafyllos Chavakis and Ioannis Mitroulis join IMBB as adjunct Researchers

New Group Leader Emmanouil Froudarakis joins IMBB



Presentations in 2019 Researcher's Night

Emeritus Professor Theodore Fotsis becomes Honorary Member of FORTH.



JUNE

George Garinis is elected member of EMBO

Nektarios Tavernarakis is elected Corresponding Member of the Academy of Athens

International FELASA course "Care of Laboratory Animals"

JULY

Onassis Foundation Lecture Series in Biology and Chemistry on the topic of Genome Editing

**AUGUST**

Panagiota Poirazi is awarded Visiting Fellowship by the Albert Einstein Foundation Berlin

SEPTEMBER

The National Academy of Sciences of the USA has published a Biographical Memoir honoring Fotis Kafatos, the Founder and first Director of IMBB

**DECEMBER**

Giorgos Chamilos is awarded an ERC Consolidator Grant

Foundation of Enzyquest, a new spinoff company of IMBB.

"Stories of Genetics" a popular science book written by Emeritus Professor Kitsos Louis is published.

NOVEMBER

Nektarios Tavernarakis is elected Member of the German National Academy of Sciences Leopoldina

George A. Garinis awarded with the Friedrich Wilhelm Bessel Research Award

Foundation of Biopix DNA Technology, a spin-off company of IMBB



TIMELINE

20
20

FEBRUARY

Alexander Domling
joins IMBB as Adjunct
Researcher



Nationwide "lockdown" to control
Covid-19 pandemic places IMBB under
emergency operation conditions

Loss of Dimitris Kafetzopoulos a
Researcher and Former Deputy
Director of IMBB

MARCH



SEPTEMBER

New Group Leader
George Gouridis
joins IMBB

NOVEMBER

Nektarios Tavernarakis is
elected Vice President of the
ERC Scientific Council

Online Presentations in 2020
Researcher's Night

JUNE

Jean-Paul Latge joins
IMBB as adjunct
Researcher

Panos Verginis joins IMBB
as a Collaborating UoC
Faculty Member

**JULY**

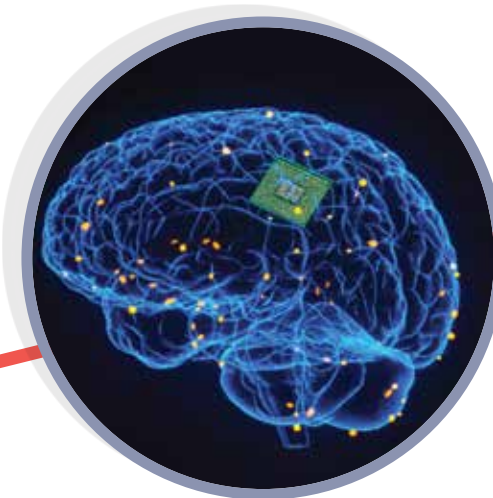
IMBB formally joins EU-LIFE
as an Associated Member

**AUGUST**

Loss of IMBB Alumni
Alekos Athanasiadis

DECEMBER

The Quanta magazine named Breakthrough
of the Year, the discovery of a new class
of calcium mediated dendritic action
potentials a research described in Science
(Vol 367, pp 83-87) paper co-authored by
IMBB Researchers Panagiota Poirazi and
Athanasia Papoutsis.





WHAT PEOPLE SAY





WHAT PEOPLE SAY

“The IMBB was my scientific home for more than a decade. The place where I first established my research lab, surrounded by colleagues and students who became friends for life. It’s a warm and supportive scientific environment, with an excellent scientific atmosphere and strong international ties on the southernmost corner of Europe.”

Michalis Averof (former IMBB Group Leader)

The IMBB-FORTH is a beacon of creativity and innovation, an institute that shows what can be done by a group of committed and passionate people.

Acino Silva – UCLA (collaborator)

It has been a great pleasure to collaborate with Yiota Poriazi’s group and others at IMBB-FORTH over the past years. During my visit at the DENDRITES meeting I had the opportunity to experience personally the unique combination of cutting-edge research, outstanding scientific community, all located in a wonderful environment at IMBB-FORTH that I consider a pinnacle of biomedical research in Greece and beyond. I am looking forward to our continued collaboration.

Attila Losonczy – Columbia University (collaborator)

I joined IMBB few years ago as a Marie Curie fellow. I pursue my career as young scientist and continue to be positively challenged by IMBB environment. It was a great experience to enjoy my career at IMBB and proud of being a member of the institute.

Chiara Currà (Post-doc)

IMBB is a unique place where the regional spirit meets the world’s class level science.

**Tomas Zelenka,
(PhD student)**

IMBB makes up a hub that provides opportunities for education and wide range, interdisciplinary research of high standards. People of IMBB constitute a vibrant, collaborative, supportive, friendly, inspiring and coherent community. I feel very fortunate I had a chance to be a member of this community.

Pawel Piwko (former PhD Student)

IMBB hosts passionate, innovative and outstanding researchers with the most welcoming multinational scientific community, creating world-class scientists that are spread across the world.

Srivathsa Magadi (former PhD student)

I am excited to be part of IMBB-FORTH, a top research institution where I can do highly collaborative projects with high-impact that would otherwise be infeasible in a traditional academic research environment.

Emmanouil Froudarakis (Group leader)

Being a PhD student at IMBB has helped me grow both professionally and personally. I will always look back on my years at IMBB with gratitude, that I was so lucky to be part of an excellent research and family-like environment which always strives to get better, no matter the circumstances

Andrea Princz (former PhD student)

Working at IMBB was a memorable experience due to the excellent working conditions, fantastic colleagues and a great work/life balance enhanced by the natural beauty of Crete!

John Strouboulis (Former IMBB Group Leader)

A highly cross-disciplinary (from molecular biology to optics, lasers and computational mathematics) environment established at FORTH is at the same time compact and highly collaborative. The key “ingredients” for stimulating biophysical research at IMBB.

Gouridis Giorgos (Group Leader)

With a fellowship from the Indonesian government (LPDP), I recently obtained a PhD from two top European Universities (Univ. of Groningen/ KU Leuven). I am excited to continue my post-doctoral scientific journey in molecular biophysics at IMBB and the sister FORTH Institutes! The multi-disciplinary environment created by the institute emerged a very positive atmosphere! Here, I learn that doing sophisticated science should be open and fun for everyone! This environment will help me to adapt and grow to be an enthusiastic scientist quickly!

Yusran Abdillah Muthahari. (Postdoctoral Researcher)

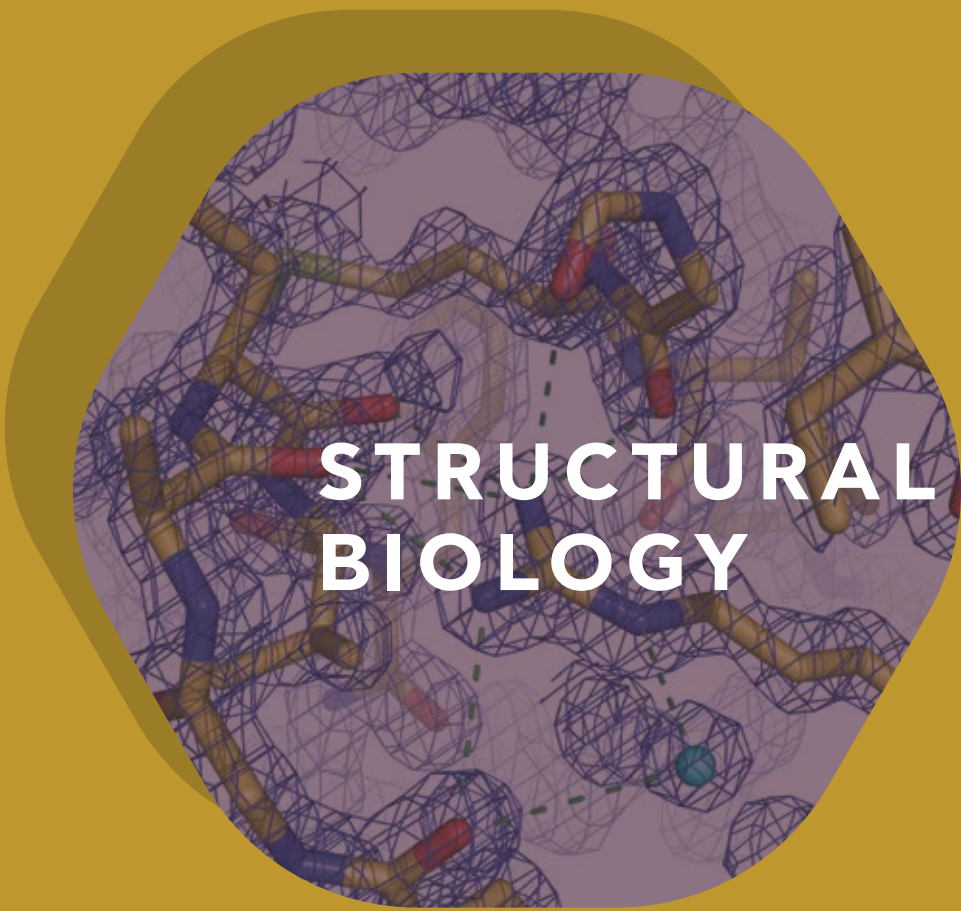
After 18 years abroad, I joined the IMBB in 1983 having accepted Fotis Kafatos' offer, and I have certainly not regretted this decision. During these 33+ years till my retirement, which initially included helping the set-up of the IMBB the labs from scratch, I was able to perform some very competitive research, especially in insect genomics before even that term became “established. I made very good friends, and my family and I enjoyed life in our final home, Crete. Even if this sounds somewhat exaggerated in a typical Greek way, the IMBB was, after all, a “dream come true”!

Kitsos Louis (Emeritus Professor)

I had a very fruitful, long term collaboration with IMBB that resulted in the successful completion of many research programs as well as Masters and PhD theses performed in my laboratory.

Basilis Bouriotis (Emeritus Professor)





STRUCTURAL BIOLOGY

STRUCTURAL DYNAMICS TO UNDERSTAND LIFE AT THE MOLECULAR LEVEL



Giorgos Gouridis

Principal Researcher (B)

GROUP MEMBERS

Senior research personnel:

Charalambos
Pozidis

Postdoctoral researcher:

Yusran A.
Muthahari

PhD student:

Ruixue Xu
(co-supervised with Prof.
Dömling)

Summary

The aim of our group to understand life and evolution at the molecular level is persuaded by studying protein folding, binding and dynamics. Our lab's holy grail is to: (i) Find the key structural elements and understand how such modulate the depth of the energy valleys of the folding funnel, (ii) Understand how such elements modulate the enthalpic-entropic factors to by-pass the transition barriers and ultimately (iii) How these elements vary during evolution to confer distinct functions and substrate specificities.

Our recent research uncovered the remarkable modularity of proteins. During evolution a conserved structural core can acquire secondary structural elements, alike lego bricks added on a core-board over time. Such elements confer multi-tier structural dynamics to the core, diversifying its function and specificity.

Current Aims

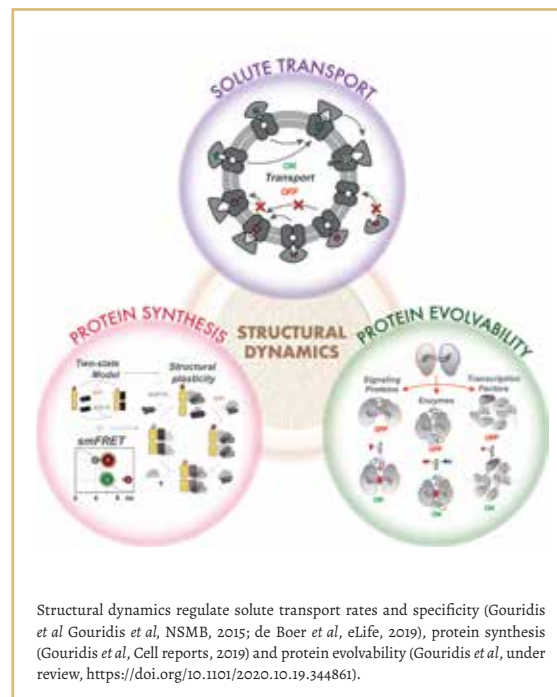
Our research line is based on a 3-pronged approach: (A) Fundamental research; (B) Drug & Biosensor development and (C) Tech transfer / High-throughput screening. For this, we are focusing on human Ras signaling and bacterial bilobed proteins. The former ones regulate many signaling pathways being altered in 30% of human cancers, whereas the later are responsible for transcriptional regulation (e.g. LysR transcription factors), pathogenicity (e.g. FbpA) or mediating the uptake of nutrients (e.g. MalE). The vision of our newly formed group at IMBB is to act alike a “seed” placed into the IMBB “soil” embedded within the ITE-FORTH “garden” to establish and develop biophysical tools (smFRET, HDX-MS, etc.) for creating a unique hub in Greece for modern dynamic structural biology related research. We aim the “harvest” to be top-level fundamental and translational science. We are grateful to the groups of Prof. Cordes (LMU-Germany) and Prof. Dömling (RUG-The Netherlands) for gifts, material-and expertise-transfer via which we are materializing our vision.

Progress in 2019-2020

- i) The biannual period was a transition period for our laboratory. Our team hosted within the laboratory of Molecular Bacteriology headed by Prof. Economou transited within IMBB-FORTH.

- ii) We elucidated the role of structural dynamics in:

- a) Protein synthesis (Gouridis *et al*, Cell reports). The two nucleotide-binding sites of the ribosome recycling factor are in a dynamic structural equilibrium regulated by nucleotides, ribosomes and release factors.
 - b) Solute transport (de Boer *et al*, eLife). The structural dynamics of the substrate binding proteins (SBPs) dictate the SBP-ligand interactions but also the transport rate and selectivity.
 - c) Protein evolvability (Gouridis *et al*, under review). Signal propagation originating from ligand binding through an ancestral and conserved structural core is modulated by distinct structural dynamics.
- iii) We also extended our single-molecule FRET toolbox (de Boer *et al*, Biophysical journal) and additionally expanded via fruitful collaborations our one-molecule-at-a-time procedures to include nanopores (Zernia *et al*, ACS nano)



Other activities

- i) Participated in 2 multi-PI grants
- ii) Five researchers the PI was (co) supervising obtained their PhDs

Web page

<https://www.imbb.forth.gr/en/research-en/structural-biology/item/5040-giorgos-gouridis>

Publications

Gouridis G, *et al*. (2020) Evolution of structural dynamics in bilobed proteins. (submitted & bioRxiv: <https://doi.org/10.1101/2020.10.19.344861>)

Gouridis G, *et al*. (2019) ABCe1 controls ribosome recycling by an asymmetric dynamic conformational equilibrium. **Cell reports** 28:723-734

de Boer M, *et al*. (2019) Conformational and dynamics plasticity in substrate-binding proteins underlies selective transport in ABC importers. **eLife** 8:pii: 44652

STRUCTURAL BIOLOGY



M. Kokkinidis

Professor Emeritus -
Collaborating Faculty
Member

GROUP MEMBERS

Post Doctora Researchers:

Dr. M. Amprazi

Dr. K. Kefala

Dr. E. Mylonas

PhD students:

A. Molfetas

A. Lemonakis

Research Assistants:

D. Kotsifaki

M. Providaki

Visiting scientist:

Dr. Rana Rehan
Khalid

Summary

Although nearly 50 years ago, Christian Anfinsen was awarded the Nobel Prize for showing that the shape of proteins is determined by their sequence of amino acids, protein folding is still poorly understood. We apply a wide range of Structural Biology techniques to develop a detailed and comprehensive understanding of protein folding. Based on this knowledge, we engineered novel proteins for bio-inspired materials applications. We are also working on the autocatalytic hydroxylation of the proline C_α atom. This Pro[®] 2-hydroxyproline (2-Hyp) conversion which we discovered, has potentially far-reaching applications in Health and Biotechnology. Furthermore, we are performing structural studies on several enzymes (e.g. the human glutamate dehydrogenase 2, hGDH2) that are relevant to human health and drugs development.

Current Aims

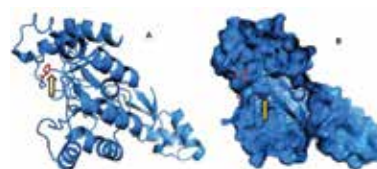
We will pursue our protein folding studies using the highly informative approach of sequence reversal. This allows us to explore folding via sequence-structure relationships not yet sampled by known natural proteins. The unusual properties of inverted proteins will be exploited in bio-inspired materials. Besides the Pro[®] 2-Hyp post-translational modification, we will explore the self-hydroxylation of the C_α atom of glycine. Autocatalytic hydroxylation is crucial to numerous bacterial pathogens, enabling the evasion of host's innate immune system. We will pursue: a) Structural/ functional studies b) Restoration of self-hydroxylation and enzymatic activities in pseudoenzymes from the carbohydrate esterase family 4 (CE4) that have evolved towards inactive forms. c) Discovery of C_α hydroxylation sites in various protein families through computational techniques d) Study of links between C_α hydroxylation and human diseases, e.g. establishment of potential connections between the Gly C_α hydroxylation and cancer proliferation. We will also advance our structure-function studies on enzymes that are important to Human Health, e.g. hGDH2, which is linked to human brain evolution, thereby acquiring unique catalytic and regulatory properties. This opens new avenues for drug design, especially for cancer therapeutics.

Progress in 2019-2020

Amino acid sequence reversal was employed in our protein folding studies. Using α-helical bundles as model systems,

we reversed the sequences of two well-characterized, highly regular four-α-helical bundle proteins and studied folding, oligomerization and physicochemical properties of their retro-proteins, using multidisciplinary approaches. A comparison between parent proteins and their retro-counterparts reveals key parameters for the 'foldability', contributing considerably to our global understanding of protein folding.

The autocatalytic hydroxylation of the C_α atom of Pro was discovered and characterized by us as a process which modifies with utmost specificity a highly conserved Pro in polysaccharide deacetylases (PDAs) active sites. There is a crucial coupling between self-hydroxylation and deacetylation activity, with the former greatly enhancing deacetylation, which in turn is critical for the defense mechanisms of pathogens (*B. anthracis*, *B. cereus* etc.), against the host innate immune system. The conversion Pro → 2-Hyp, opens up previously unanticipated prospects for human diseases, biotechnology, etc. We also discovered a C_α-hydroxylation activity targeting Gly residues, a finding which is potentially relevant to cancer research. Self-hydroxylation stimulated also our interest on evolutionary aspects of "pseudoenzymes" from bacterial pathogens. These molecules, although structurally highly similar to PDAs, lack C_α-hydroxylation and deacetylation activities. We determined several pseudoenzyme structures at very high resolutions. Subsequent structure-guided mutagenesis enabled us to roll back evolution and restore their original activities.



A) Structure of the *B. anthracis* pseudoenzyme Ba3943 (PDB ID 6HPA) with a self-hydroxylation site (arrow), which in pseudoenzymes is inactive. B) The molecular surface of Ba3943 showing structural changes (arrow) associated with the loss of self-hydroxylation activity.

Attempts by several research groups to determine the hGDH2 structure had long remained unsuccessful. We were able to crystallize the enzyme and solve its structure at a resolution of 2.9 Å. hGDH2 adopts a novel semi-closed conformation which is an intermediate between known open and closed GDH1 conformations, differing from both. Our data provide insights into the structural basis of hGDH2 properties, the functional evolution of hGDH isoforms, and open new avenues for drug design, especially for cancer therapeutics.

Other activities

Guest editor for Special Issues of International Journal of Molecular Sciences.

Web page

<https://www.imbb.forth.gr/en/research-en/structural-biology/item/28-michael-kokkinidis>

Publications

Kokkinidis M, et al. (2020) Catalytic activity regulation through post-translational modification: the expanding universe of protein diversity. **Adv Protein Chem Struct Biol.** 122:97-125 doi: 10.1016/bs.apcsb.2020.05.001

Khalid RR, et al. (2020) Probing the Structural Dynamics of the Catalytic Domain of Human Soluble Guanylate Cyclase. **Sci. Rep.** 10:9488 doi: 10.1038/s41598-020-66310-4

Khalid RR, et al. (2019) Dynamic Characterization of the Human Heme Nitric Oxide/Oxygen (HNOX) Domain under the Influence of Diatomic Gaseous Ligands. **Int J Mol Sci.** 20:698-713 doi: 10.3390/ijms20030698

STRUCTURAL BIOLOGY-PROTEIN CRYSTALLOGRAPHY



Kyriacos Petratos

Principal Researcher (B)

GROUP MEMBERS

Research Assistants:

Dr. Renate Gessmann

Mrs. Maria Papadovasilaki

Summary

Our interests stand in the area of the structure and function of biological molecules. In particular, we employ X-rays to determine the crystal structure of proteins and biologically active peptides. The elucidation of the catalytic mechanism of enzymes remains in the core of our interests. Moreover, we have investigated the cold-adaptation of enzymes from psychrophilic organisms via structure comparisons, kinetic measurements and molecular dynamics simulations at varying temperatures. Results include the structure and dynamics of a thermostable alcohol dehydrogenase from the antarctic psychrophile *Moraxella* sp. TAE123 (MoADH) and the atomic resolution structure of an amino-terminus protected tetrapeptide containing alternating residues of glycine (Gly) and α -amino-isobutyric acid (Aib).

Current Aims

At present, we aim at the biochemical and functional characterization of a sterol oxidase from a pathogenic Gram-negative bacterium. The ultimate goal is the high-resolution crystal structure of the enzyme and its complexes with substrates and inhibitors. We continue the efforts to produce adequate amounts of soluble active enzyme. Also, we continue to share our expertise in protein purification with other groups of the Institute.

In parallel, we carry on with the crystallization and structure determination of natural antibiotic peptides (peptaibiotics) and synthetic peptides containing a higher order of Gly-Aib residue repeats in order to explore further the relation between sequence and structure of this interesting class of molecules, which occurs frequently in sections of the known natural peptaibiotics.

Progress in 2019-2020

The analysis of the crystal structure of the homo-tetrameric MoADH along with Molecular Dynamics simulations of its protomer at three different temperatures (280, 310 and 340 K) have been completed recently (Petratos *et al.*, 2020). The work included thermal unfolding of the enzyme probed with circular spectropolarimetry and kinetic measurements in a wide range of temperatures. The latter experiments led to the

conclusion that the enzyme is thermostable (melting temperature approximately 89 °C) and operates from 10 up to at least 53 °C, which is uncommon for an enzyme from a psychrophilic organism. The derived, relatively small value of activation enthalpy ($\Delta H^\ddagger$ 30 kJ/mol) dictates a small dependence of the reaction rates on temperature, which may be the most significant part of the cold adaptation of the *Moraxella* enzyme. The MD simulations were carried out in parallel with the closely related GsADH from a thermophilic bacterium *Geobacillus stearothermophilus*. The results of the latter analyses led to the observation of an enhanced wide-ranging mobility of MoADH at high temperatures and generally lower but more distinct and localized mobility for GsADH. Furthermore, Principal Component Analysis of the fluctuations of both ADHs resulted in a prominent open-close transition of the structural domains mainly at 280 K for MoADH and 340 K for GsADH.

We have also continued our long-standing efforts of crystallizing and solving the structures of peptides with antibiotic activity and of peptides containing Gly-Aib residue repeats of variable multiplicities. The latter peptide structures are analysed in the presence or absence of protecting groups at their amino and carboxyl termini. The latter work allowed among other the observation of the critical role of hydrogen bonding in crystal quality and size (Gessmann *et al.*, 2020).

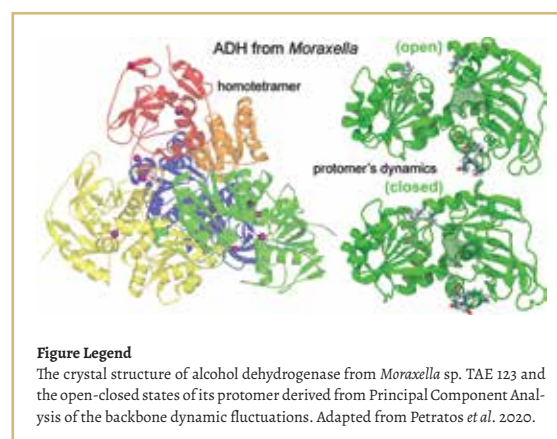


Figure Legend

The crystal structure of alcohol dehydrogenase from *Moraxella* sp. TAE 123 and the open-closed states of its protomer derived from Principal Component Analysis of the backbone dynamic fluctuations. Adapted from Petratos *et al.* 2020.

Other activities

Reviewing activity (K.P.) on behalf of *Chemical Reviews* published by the American Chemical Society. Review identifier: ACS-19-57775

Participation (R.G.) in the European Researchers Night event organized by the Science and Technology Park of FoRTH in September 2019

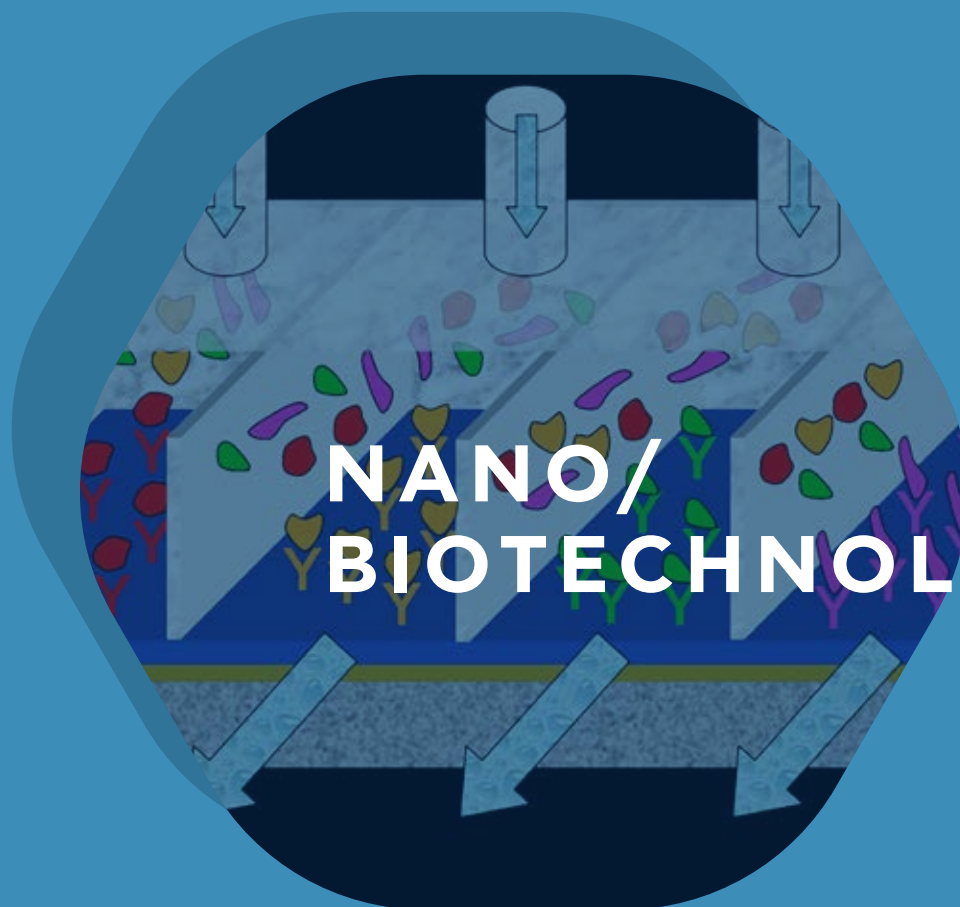
Web page

<https://www.imbb.forth.gr/petratos>

Publications

Petratos K, *et al.* (2020) Structure and Dynamics of a Thermostable Alcohol Dehydrogenase from the Antarctic Psychrophile *Moraxella* sp. TAE123. **ACS Omega** 5(24):14523–14534 PMID: 32596590

Gessmann R, *et al.* (2020) Removing the C-terminal protecting group enlarges the crystal size: Z-(Gly-Aib)₂-OH·H₂O. **Acta Crystallogr. C** 76 (12):1057–1061



NANO/ BIOTECHNOLOGY

BIOSENSORS AND MICRO/NANO-BIOTECHNOLOGY: BASIC RESEARCH TOOLS AND INNOVATION IN MOLECULAR DIAGNOSTICS



Electra Gizeli

Professor - Collaborating
Faculty Member

GROUP MEMBERS

Researcher/Staff Scientist:
Dr. Achilleas Tsortos

Post-Doctoral Researchers:

Dr. George Papadakis

Dr. Alexandros Pantazis

Dr. Pablo Mateos-Gil

Dr. Ilya Reviakine

Dr. Vasilios Raptis

Dr. Dimitra Milioni

Researcher Assistants:

Maria Megariti

(laboratory manager)

Vaia Tsiakalou

Other:

Nikos Fikas

(software developer)

Sergios Katsaros

(business developer)

PhD students:

Dimitra Chronaki

Nikoletta Naoumi

Anastasios Samarentsis

Fotini Papagavriil

Visiting Researchers:

Prof. Rafael Delgado-Buscalioni

(Univ. Autonoma Madrid,
Jan. 2019)

Dr. Monica Araya-Farias

(Curie Institute, Summer 2019)

Dr. Ceyhan Kirmli,

Erasmus visitor (Acibadem

Mehmet Ali Aydinlar

University, May 2019)

Summary

Multidisciplinary research forms the “heart” of Biosensors group, with biophysics, micro-engineering and biotechnology/ molecular biology combined in a variety of research projects. We mainly employ acoustic (QCM, SAW) and optical (SPR, spectroscopic Ellipsometry) biosensors to understand and quantitate biomolecular interactions involving e.g. protein/ DNA, protein/lipid membranes/endosome and DNA/nanoparticle systems. Newly developed biophysical concepts are used in molecular diagnostics combined with integrated lab-on-chip platforms for automated analysis of genetic markers in crude samples (food, plant tissue, blood, swab, saliva). We are also employing 3D-printing to develop fully autonomous systems for point-of-care applications (healthcare) and field testing (food/plants).

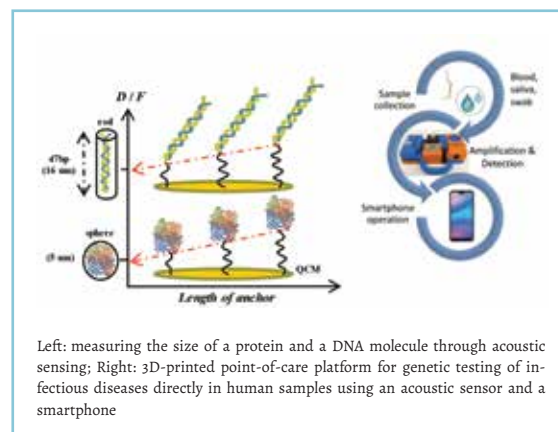
Current aims

One of our main aims is to continue our efforts to understand how acoustic waves interact with biomolecules and nanoparticles. We want to expand our novel hydrodynamic theory which correlates the acoustic signal to the size and shape (conformation) of the molecule to other nano-entities such as liposomes and extracellular vesicles. More complex molecular recognition events such as processes involving protein-exosome recognition and the mechanical characterization of vesicles are under study. Our fascination to combine acoustic biophysics with nano/micro technology is ongoing; we aim to translate our new findings to platforms for clinical diagnostics, always in collaboration with clinical partners and end users in Greece, Europe and globally.

Progress in 2019-2020

- We developed an experimental methodology to enhance the acoustic signal and thus lower the limit of detection for ultrasensitive DNA detection; this is an obvious necessity in case the analytes are of diagnostic importance.

- Our earlier-developed hydrodynamic theory was elaborated on, modeling anchored/suspended particles and their movement with the sensor in the surrounding liquid as a damped harmonic oscillator. This allowed for new insights on the effect of the linker stiffness, mode of immobilization and size/shape of the particle on the measured acoustic signal.
- We 3D-printed in-house two novel platforms for DNA detection using either acoustic or colorimetric means for verifying the presence of the target. Isothermal amplification (LAMP) performed inside a microfluidic channel or a plastic tube allowed the detection of food/plant-borne pathogens (*Salmonella*, PSTVd) and viruses (SARS-CoV-2, influenza) in unprocessed samples. We are currently excited to test one of our platform in S. Africa for HIV and SARS-CoV-2 detection
- With our clinical and industrial partners, we developed an acoustic array-based method for performing both tissue and liquid biopsy for BRAF-V600E and KRAS-G12D mutations-detection.



Left: measuring the size of a protein and a DNA molecule through acoustic sensing; Right: 3D-printed point-of-care platform for genetic testing of infectious diseases directly in human samples using an acoustic sensor and a smartphone

Other activities

- I gave a Keynote presentation at “Smart Bioelectronic and Wearable Systems” EU workshop on 22-23 October 2019 in Brussels
- PhD student Nikoletta Naoumi was invited speaker at the 8th Annual Diagnostics Innovation Summit on Developing Rapid Tests & Liquid Biopsies, Portugal May 2020
- With my colleagues Dr. George Papadakis, Dr. Alexandros Pantazis and Nikos Fikas we founded BIOPIX DNA Technology, a FORTH spin off company, for transferring innovations from the lab to the point-of-care
- Our lab contributes extensively in the fight against Covid19: MSc. student Stella Chatziioannidou demonstrated in April 2020 that our portable point-of-care platform can detect SARS-CoV-2 with excellent sensitivity (97.4%) and specificity (100%) using patients’ samples (89).
- The group attracts total funding of >7M Euros through nine competitive grants of which I coordinate five (1 HFSP, 2 FET-OPEN, 1 industrial and 1 ESPA/Region of Crete)

Web page

<http://biosensorslab-forth.gr/>

Publications

Milioni D, et al. (2020) An acoustic methodology for selecting highly dissipative probes for ultra-sensitive DNA detection.

Analytical Chemistry 92(12), 8186-8193

doi.org/10.1021/acs.analchem.0c00366

Papadakis G, et al. (2019) 3D-printed point-of-care platform for genetic testing of infectious diseases directly in human samples using acoustic sensors and a smartphone. **ACS Sensors** 4(5), 1336 doi.org/10.1021/acssensors.9b00264

Raptis V, et al. (2019) Theoretical aspects of a discrete-binding approach in quartz-crystal microbalance acoustic biosensing.

Physical Review Applied 11, 034031

POST-GENOMIC APPLICATIONS LAB



Dimitris Kafetzopoulos
Research Director (A)

GROUP MEMBERS

Research associate:

Despoina Vassou

Postdoctoral Researchers:

Emmanouela

Linardaki

Nikolaos Psonis

Anastasios Batas

Androniki Bibi

Stella Maxouri

Eumorfia

Tzagkaraki

Master students:

Maria Astrinaki

Giannis Patramanis

Research assistants:

Eugenia Tabakaki

Anastasios Kollias

Effie Kolotourou

Katerina Sirigonaki

Summary

The laboratory has a strong background in Molecular Biotechnology and a clear inclination for multidisciplinary and applied research. Our main research interests for more than ten years now, remain within the field of Post-Genomic analysis. By utilizing our experience and the power of high-throughput sequencing technologies, the lab is currently oriented towards three main research goals:

- **Ancient DNA analysis:** The ancient DNA lab of IM-BB-FORTH started its operation in 2016 and is focused on the analysis of ancient biological specimens, taking advantage of the rapid technological advances of Next Generation Sequencing and the rich (bio)archaeological treasure of Greece. Our main scientific interests include the study of the origin of human populations, the genetic identification of individuals and phenotype determination, the reconstruction of the evolutionary history of animal species and species molecular identification and the tracing of ancient pathogens.
- **Personalized medicine:** Our lab initiated the Precision Medicine Unit (PMU) of Crete, a member of the three Greek Precision Medicine Networks (Oncology, Cardiology and Neurodegenerative Diseases). As part of these networks the lab focuses on the identification of tumor molecular markers of diagnostic and prognostic significance, as well as clinically actionable genetic variants and the genetic profiling of neurodegenerative and hereditary cardiological diseases in the Greek population and the identification of specific molecular markers associated with them.
- **Agriculture genomics:** Our lab, identified the necessity of Genomics in Agriculture for the identification, preservation and certification of indigenous varieties of olive and grapevine, both of high value for Greek economy.

tification of animal and human findings.

Furthermore, the lab established the Precision Medicine Unit which is dedicated in identifying molecular markers in solid tumors, hematological malignancies, cardiopathies and more recently neurodegenerative diseases via the utilization of cutting-edge sequencing technologies. Key milestones were the establishment of all necessary experimental and bioinformatics protocols, participation and successful completion of inter-laboratory as well as external quality assessment scheme (EMQN OncoPanel 19), analysis of more than 250 oncology samples and more than 50 cardiological samples and the initiation of necessary procedures for the certification with ISO 15189 standard for medical laboratory quality and competence and ISO 27001, standard for information security management, aiming to receive accreditation for both within 2021.

Finally, in the field of Agriculture, the lab is involved in several projects, regarding mainly the whole genome and transcriptome sequencing of selected cultivars (olive and grapevine) of high economical value, in collaboration with experts and private companies active in the field of Agriculture. Our ultimate aim is the establishment of a traceability, identification and certification system for propagating material and Greek products.



Progress 2019-2020

During 2019-2020 the lab managed to initiate collaborations with several Greek and European Institutes in the field of ancient DNA, to successfully establish challenging optimized experimental and computational protocols for ancient DNA analysis and to implement research regarding molecular iden-

Other activities

- Founder of the Ancient DNA laboratory and leader of the facility till early 2020
- Establishment of the Precision Medicine Unit in Oncology in Crete
- Teaching: *Molecular & Cellular Biology*, graduate program in bioinformatics, COMP 101, School of Medicine, University of Crete (2019, 2020)

Web page

<http://www.imbb.forth.gr/kafetzopoulos>

Publications

Psonis N, et al. (2020) Molecular identification and geographic origin of a post-Medieval elephant finding from southwestern Portugal using high-throughput sequencing. *Sci. Rep.* 10, 19252. doi.org/10.1038/s41598-020-75323-y

Gastineau R, et al. (2020) Two new bacilladnaviruses associated with the diatom *Haslea ostrearia*. *European Journal of Phycology* 55:4, 444-453, DOI: 10.1080/09670262.2020.1748723

Astrinaki M, et al. (2019) Zazz: Variant Annotation and Exploration of Next Generation Sequencing Variants. *IEEE 19th International Conference on Bioinformatics and Bioengineering (BIBE)* Athens, Greece pp. 856-860, doi: 10.1109/BIBE.2019.00159

MOLECULAR ENTOMOLOGY GROUP



John Vontas
Professor - Collaborating
Faculty Member

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Jason Charamis

Summary

The Molecular Entomology group, led by Prof John Vontas, studies the mechanisms by which disease vectors and agricultural pests develop resistance to insecticides, as well as explores novel insecticide targets and biotechnology based approaches to increase the efficiency and sustainability of insect control interventions.

Current Aims

Some of the most acute challenges that the world faces now and in the foreseeable future are caused by insecticide resistant arthropods that seriously threaten human health and food security. For example, malaria prevalence has halved since 2000, primarily due to vector control interventions, saving 660 million lives, with 80% of the reduction being attributable to the use of insecticides. However, the evolution of insecticide resistance and the limited availability of highly selective and safe insecticides represent a major threat to human health and food security. Our research focusses on the molecular analysis of the mechanisms by which insects develop resistance to insecticides, aiming to develop means of managing and overcoming this resistance. Currently we also focus, on the discovery and the functional characterization of novel insecticide targets in the guts of major agricultural pests and the legs of *Anopheles* mosquitoes.

Progress in 2019-2020

Several insecticide resistance mechanisms in mosquitoes and agricultural pests were functionally elucidated recently in our lab, using genome modification technology CRISPR/Cas9 and other molecular approaches. The contribution of individual mechanisms when present alone or in combination with other mechanisms and their synergistic interactions were also functionally validated (**Figure 1**).

Other activities

- In the frame of the Horizon2020 EU Projects INFRA-VEC2 and DMCMALVEC, we offered a large number of mosquito molecular genotyping "service" (Transnational Access, TNA) and provided support and training in researchers from >20 countries worldwide (primarily Africa) to advance their research in the field.
- In the frame of national and regionally programs, we provide scientific support and training for the implementation of control programs, against mosquitoes and major agricultural pests in Greece.
- ~20 undergraduate and master students received training and performed their thesis in the lab of Molecular Entomology

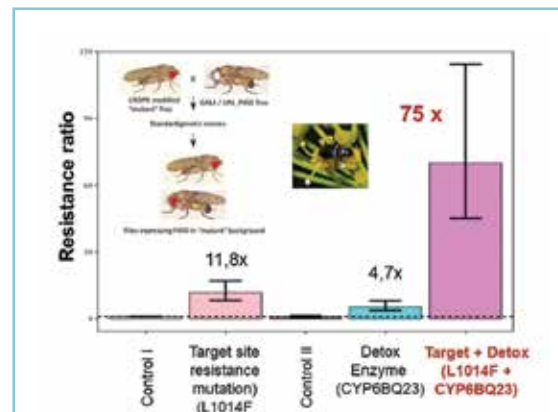


Figure 1. Functionally validated synergism of metabolic and target site insecticide resistance.

By combining genetic transformation and CRISPR/Cas9 genome modification, we generated transgenic insects expressing insecticide metabolizing P450 enzymes in a genetic background along with engineered mutations in the insecticide target site, and measured the contribution of each individual or combined mechanism.

In close collaboration with researchers at the Liverpool School of Tropical Medicine, we revealed novel insecticide resistance mechanisms expressed in mosquito legs, the most relevant insect tissue for contact insecticide uptake. Modification of epicuticular hydrocarbons and remodeling of leg cuticles, as well as the overexpression of leg-enriched sensory appendage proteins (SAP2) confers pyrethroid resistance in *Anopheles gambiae*, the major malaria vector in Africa. SAP2 expression is elevated in insecticide-resistant populations and is further induced upon mosquito contact with pyrethroids. SAP2 silencing fully restores mosquito mortality, whilst its overexpression results in increased resistance, likely due to the high-affinity SAP2 binding to pyrethroid insecticides. Mining of genome sequence data reveals a selective sweep near the SAP2 locus in three West African countries, with the observed increase in haplotype associated SNPs mirroring increasing resistance reported in Burkina Faso.

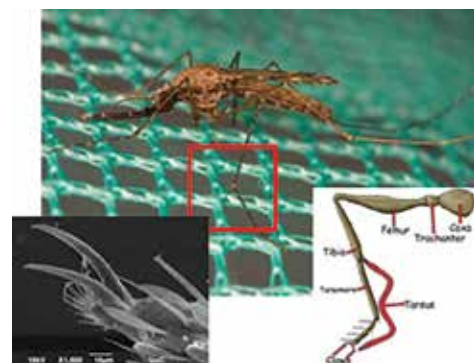


Figure 2. Insecticide resistant *Anopheles gambiae* "walking apathetic" on impregnated bednets. Modification of epicuticular hydrocarbons and remodeling of leg cuticles, as well as the overexpression of leg-enriched sensory appendage proteins (SAP2) confers pyrethroid resistance, by sequestering and substantially delaying insecticide uptake, giving time to detoxification mechanisms to detoxify them, thus producing striking insecticide resistance phenotypes.

<https://www.aua.gr/vontas/>

<https://www.imbb.forth.gr/en/research-en/biotechnology/item/2057-john-vontas>

Web page

Publications

Ingham VA, et al. (2020) A sensory appendage protein protects malaria vectors from pyrethroids. **Nature** 577(7790):376–380 doi:10.1038/s41586-019-1864-1. PMID: 31875852

Samantsidis GR, et al. (2020) 'What I cannot create, I do not understand': Functionally validated synergism of metabolic and target site insecticide resistance. **Proc R Soc B** 287 (1927):20200838 DOI:10.1098/rspb.2020.0838. PMID:32453986

Balabanidou V, et al. (2019) Mosquitoes cloak their legs to resist insecticides. **Proc R Soc B** 286(1907):20191091 DOI:10.1098/rspb.2019.1091. PMID: 31311476



NEUROSCIENCES

REGENERATIVE PHARMACOLOGY - NEUROSCIENCES DIVISION



Ioannis Charalampopoulos

Associate Professor -
Collaborating Faculty
Member

GROUP MEMBERS

Post-doctoral fellows:

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Aikaterini Kalemaki

PhD students:

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Marianna Papadopoulou
Despoina Charou
Alexandros Tsimpolis

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Dimitris Lypitkas
Ellie Karamoustou
Vasilina Gavra
Erica Saridaki
Konstantina Mylonaki

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Vana Nalmpanti
Evtixia Siggeri

Summary

Our research group is investigating the role of neurotrophins and their receptors in adult neurogenesis and neuroprotection under neurodegenerative conditions, like Alzheimer's Disease (AD). Using *in vivo* animal models of AD (5xFAD mice) and 2D/3D *in vitro* co-cultures of neuronal progenitors, mature neurons and glia cells of human and mouse origin, we study the neurotrophin signaling and cellular effects of endogenous neurotrophins and synthetic, micromolecular neurotrophic analogs, as potential therapeutic agents against neurodegenerative diseases.

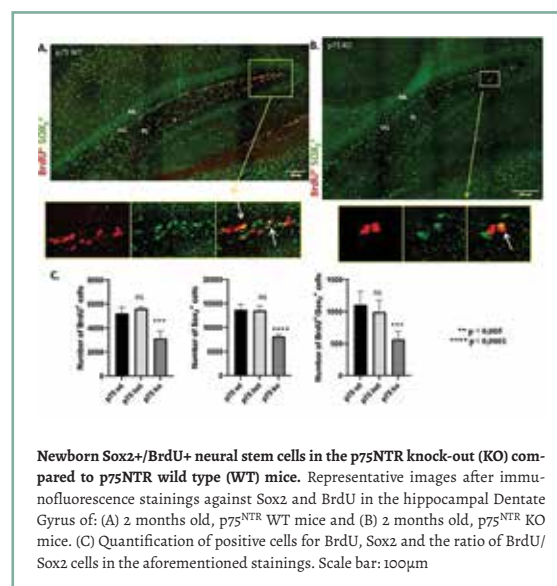
Current Aims

Neurotrophic factors consist key molecules for the development, survival and plasticity of the nervous system. Despite neurotrophins well-documented importance as neuroprotective and neurogenic molecules, the neurotrophin receptors exact expression and signaling properties are still largely unknown, especially for the pan-neurotrophin p75 receptor. We aim to reveal the role of neurotrophin receptors and their partners in neural stem cells during adult neurogenesis under neuropathological conditions, ranging from molecular to neural network level, emphasizing on Alzheimer's Disease.

Progress 2019-2020

With the support of the Hellenic Foundation of Research and Innovation, the General Secretary of Research & Technology and FORTH Institute grants, we obtained the necessary animal models for studying Alzheimer Disease (the 5xFAD mouse) and neurotrophin signaling (p75^{NTR} KO and p75^{NTR-flox} mice). We have completed the study of a novel synthetic neurotrophin analog in the AD mouse model, and we are developing humanized models of AD using human iPSCs-derived neuronal and glial cells, in parallel with the screening and biological characterization of new synthetic neurotrophin analogs.

In collaboration with Professors Maria Papadopoulou and Stelios Smirnakis, we are expanding the molecular studies of AD on the neuronal network level. Finally, we have started the chronopharmacological evaluation of the ultradian rhythmicity of glucocorticoids on neurotrophins' effects in neurological disorders.



Other activities

- As partners of a Marie Skłodowska-Curie European program, two PhD students were visited and worked for few months in other European laboratories, while all PhD students participated with oral or poster presentations in many international and national conferences (EMBO courses, FENS meeting, etc). Ioannis Charalampopoulos was invited as a speaker in the 11th panhellenic Conference of Alzheimer's Disease, the 4th Mediterranean Conference on Neurodegenerative Diseases and the 70th panhellenic conference of Hellenic Society for Biochemistry and Molecular Biology.
- Undergraduate and graduate students were performed their diploma thesis at our lab within our participation in Biomedical and Neuroscience Graduate Programs at the University of Crete.
- Our lab organized and participated in many events for providing educational and professional information in high-school students, welcoming them at the lab for live exhibition of experimental protocols and discussion for the present and future of the biomedical sciences.

Web page

<https://www.imbb.forth.gr/en/research-en/neurosciences/item/3677-ioannis-charalampopoulos>
http://regenera-pharm.med.uoc.gr/regenerative_pharmacology/Main_Page.html

Publications

- Kourgiantaki A, et al. (2020) Neural stem cell delivery via porous collagen scaffolds promotes neuronal differentiation and locomotion recovery in spinal cord injury. **NPJ Regen Med.** 5:12
- Delivanoglou N, et al. (2020) Spatio-temporal expression profile of NGF and the two-receptor system, TrkA and p75NTR, in experimental autoimmune encephalomyelitis. **J Neuroinflammation** 17(1):41
- Yilmaz C, et al. (2019) Neurosteroids as regulators of neuroinflammation. **Front Neuroendocrinol.** 55:100788

SYSTEMS NEUROSCIENCE



Emmanouil Froudarakis

Assistant Researcher (C)

GROUP MEMBERS

Postdoctoral researcher:
Maria Diamantaki,
Ph.D.

Summary

Our lab investigates how cortical circuits across different brain areas interact to form multimodal object representations that can guide behavior. Natural scenes contain large number of objects, and our brain is capable of using information from different sensory modalities to extract their identities with ease. Yet, despite extensive research in the last few decades, we are still far from having a complete understanding of how the brain creates untangled (transformation-invariant) object representations. If we understood how brains achieve this extraordinary ability at the algorithmic level, this would represent a significant advance in our understanding of cortical computation. To address this question, we combine advanced imaging techniques for recording neural activity with high-throughput behavioral training and computational modeling to study how the activity of large neuronal populations across different cortical regions enables behaving animals to identify and isolate objects in different contexts.

Current Aims

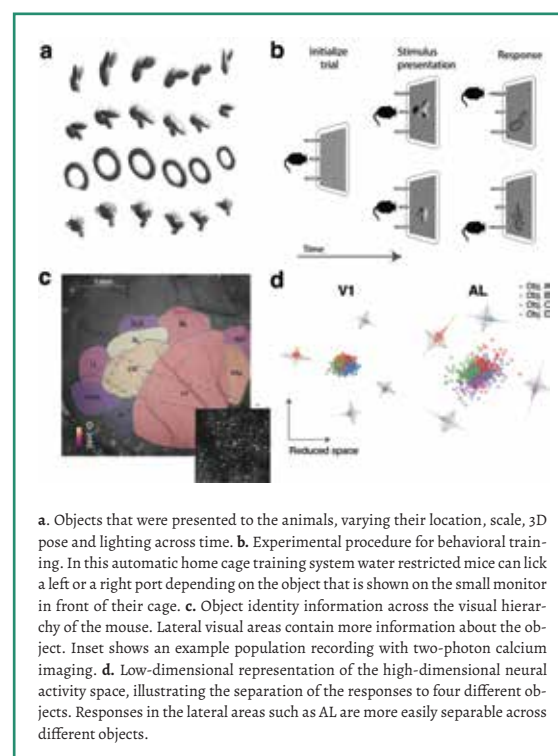
The group's research focuses on understanding:

1. How neural representations of objects evolve across the cortical hierarchy and how circuits in these areas interact in order to optimize the computations necessary for different behaviors.
2. How multisensory input that carries information about both the identity and the context of the objects, affects the neural representations in the visual system.

Progress in 2020

In order to address these questions, we have successfully implemented an automatic, high-throughput, low-cost behavioral training system in which animals are trained to discriminate objects in their home cage. We have adapted this system to perform multimodal two-alternative forced choice training and we are working on expanding the capabilities to include match-to-sample tasks that are typically used for object dis-

crimination in higher mammals. We are developing a virtual environment in which animals can navigate based on the information from multiple sensory inputs, which will allow us to study how context can affect the cortical representation of objects. Together with the behavioral training, we are using both wide-field imaging methods and chronic recordings with miniature microscopes to map the re-organization of the visual circuits that occurs with visual experience. Finally, we have implemented computational methods to study the state-dependent dynamic interactions between all cortical visual areas of the mouse.



Other activities

Teaching:

- *Multicellular Organization of Life*, Graduate Course, BIO-1405, School of Medicine, University of Crete (2019, 2020)
- *Analysis and Modeling of Brain Circuits*, Graduate Course, HY-590.21, University of Crete (2019)

Web page

<https://www.imbb.forth.gr/imbb-people/en/froudarakis-members/item/3001-emmanouil-froudarakis-eflab.org>

Publications

- Froudarakis E, et al. (2020) Object manifold geometry across the mouse cortical visual hierarchy. **bioRxiv** 2020.08.20.258798
- Liu G, et al. (2019) Target specific functions of EPL interneurons in olfactory circuits. **Nature Communications** 10, 1–14
- Froudarakis E, et al. (2019). The Visual Cortex in Context. **Annual Review of Vision Science** 5, 317–339

NEUROPHARMACOLOGY



**Achilleas
Gravanis**

Professor - Collaborating
Faculty Member

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Chanoumidou**

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Thanassis Rogdakis
George Stamatiadis
Antonio Varone
Konstantia Kodella

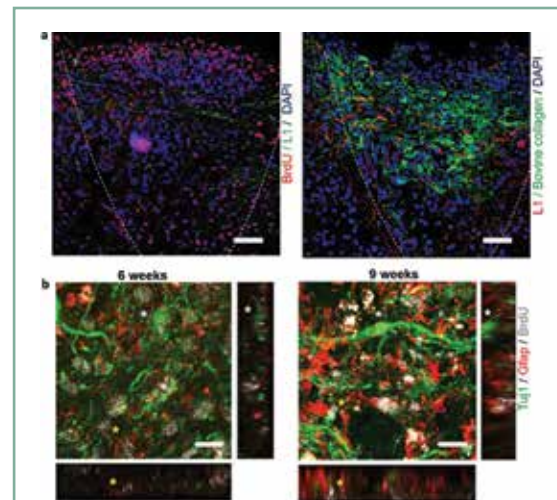
Summary

Our research group is developing synthetic compounds, agonists of neurotrophin receptors, with neuroprotective and neurogenic properties and potential applications in therapeutics of neurodegenerative diseases and brain ageing. Additionally, our group is focusing on organ-on-a-chip technologies and 3D micro scaffold bioengineering, hosting neural stem cells to develop neuroimplants for spinal cord and brain injury and neurobiosensors for drug screening.

Current Aims-Progress 2019-2020

Neural stem cell (NSC) grafts have demonstrated significant effects in animal models of spinal cord injury (SCI), yet their clinical translation remains challenging. We demonstrated that neuroimplants based on porous collagen-based scaffolds (PCSs), similar to biomaterials utilized clinically in induced regeneration, can deliver and protect embryonic NSCs at SCI sites, leading to significant improvement in locomotion recovery. Our neuroimplants induce regeneration in SCI lesions including enhancing NSC neuronal differentiation and functional integration in vivo, enabling robust axonal elongation, and reducing astrogliosis. Our findings suggest that the efficacy and translational potential of emerging NSC-based SCI therapies could be enhanced by delivering NSC via scaffolds derived from well-characterized clinically proven PCS.

Dopaminergic system is compromised in schizophrenia. It is of note that the mixed dopamine (DA) D1/D2 receptor agonist apomorphine induces schizophrenia-like symptoms in rodents, including disruption of memory abilities. BNN27, our microneurotrophin derivative, was shown to counteract schizophrenia-like behavioural deficits produced by glutamate hypofunction in rats. Our findings suggest that BNN27 is effective to dopamine dysfunction caused by apomorphine, attenuating cognitive impairments induced by this D1/D2 receptor agonist in rats.



a: Immunofluorescence images of sagittal sections stained for bovine collagen I, BrdU, and L1 at the lesion epicenter, of 6 weeks post injury mouse spinal cord with our collagen-neural stem cell neuroimplant. Dashed line indicates the approximate lesion boundary. Scale bars, 50 μm. **b** High-magnification immunofluorescence images and orthogonal views at the lesion epicenter, 6 and 9 weeks post injury. Cellular colocalization of BrdU⁺ nuclei with cytoplasmic Tuji or GFAP are marked by white and yellow asterisks, respectively (Kourgiantaki et al, Nature Reg Med 2020)

Other activities

- efforts to translate our neuroimplant in patients with spinal cord injury in collaboration with Orgenesis SA (NY) and Spaulding Rehabilitation Hospital (Harvard MS)
- developing a Covid19-human brain-on-chip platform to study ex vivo the interactions of SARS-CoV-2 virus with human neural tissue and test potential therapeutics

Web page

<http://gravanis.med.uoc.gr>

Publications

Kourgiantaki A, et al. (2020) Neural stem cell delivery via porous collagen scaffolds promotes neuronal differentiation and locomotion recovery in spinal cord injury. **Nature Regenerative Medicine** 15:51

Pitsikas N, et al. (2020) The novel dehydroepiandrosterone (DHEA) derivative BNN27 counteracts cognitive deficits induced by the D1/D2 dopaminergic receptor agonist apomorphine in rats. **Psychopharmacology (Berl)** 238(1):227-237 doi: 10.1007/s00213-020-05672-z. Epub 2020 Oct 2

Gampierakis IA, et al. (2020) Hippocampal neural stem cells and microglia response to experimental inflammatory bowel disease (IBD). **Molecular Psychiatry** 2020 Jan 22 doi: 10.1038/s41380-020-0651-6

DEVELOPMENTAL NEUROBIOLOGY-MYELIN BIOLOGY



Domna Karagogeos

Professor - Collaborating
Faculty Member

GROUP MEMBERS

Staff scientist:

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Senior Postdoctoral

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Postdoctoral Researcher:

Theodora Velona

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Danai

Nikolantonaki

Anna Tsiamanta

Alexandros Patellis

Sofia Maragkou

Summary

Myelinated fibers are segregated into functionally distinct domains due to interactions between myelinating glial cells and the adjacent axon. These domains ensure the rapid propagation of action potentials and are particularly vulnerable in demyelinating pathologies such as multiple sclerosis. Our work focuses on a) *elucidating axoglial interactions in health and demyelinating pathologies* b) *testing molecules that may influence demyelination/remyelination* c) *investigating the role of autophagy in CNS myelin*.

Neurodevelopmental pathologies originate often as a result of perturbed neuronal migration in the developing embryo.

Cortical interneurons (CINs), provide the balance of excitation and inhibition and are implicated in many neurodevelopmental pathologies. *We have contributed towards the understanding of the molecular, cellular and functional changes of CINs in two mouse models of decreased inhibition that we have generated by ablating the small RhoGTPases Rac1 and 3.*

Current Aims

a) CNS myelin homeostasis and disruption

We are focusing on the role of autophagy in CNS myelin as an essential mechanism of myelin homeostasis by *in vivo* and *in vitro* approaches. We are analyzing the mechanism by which autophagy affects oligodendrocyte maturation as well as identifying the autophagic cargo in oligodendrocytes. Using demyelination/remyelination *in vivo* and *ex vivo* models we are testing selected compounds in these processes.

b) Molecular and functional analysis of models of decreased inhibition in the mouse cerebral cortex.

We aim to understand the role RhoGTPases Rac1 and 3 in cell cycle and morphology of interneurons by using animals where Rac1 and Rac1/3 were ablated specifically in CINs. Currently we focus on the characterization of the molecular nature of their defects via novel, state-of-the-art approaches.

Progress in 2019-2020

a) Myelin

We investigated the effect of a microneurotrophin on glial populations *in vitro* and *in vivo*, in a focal demyelination

model. Treatment leads to reduced myelin loss, increased oligodendrocyte maturation and diminished astrocytic accumulation leading to a faster remyelination process through the neurotrophin receptor TrkA. This microneurotrophin could serve as a potential therapeutic agent.

We showed that autophagy is active in mature oligodendrocytes and its pharmacological inhibition results in severely altered oligodendrocyte morphologies, strongly supporting the hypothesis that autophagy is an essential mechanism for central myelin formation and maintenance.

b) Interneurons

We investigated the developmental cellular processes in the mouse medial prefrontal cortex during a critical postnatal period and highlighted the role of CINs. We showed that basal synaptic transmission decreases due to increased spontaneous inhibitory currents and reduced excitatory ones. Our data suggest that GABA_A function in the neonatal mPFC is non-inhibitory.

We have characterized the migratory defects of Rac1/3 double mutant interneurons via time-lapse video microscopy and *ex vivo* cultures. Defects in centrosome, Golgi and ER positioning have been identified. RNA seq experiments indicated a number of affected molecules which are currently under investigation.

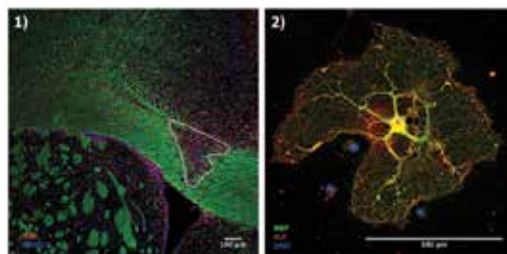


Figure Legend

1. Section of mouse brain showing a demyelinating lesion (area within white dotted lines)
2. Mature oligodendrocyte in culture making myelin proteins MBP, PLP

Other activities

- Prof Domna Karagogeos has served as the chair of the Committee on Higher Education and Training (CHET) of FENS (Federation of European Neuroscience Societies) during 2018-2020
- Prof Karagogeos co-organized and participated in the Virtual FENS Forum 2020 ; edited the chapter on the development of the nervous system in the Greek version of the latest edition of the book "Principles of Development" by L. Wolpert; co-authored with Dr Denaxa an article entitled "How Cortical Interneurons Develop: Current and Future Research" that appears on the Soc. for Neuroscience, U.S.A. (SFN) webpage (Neuronline article).

Web page

<https://www.imbb.forth.gr/imbb-people/en/publications-karagogeos>

Publications

Kalemaki, K., et al. (2020) Early postnatal inhibitory circuit development in the mouse medial prefrontal and primary somatosensory cortex. *bioRxiv* doi: <https://doi.org/10.1101/2020.01.21.913913>

Kalafatakis, I., et al. (2020) Using the Allen gene expression atlas of the adult mouse brain to gain further insight into the physiological significance of TAG 1/Contactin 2. *Brain Structure and Function* Sep;225(7):2045-2056. <https://doi.org/10.1007/s00429-020-02108-4>

Kastriti ME, et al. (2019) Ablation of CNTN2+ Pyramidal Neurons During Development Results in Defects in Neocortical Size and Axonal Tract Formation. *Front Cell Neurosci.* 2019 Nov 1; 13:454. doi: 10.3389/fncel.2019.00454

NEURONAL CELL FATE AND FUNCTION



Maria Monastiriotti
Senior Staff Scientist (A)

GROUP MEMBERS

MSc student:
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(2019)

Diploma Thesis Student:
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(2020)

Visiting Student:
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(2020)

Summary

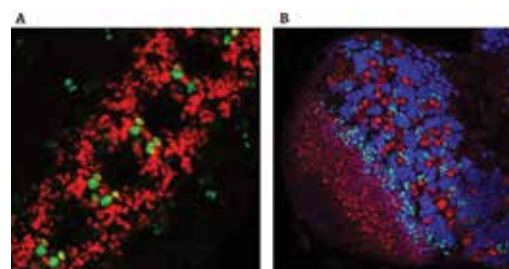
Establishment of the characteristic anatomical and/or functional properties of a cell type takes place primarily during development and it is accomplished by the concerted action of signalling and regulatory molecules. We are interested in mechanisms involved in the determination of cellular fate and function within the nervous system aiming to understand their impact in the viability, physiology and behaviour of organisms. Our research lines focus on intercellular communication pathways underlying neuronal cell fate determination and on the development of neurochemical specificity of distinct neuronal cell types. For our studies we use the fruit fly *Drosophila melanogaster*, a model organism with a wide repertoire of technical advances that allow genome manipulation as well as molecular, genetic, imaging and behavioural analysis.

Current aims

Our current work relates to the asymmetric divisions of stem cells and /or precursor cells taking place during *Drosophila* neurogenesis. This process is based on a multiple series of two consecutive asymmetric divisions, one of the neural stem cells (neuroblasts) and one of their mitotic progeny, the Ganglion Mother cells (GMCs). The Delta-Notch intercellular signalling pathway is involved in the regulation of both divisions and instructs different post mitotic cell identities within the same neuronal lineage. **In collaboration with Delidakis group**, we investigate the role of bHLH-O transcription factors (TXFs) acting downstream of Delta-Notch signalling during the two mitotic events and especially the GMC division when establishment of an initial dichotomy of newborn neurons into "A" type (Notch responsive) versus "B" type (Notch unresponsive) takes place. We want to elucidate how Notch and other signals regulate the expression of those TXFs, any putative interactions among them and with other factors in the course of the above process as well as their transcriptional targets and the gene expression programs they control.

Progress in 2019-2020

- We concentrated on the bHLH-O protein HEY which we have previously identified as a transcriptional target and effector of Notch signalling during the asymmetric division of GMCs. We defined genomic regions that confer to the transcriptional and post-transcriptional regulation of *Hey* gene and we establish how these contribute to the time and cell specific mode of its expression.
- We also initiated a study on cell fate determination within *Drosophila* intestine. We aim to elucidate the Notch dependent expression and the functional role of the TXF HEY in the developing fly midgut as well as during proliferative activity that maintains homeostasis in the functional intestine.



Developing CNS of *Drosophila* embryo (A) and 3rd instar larva (B)

- TxF Hey (RED) is expressed in "A" type (vMP2-YELLOW) neurons of the MP2 neuronal lineage (GREEN)
- Third instar larva Neuroblasts (RED) and their neuronal lineages (BLUE). Only new-born neurons express Hey (GREEN).

Other activities

- Teaching courses in two Graduate Programs of University of Crete (UOC) ("Molecular Biology and Biomedicine" and "Neurosciences")
- We hosted rotator students from UOC Graduate programs and an Erasmus MSc trainee for research training
- Represented IMBB in the Organization committees of the 12th biannual FORTH retreat at Patras (October 15-16, 2019) and the 1st FORTH YouR Night event (December 13, 2019).

Web page

www.imbb.forth.gr/en/research-en/neurosciences/item/72-maria-monastiriotti

Publications

Flint Brodsky N, et al. (2019) The transcription factor Hey and nuclear lamins specify and maintain cell identity. *Elife* 8: e44745. doi: 10.7554/eLife.4474

AUTOPHAGIC MECHANISMS OF PROTEIN HOMEOSTASIS IN NEURONS



Vassiliki Nikolettou*

Assistant Researcher (C)
*Currently: Assistant Prof.,
Université de Lausanne

GROUP MEMBERS

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Postdoctoral fellow:

Emmanouela Kallergi

PhD students:

Akrivi Daskalaki

Theodora Chalatsi

Summary

The main interest of our group is to investigate autophagic mechanisms of protein homeostasis in neurons. Autophagy is an evolutionarily conserved process that delivers macromolecules and damaged or superfluous organelles to the lysosome for degradation. In addition, autophagic intermediates are also involved in non-degradative processes, participating in the unconventional secretion of proteins or their targeting to the plasma membrane. In line with these crucial functions, autophagy is indispensable for neuronal integrity and its deregulation causes severe synaptic defects that perturb behavior. However, the collective contribution of different autophagic processes to the homeostasis of synaptic proteins and to the function of neuronal subpopulations remain largely elusive.

Current aims

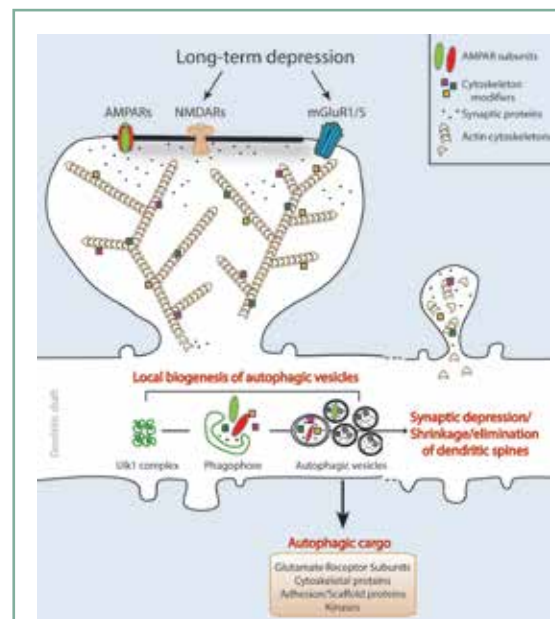
Many forms of long-term synaptic plasticity that underlie key cognitive functions, such as memory formation, memory erasure and behavioral flexibility, require protein turnover, meaning both protein synthesis and degradation. Autophagy is a major pathway for protein degradation, yet its requirement in synaptic plasticity remains unknown. Our recent work demonstrated that Brain derived neurotrophic factor (BDNF), the main neurotrophic factor in the adult brain and a major regulator of synaptic plasticity, suppresses autophagy in neurons. Following up on these findings, we aim to explore the regulation of autophagy by neurotrophic factors and its relevance to synaptic function and life-death decisions in the brain.

Progress in 2019-2020

My activity at the IMBB concerns the first 6-months of 2019. In July 2019 my lab relocated to the University of Lausanne in Switzerland.

During this period, we made progress in characterizing the contribution of autophagy in long-term synaptic depression. Our work demonstrated that the two major types of long-term

synaptic depression (LTD), mediated by activation either of NMDA or group 1 metabotropic glutamate receptors, trigger the rapid and local biogenesis of autophagic vesicles (AVs) in post-synaptic dendrites. In return, autophagy is indispensable for LTD, as either genetic ablation of *atg5* in pyramidal neurons or acute pharmacological inhibition of AV biogenesis totally prevents LTD induction. Using quantitative proteomic profiling of purified AVs, we revealed that upon LTD the autophagic cargo is significantly enriched for synaptic proteins, as well as modulators of the actin cytoskeleton and autism-impaired proteins. In line with these findings, a mild autophagy deficit is sufficient to impair behavioral flexibility, a cognitive function that requires efficient LTD. Therefore, local synthesis and assembly of the autophagic machinery in dendrites ensure the elimination of synaptic structures via degradation of their components, facilitating plasticity and associated behaviors.



"Schematic representation of autophagic vesicle biogenesis in postsynaptic dendrites following long-term depression. Adapted from Kallergi, Daskalaki et al., 2020 (Biorxiv)"

Other activities

- FENS-Kavli Network of Excellence: PhD student prize, awarded during FENS 2020
- FENS Forum 2020: Environmentally friendly Neuroscience workshop

Web page

<https://wwwfbm.unil.ch/dnf/group/autophagic-mechanisms-of-protein-homeostasis-in-neurons>

Publications

Kallergi E, et al. (2020) Long-term synaptic depression triggers local biogenesis of autophagic vesicles in dendrites and requires autophagic degradation.
BioRxiv doi.org/10.1101/2020.03.12.983965

INVESTIGATING THE ROLE OF DENDRITES IN BRAIN FUNCTION



Panayiota Poirazi

Research Director (A)

GROUP MEMBERS

Principal Staff Scientist:
Athanasia Papoutsis

Postdoctoral fellows:
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Spyridon Chavlis

PhD students:
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Kostas Petousakis
Ioanna Pandi
Stefanos Stamatiadis
Alexandra Tzilivaki
(co-supervised with D. Schmitz,
Charite University, Berlin)
Panos Bozelos
(co-supervised with Tim Vogels,
IST, Austria)

Master student:
Nikos Malakasis

Undergraduate student:
Iasonas Sifaki

Summary

Dendrites are thin processes that emerge from the cell body of neurons. They receive over 90% of the synaptic inputs to neurons, integrate them in non-linear ways thus expanding the processing power of neurons and their properties are altered in neurodegenerative conditions, making them targets for new treatments. We are interested in understanding how dendrites contribute to brain functions in health and disease and whether dendritic features can help advance artificial intelligence. We tackle this problem using a multidisciplinary approach that includes computational modeling, experiments in mice and machine learning.

Current Aims

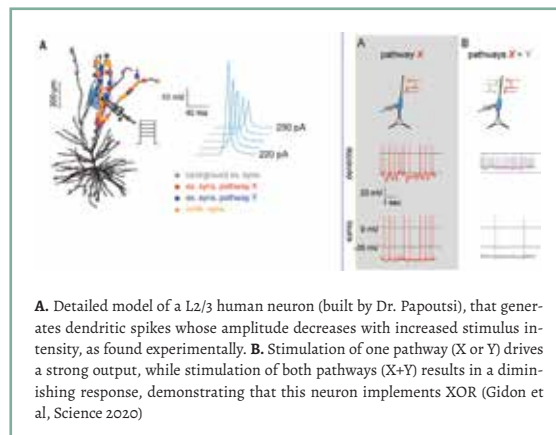
We currently investigate how dendrites of different cell types and species contribute to neuronal and network computations that determine behavior. We build computational models of various complexity levels and combine them with experimental data to study dendritic contributions to neuronal arithmetic, circuit computations and functions like learning, memory and decision making. Based on our findings, we transfer dendritic features to Deep Learning architectures aiming at advancing their performance. Finally, we develop a drug discovery platform, whereby Alzheimer's network models drive cultured neurons on nanowire arrays while applying new drugs.

Progress in 2019-2020

In the past two years we made several contributions to the dendrites field:

- We showed that active dendrites may exist in interneurons and expand their processing power, challenging the point neuron dogma (Tzilivaki et al, 2019).
- Our models demonstrated that a new type of dendritic spike (discovered by the Larkum lab, at Humboldt Univ.) allows human neurons to solve the XOR problem, a computation believed solvable only by large networks (Gidon et al, Science 2020).

- Our models revealed that active dendrites of interneurons allow a circuit to form memories using fewer resources, thus providing important savings (Tzilivaki et al, 2019).
- Together with A. Losonczy at Columbia Univ., we explained how specific subtypes of VIP cells underlie reward-learning in mice, a finding impossible to reach with experimental tools alone (Turi et al, 2019).
- The same model also pointed to neuronal de-synchronization as opposed to the widely believed interneuron loss as the underlying mechanism of spatial deficits in epileptic mice (Shuman et al, 2020).
- We developed novel Deep Learning methods for the automated classification of neuronal cell types and demonstrated their potential to replace current, painstaking approaches (Geiller et al, 2020; Troullinou et al, 2020).
- We discussed how models have furthered our understanding of dendritic functions and their potential to advance machine learning in various review articles (Poirazi and Papoutsis, 2020; Kastellakis and Poirazi 2019; Richards et al, 2019).



A. Detailed model of a L2/3 human neuron (built by Dr. Papoutsis), that generates dendritic spikes whose amplitude decreases with increased stimulus intensity, as found experimentally. **B.** Stimulation of one pathway (X or Y) drives a strong output, while stimulation of both pathways (X+Y) results in a diminishing response, demonstrating that this neuron implements XOR (Gidon et al, Science 2020)

Other activities

- Our collaborative work on human neurons was featured by Quanta Magazine, including an article and a Podcast featuring Dr. Poirazi.
- In 2019, Dr. Poirazi was awarded the Wilhelm Bessel Research Award of the Alexander von Humboldt Foundation and a Visiting fellowship from the Einstein Foundation in Berlin, in recognition of her pioneer work on dendrites.
- Dr. Poirazi was a Keynote Speaker at COSYNE 2019, which took place in Lisbon, Portugal.

Web page

<http://dendrites.gr/>

Publications

Poirazi P, and Papoutsis A. (2020) Illuminating dendritic function with computational models. **Nat Rev Neurosci.** 21(6):303-321

Gidon A, et al. (2020) Novel dendritic action potentials shape the computational properties of human layer 2/3 cortical neurons. **Science** 367(6473):83-87

Tzilivaki A, et al. (2019) Challenging the point neuron dogma: FS basket cells as 2-stage nonlinear integrators. **Nat Commun.** 10(1):3664

NEUROPHYSIOLOGY AND BEHAVIOR LABORATORY



Kyriaki Sidiropoulou

Assistant Professor-
Collaborating Faculty Member

GROUP MEMBERS

Ph.D. students:

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Maria Plataki

Vasiliky Stavroulaki

Lida Vagiaki

Research Assistant:

Chrysoula

Iordanidou

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Lito Parapera-

Papantoniou

Undergraduate thesis students:

Alexandros

Georgilis

Marouso Velissariou

Anastasios Cholevas

Illiana Mantouka

Summary

Research in the "Neurophysiology and Behavior" laboratory (www.sidiropouloulab.com) focuses on two main topics: 1) investigating the cellular mechanisms involved in the function of the prefrontal cortex and the hippocampus and 2) understanding the early-life neurobiological alterations that occur in neurodevelopmental mouse models of schizophrenia.

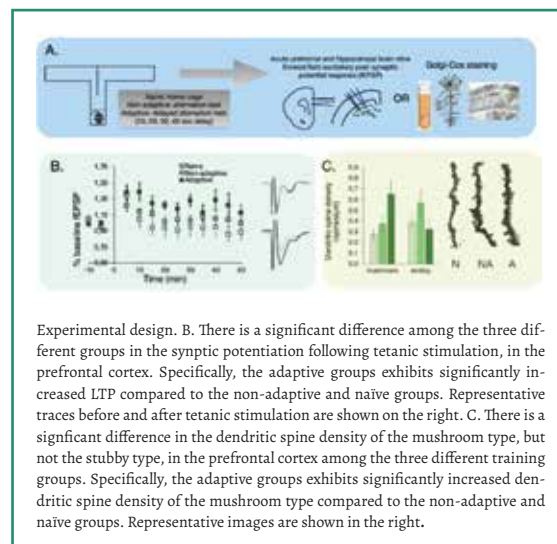
Current Aims

Our current aims include: 1) to delineate the biophysical mechanisms involved in critical periods of development of the prefrontal cortex, i.e. the juvenile and adolescent period, 2) dissect the biophysical mechanisms that underlie learning in working memory tasks, and 3) changes in biophysical mechanisms during the juvenile and adolescent periods in the MAM mouse model of schizophrenia.

Progress in 2019-2020

With regards to our first research topic, we have conducted studies showing that learning working memory tasks results in increased synaptic plasticity in the prefrontal cortex and increased synaptic response in the hippocampus. In addition, learning working memory tasks results in increased dendritic spine density in both the prefrontal cortex and the hippocampus. In addition, we investigate the sexual dimorphic effects of acute stress in the function of the prefrontal cortex.

With regards to our second research topic, we have published the neurodevelopmental animal model of schizophrenia, the MAM model, in mice (2019). Since then, we are conducting experiments to identify how neuronal activity and synaptic plasticity is altered in the juvenile and adolescent period. In addition, we are conducting experiments during the juvenile and adolescent periods to investigate the developmental profile of various biophysical mechanisms.



Other activities

Dr. Sidiropoulou serves as the President of the Hellenic Society for Neuroscience in 2019-2020 and is a council member of the European Brain and Behavior Society. She served as the chair of the organizing committee for the 28th meeting of the Hellenic Society for Neuroscience, which was held in Heraklio, Greece in October 2019. The Neurophysiology and Behavior Laboratory participated in outreach activities in 2019 and 2020, including the Brain Awareness Week and the Marie-Curie Researcher's Night.

Web page

www.sidiropouloulab.com

Publications

Stavroulaki V, et al. (2020) Working memory training effects across the lifespan: Evidence from human and experimental animal studies. **Mech Ageing Dev.**194:111415
doi: 10.1016/j.mad. 2020.111415

Kourgiantaki A, et al. (2020) Neural stem cell delivery via porous collagen scaffolds promotes neuronal differentiation and locomotion recovery in spinal cord injury. **NPJ Regen Med.** 5:12 doi: 10.1038/s41536-020-0097-0

Chalkiadaki K, et al. (2019) Development of the MAM model of schizophrenia in mice: Sex similarities and differences of hippocampal and prefrontal cortical function. **Neuropharmacology** 144:193-207 doi: 10.1016/j.neuropharm.2018.10.026

A large, rounded hexagonal graphic containing a microscopic image of cells. The cells are stained with green and blue fluorescent dyes, showing various cellular structures and organelles. The background of the entire page is a solid blue color.

INFECTIONS & IMMUNITY

LABORATORY OF RHEUMATOLOGY, AUTOIMMUNITY AND INFLAMMATION - SYSTEMIC AUTOIMMUNITY AND GENE REGULATION UNIT



George Bertias

Assistant Professor -
Collaborating Faculty
Member

GROUP MEMBERS

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PhD candidates:

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Despoina Kosmara,
MSc

Dimitra Nikoleri,
MSc

Sofia Papanicolaou,
MSc

Summary

Our focus is on the study of immune regulation of innate and adaptive immunity, and cellular metabolic pathways implicated in the pathogenesis of autoimmunity using SLE as a disease-model. We apply high-throughput technologies in tissues from selected patient groups in order to delineate the genomic/epigenetic basis of specific phenotypes (such as gender bias) or outcomes (response or failure to treatments) of the disease.

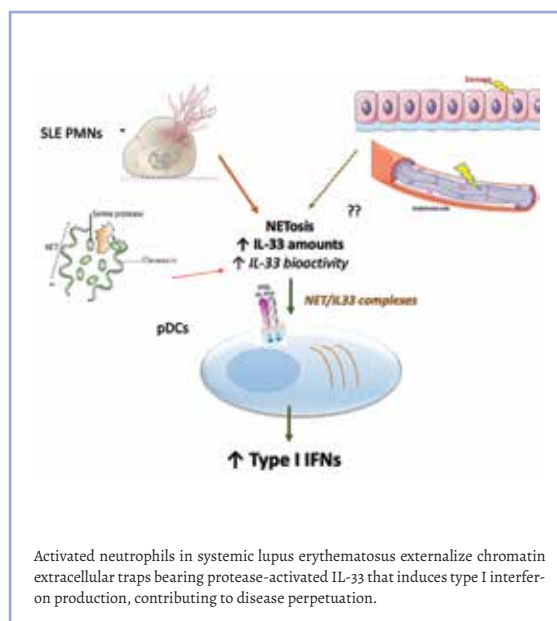
Current Aims

Driven by our results in a genome-wide gene expression study in patients with lupus, we are exploring the implication of activated neutrophils and death by NETosis in deregulated type I interferon production in the disease. We also study the role of chromatin regulators such as the cohesin complex in the genomic perturbations and monocytes activation in males and females with SLE, as well as the contribution of distinct metabolic pathways in inducing their inflammatory phenotype.

Progress in 2019-2020

- We concluded an RNA-seq study in a large cohort of patients with SLE and healthy individuals, which yielded significant insights into the molecular characterization of the disease and revealed the impact of genetic variation in deregulated gene expression and disease susceptibility.
- In evaluating the role of neutrophil activation and NETosis in autoimmunity, we have revealed a major contribution of the alarmin IL-33 in activating plasmacytoid dendritic cells to produce high amount of interferon (Figure legend).

- Driven by these data and a collaborative analysis with the group of Dr. Nikolaou, we identified a possible role for the cohesin complex in genomic alterations in the context of SLE. This project is currently being funded by the ELIDEK.
- Preliminary data suggest also that lupus monocytes, under the effect of interferon, induce glycolytic pathways which drive the acquisition of inflammatory phenotype possibly through changing the epigenetic landscape (funded by Fondation Santé research grant).



Web page

<https://www.imbb.forth.gr/en/research-en/infections-immunity/item/4187-george-bertias>

Publications

Panousis NI, et al. (2019) Combined genetic and transcriptome analysis of patients with SLE: distinct, targetable signatures for susceptibility and severity. **Ann Rheum Dis.** 78(8): 1079-1089

Ntasis VF, et al. (2020) Extensive fragmentation and re-organization of transcription in Systemic Lupus Erythematosus **Sci Rep.** 10(1): 16648

FUNGAL IMMUNOLOGY GROUP



Georgios Chamilos

Professor - Collaborating
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GROUP MEMBERS

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Stavroula Baiba

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Marina

Gourtzinopoulou

Research Assistant:

Alexandra Vatikioti

Summary

Airborne filamentous fungi (molds) are major causes of respiratory diseases in an expanding population of patients with complex immune and metabolic defects. Invasive mold infections (IMI) are associated with substantial mortality and enormous economic impact. Understanding pathogenesis of IMI is an unmet need for design of better therapies. Our group is interested to explore (a) the physiological host and pathogen molecular determinants of the intracellular fate of fungi inside phagocytes, (b) mechanisms of immunodeficiency that drive the development of fungal diseases, as well as to (c) select novel targets for host-directed therapies with the ultimate goal to improve IMI outcome. In order to dissect the complexity of host-fungal interplay we utilize genetic approaches in *Drosophila melanogaster*, murine and human primary immune cells, and animal models of fungal diseases in transgenic animals.

Current aims

Nutritional immunity, an ancient host defense strategy of iron limitation to pathogens, is incompletely understood at the molecular level. Iron metabolism also has a major role in physiological immunity. In macrophages, the master regulators of iron homeostasis, iron orchestrates metabolic, inflammatory and immune effector pathways via mechanisms that remain largely unexplored. Physiological immunity against molds is mainly conferred by macrophages. Therefore, molds are model pathogens to dissect molecular regulation of nutritional immunity in macrophages and identify mechanisms of cross-talk of iron metabolism with other antifungal effector pathways.

To date, there was no pathogenetic model to explain how abnormalities in iron metabolism result in development of *invasive mold infections (IMI)*. *Studies from our group put forward a novel mechanism for the pathogenesis of IMI, according to which development of IMI requires two discrete mechanisms (a) phagosome maturation arrest (via inhibition of LC3-associated phagocytosis, LAP), which allows intracellular persistence of fungal conidia (spores), and (b) alteration in iron homeostasis, resulting in invasive fungal growth and lysis of the macrophage.* According to this model, because LAP is targeted by fungal melanin and most immunosuppressive conditions predisposing to IMI, *iron regulation inside macrophages is the last and most critical line of host defense against molds.* On the pathogen

site, our pilot studies imply that fungal melanin profoundly alters macrophage metal homeostasis. Collectively, these findings put forward the hypothesis that mold infection triggers novel regulatory schemes influencing iron metabolism in macrophages.

Aim of our research is to identify the key regulators involved in fungal infection-triggered iron metabolism reprogramming and elucidate its role in the pathogenesis of IMI. These studies will set the basis for future research that will address long-standing fundamental questions on macrophage iron biology, metabolism and inflammation.

Progress in 2019-2020

Completed Projects:

Our work on a newly identified role of IL-6 signaling on regulation of microtubule-dependent endosomal trafficking and activation of LAP with major physiological importance in development of sepsis-induced immunoparalysis is about to be published in *Cell Host and Microbe* (accepted with minor revisions).

In collaboration with Agostinho Carvalho lab we have revealed the inhibition of calcium signaling by fungal melanin triggers an immunometabolic signaling axis towards glycolysis via activation of HIF-1 α and phagosomal recruitment of mTOR, which is essential for antifungal host defense.

In collaboration with Ashraf Ibrahim lab, we identified the important role of a novel ricin-like toxin produced by *Mucorales* molds in pathogenesis of invasive fungal disease and we identified host lipid effectors that target the expression of this toxin to block fungal pathogenicity *in vivo*.

Ongoing projects:

Exploring the role of lipid metabolism on antifungal host defense.

Identifying novel antifungal lipid effectors in human serum.

Exploring the role of novel regulators of macrophage iron homeostasis in antifungal immunity.

Exploring the role of LAP defects in pathogenesis of macrophage activation syndrome



Proposed model on the central role of iron in antifungal immunity. Apart from regulating nutritional immunity, iron controls macrophage immunometabolism, inflammation and phagosome responses (LAP)

Other Activities

- Hosted undergraduate and rotation students for training.
- The lab organized a clinical protocol for treatment of patients with COVID-19 with convalescent serum from patients who have recovered from the disease.

Web page

<https://cmmp.med.uoc.gr/index.php/intro-hdhp>

Publications

Gonçalves SM, et al. (2020) Phagosomal removal of fungal melanin reprograms macrophage metabolism to promote antifungal immunity. **Nat Commun.** 11(1): 2282

Schmidt F, et al. (2020) Flotillin-Dependent Membrane Microdomains Are Required for Functional Phagolysosomes against Fungal Infections. **Cell Rep.** 32(7):108017

Soliman SSM, et al. (2020) Selective inhibition of Rhizopus eumelanin biosynthesis by novel natural product scaffold-based designs caused significant inhibition of fungal pathogenesis. **Biochem J.** 477(13):2489-2507 doi: 10.1042/BCJ20200310. PMID: 32538426

INVESTIGATING THE BIOLOGY OF THE MALARIA PARASITE IN THE MOSQUITO VECTOR



Inga Siden-Kiamos

Principal Researcher (B)-
Emeritus since May 2019

GROUP MEMBERS

Post-doctoral researchers:

Chiara Currà

Maria Andreadaki

Elena Deligianni

Special Research Assistant:

Lefteris Spanos

M. Sc. Student:

Ioanna Kavelaki

Diploma thesis students:

Andriani
Geronikolou

(left in 2020)

Danae Bemplidaki

(left in 2019)

Sofia Tzagkaraki

(left in 2020)

Visiting students:

Matteo deGennis
(2019)

Arnau Biosca
(2019)

Karunanithi
Nivedita Aditya
(2019)

Summary

Malaria remains a devastating disease leading to 250-400 million infections and approximately half a million deaths every year, primarily in sub-Saharan Africa. Malaria is caused by *Plasmodium* parasites, which are evolutionary divergent organisms with a unique life style. The parasites are transmitted by mosquitoes and we are especially interested in understanding the basic biology of the parasite during its complex development in the insect. We investigate function of genes essential to the parasite during these stages using reverse genetics approaches. In the long term, our research may lead to new strategies to control the disease. However, we also contribute directly to the development of novel approaches to target the disease by blocking transmission through the mosquito.

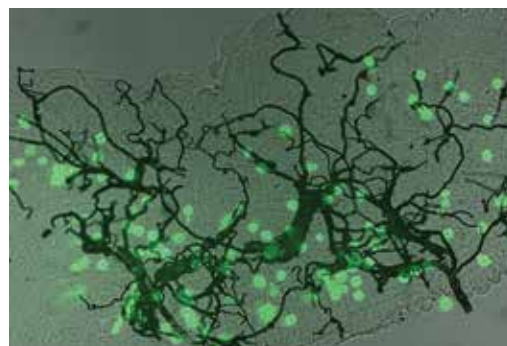
Current Aims

- We have discovered that malaria parasites contain proteins with similarities to the subunits of the NF-Y transcription factor in higher eukaryotes. However, in the parasite these proteins, called ORPs, are not involved in regulation of gene expression. Instead, they are found in the periphery of the so called oocyst, a stage of the parasite found on the mosquito midgut. These proteins function in the rupture of the oocyst, which leads to the release of the infectious form of the parasite. We are interested in understanding how these proteins interact as well as identifying other factors involved in oocyst rupture. The aim is the identification of molecules able to block oocyst rupture and the transmission of the parasite.
- Actin dynamics is important in many processes in the parasite. We are investigating the role of a divergent actin that is exclusively found in the malaria parasite during the early stages in the mosquito. This protein is essential for completion of the sexual stage of the parasite but also has a function during meiosis. Our work aims to understand the function of this actin on the molecular level as well as how it interacts with other proteins.
- Our laboratory is involved in an international collaboration aiming at developing polymeric nanovectors for the delivery of antimalarial agents to the mosquitoes with the goal to reduce transmission of the parasite. Our part of this project is to evaluate the effect of drugs encapsulated in nanoparticles in blocking parasite proliferation in the mosquito.

Progress in 2019-2020

An important milestone was achieved by the development of a method to purify oocysts from infected mosquitoes. This parasite stage develops attached to the epithelial cells of the mosquito midgut and the oocysts are in a small number compared to the insect cells of the midgut. This has hampered efforts to study this parasite stage as isolation of the oocysts without huge contamination of insect material has not been possible. The method that we developed now opens the way to study this stage in more detail using proteomics techniques as well as performing *in vitro* experiments.

Our research depends on the ability genetically modify malaria parasites. Until now, we have used a method based on homologous recombination, which due to its complexity is limiting the experiments that can be performed. In addition this method necessitates the introduction of unrelated genetic material, such as antibiotic resistance markers and plasmid DNA, into the genome of the parasite. This sometimes leads to interference with the function of the gene of interest. To circumvent this we have now developed a system based on CRISPR/Cas9 technology that simplifies the generation of mutants. Importantly this new method allows the generation of sequential mutations that has not previously been possible as well as the fact that the CRISPR technology allows genetic modifications with surgical precision.



Mosquito midgut with oocysts expressing Green Fluorescent Protein

Other activities

- In 2019 our laboratory participated in the European outreach activity Researcher's Night (funded by Marie Skłodowska Curie action) presenting our work on mosquitoes and malaria.
- During 2019 we received two Erasmus exchange students as well as a visiting student from ISGlobal, Barcelona for three months training.

Web page

<http://www.imbb.forth.gr/siden-kiamos>

Publications

Andreadaki M, et al. (2020) *Plasmodium berghei* Gamete Egress Protein is required for fertility of both genders. **Microbiology Open** doi: 10.1002/mb03.1038

Siden-Kiamos I, et al. (2020) A method for purification of *Plasmodium* oocysts from mosquito midguts. **Sci Rep**. doi: 10.1038/s41598-020-64121-1

Currà C, et al. (2019) Malaria transmission through the mosquito requires the function of the OMD protein. **PLoS One** doi: 10.1371/journal.pone.0222226

LABORATORY OF RHEUMATOLOGY, AUTOIMMUNITY AND INFLAMMATION



Prodromos Sidiropoulos

Professor - Collaborating
Faculty Member

GROUP MEMBERS

Postdoctoral researchers:

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PhD

Eirini Flouri, MD,
PhD

PhD students:

Panagiota Goutakoli

Elpida Neofotistou
Themeli

Summary

The *Laboratory of Rheumatology, Autoimmunity and Inflammation of the Medical School* (<https://www.rheumatology-uoc.gr/en/research-laboratory-2>) together with the *Rheumatology Clinic (Clinical Research Unit) at the University Hospital of Heraklion*, represent an interdisciplinary group of physician scientists, bio-scientists, clinicians and nurses, which investigate *inflammation* in the context of *autoinflammatory-autoimmune rheumatic diseases*. The laboratory explores mechanisms contributing to dysregulated immune responses with the ultimate goal of developing *novel biomarkers and therapies*. We use animal models of rheumatoid arthritis as well as tissues obtained from humans (blood, bone marrow, skin, synovium) to investigate innate and adaptive immune responses under the premise that they share common effector pathways for tissue injury. Importantly, we explore the relative contribution of these pathways in human diseases by studying well characterized patient cohorts seeking to identify *molecular biomarkers for diagnosis and monitoring*. We seek to understand how novel therapies work and explore molecular or *genetic biomarkers that predict response or toxicity to therapy*.

Current Aims

- 1. Mechanisms contributing to dysregulated immune response in inflammatory arthritis (RA).**
 - 1.A.** NETosis has been proved by us and others as a mechanism contributing to inflammation and autoimmunity in RA. In this context we explore molecular mechanisms inducing dendritic cell activation via NETosis.
 - 1.B.** Among the cellular mechanisms controlling immune responses are tolerogenic dendritic cells (tolDCs), and interestingly in RA pDCs. We currently investigating molecular pathways and cellular metabolism operable in pDCs and tolDCs.
 - 1.C.** Fibroblasts are important contributors to the articular inflammation in RA. We thus will further explore their cellular interaction with synovial immune cells and we will characterize intracellular molecular and metabolic function in the context of RA. Transgenic mouse models for fibroblast's depletion-isolation will be applied.

Other activities

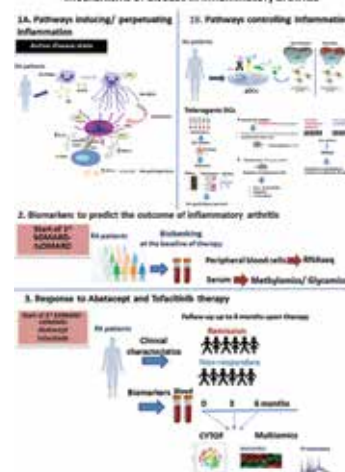
- We are actively involved in the management and support of the post-graduate program of the medical school ("Molecular Basis of Human Diseases"). We train 4-6 master students every year (rotators, master thesis).
- Drs P Sidiropoulos and G Bertias are involved in national and international medical bodies.
- We organize every 2 years a national conference, unique as a cross-fertilization stage between basic scientists and medical doctors ("Immunology Workshop for Clinicians").

- 2. Biomarkers to predict response to therapy.** 50% of patients with RA receiving treatment with biologic agents (TNF α inhibitors, CTLA4-Ig, Jak-inhibitors) respond to therapy. Personalized prediction of response is not yet available. To this end we apply combined immunophenotyping, single cell analysis and proteomics, in order to identify a molecular "fingerprint" to predict individually clinical responses.
- 3. Biomarkers to predict the outcome of inflammatory arthritis.** The long-term outcome of inflammatory arthritis cannot be predicted. In a cohort of patients with early disease we explore for a molecular "fingerprint" to predict disease's outcome. For that we apply high-throughput technologies (RNAseq, methylomics, glycomics) in peripheral blood cells of patients.

Progress in 2019-2020

- 1.A.** The proteinic cargo of NETs derived from RA patients has been characterized, as well as preliminary experiments for the molecular effects of NETosis-induced DCs' activation.
- 1.B.** We have analyzed the intracellular pathways inducing the "anti-inflammatory" function of pDCs upon IL-6R activation. Moreover, RNAseq analysis revealed pathways dysregulated upon tolDCs induction by CTLA4-Ig.
- 2.** Immunophenotypic and proteomic analysis of peripheral blood immune cells and serum respectively has been done, for RA patients treated with CTLA4-Ig (abatacept). We have further expanded the well characterized human cohorts of patients with inflammatory arthritides (rheumatoid and spondylarthropathies).
- 3.** RNAseq analysis in peripheral blood cells as well as glycomic's analysis of peripheral immunoglobulins in early RA patients has been accomplished.

Mechanisms of disease in inflammatory arthritis



Graphical outline of the different projects of rheumatoid arthritis group in the Laboratory of Rheumatology, Autoimmunity and Inflammation.

<https://www.rheumatology-uoc.gr/el/>
<https://www.rheumatology-uoc.gr/el/ereunhtiko-ergasthrio>

Web page

Publications

Panousis NI, et al. (2019) Combined genetic and transcriptome analysis of patients with SLE: distinct, targetable signatures for susceptibility and severity. *Ann Rheum Dis*. 78(8):1079-1089 doi: 10.1136/annrheumdis-2018-214379

MECHANISMS SHAPING INNATE IMMUNE RESPONSIVENESS



Christos Tsatsanis

Professor - Collaborating
Faculty Member

GROUP MEMBERS

Senior Research Associate:
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Elina Pafioti
Ioanna Pantazi

E. Deiktakis
(until Oct. 2020)
Ioanna Lapi
(started Oct. 2020)

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Christina Eframoglou
(until May 2020)
Ioanna Lapi
(until Oct. 2020)
Sevi Xenikaki

Summary

Innate immune responses are modified by pathogenic and non-pathogenic stimuli. Macrophages, the central mediators of innate immune responses, obtain different activation phenotypes in the context of metabolic disease, infections and inflammatory diseases. Our work focuses on understanding the role of insulin signaling and insulin resistance in shaping innate immune responses and how these changes may contribute to disease pathogenesis.

Current Aims

Aim of our work is to delineate how insulin, metabolic or inflammatory signals change the responsiveness of macrophages, fibroblasts as well as the differentiation and activation of adipocytes. For our studies we utilize mouse models of inflammation and infection, focusing on bacterial infection and sepsis. How insulin signaling and insulin resistance modulate macrophage responsiveness to infection is being investigated. The lab has long-standing expertise on Akt signaling and the contribution of Akt-mediated signals in innate immunity. The crosstalk of insulin signaling with epigenetic changes at the level of histone methylation that occur in the context of obesity and metabolic disease is currently analyzed. In addition, we study how these changes affect macrophage metabolism and, in turn, responsiveness to inflammatory stimuli.

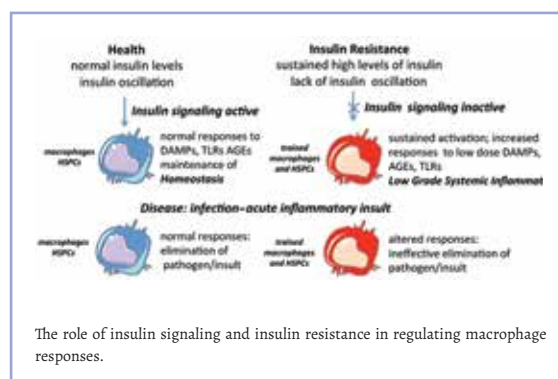
The impact of metabolites and nutritional products on innate immune responses and the gut microbiome is also investigated. Dietary metabolites can either directly or indirectly, via altering the gut microbiome, affect metabolic inflammation, inflammatory diseases and macrophage responses. To this end, using mouse models we are analyzing a series of metabolites and nutritional products on the gut microbiome and how these can modulate inflammatory responses in the context of metabolic and inflammatory diseases.

Progress in 2019-2020

We have shown that insulin resistance occurs in macrophages, which obtain an M2-like phenotype that renders them hypo-responsive to pro-inflammatory stimuli. Accordingly, obese mice

or Akt2 deficient mice that harbor insulin resistant macrophages, have reduced inflammatory responses in polymicrobial sepsis, protecting mice from developing endotoxin shock, but at the same time they do not effectively clear bacteria. We investigated the underlying mechanism and showed that insulin resistance changed the metabolic profile of macrophages, an effect that was differentially mediated by Akt1 and Akt2 kinases and mTORC1 (J. Immunol. 2019). The results have established a new concept, this of trained immunity that is shaped by insulin signaling and insulin resistance. According to this concept, active insulin signaling supports a healthy immune response while resistance to insulin signals results in altered immune responses, contributing to pathogenesis of an array of diseases associated with insulin resistance. Altered macrophage responses are attributed to metabolic and epigenetic changes that are under the control of Akt/mTOR signaling. This concept was presented in a review article (Front. Immunol. 2019).

Our work also focused on the impact of metabolic and nutritional products in regulating inflammation and the gut microbiome. We showed that a series of terpenes and disulfides modulate macrophage responses and can suppress inflammatory diseases such as inflammatory bowel disease, through modulation of Akt signaling and macrophage metabolism (Mar. Drugs 2019; Mar. Drugs 2020). These findings support the crosstalk of dietary metabolites and innate immune responses.



Other activities

- We have contributed to the organization of the 2020 Annual Conference of Clinical Chemistry and Laboratory Medicine, Greece.
- International Medical students from Brasil and Malaysia were hosted under the IFMSA program to obtain research expertise.
- A researcher from Poland was hosted as part of the ERASMUS exchange program to conduct research.
- We presented our work in national and international scientific conferences receiving best poster award at the Annual conference of the Hellenic Society of Biochemistry and Molecular Biology.

Web page

<https://www.imbb.forth.gr/imbb-people/en/tsatsanis-overview>

Publications

Daskalaki MG, et al. (2020) Disulfides from the Brown Alga Dictyopteris Membranacea Suppress M1 Macrophage Activation by Inducing AKT and Suppressing MAPK/ERK Signaling Pathways. *Mar Drugs* 18(11): E527 doi:10.3390/md18110527

Ieronymaki E, et al. (2019) Insulin Resistance in Macrophages Alters Their Metabolism and Promotes an M2-Like Phenotype. *J Immunol.* 202(6):1786-1797 doi:10.4049/jimmunol.1800065

Ieronymaki E, et al. (2019) Insulin Signaling and Insulin Resistance Facilitate Trained Immunity in Macrophages Through Metabolic and Epigenetic Changes. *Front Immunol.* 10:1330 doi:10.3389/fimmu.2019.01330

**CELLULAR &
DEVELOPMENTAL
BIOLOGY**

GENE REGULATION DURING DEVELOPMENT, HOMEOSTASIS AND TUMORIGENESIS IN *DROSOPHILA*



Christos Delidakis

Professor - Collaborating
Faculty Member

GROUP MEMBERS

Senior postdocs:

Vasiliki Theodorou
Evanthia Zacharioudaki
Zoe Veneti

PhD students:

Pawel Piwko
(graduated 2019)
Konstantina Kalodimou
Chrysanthi Voutyraki
Virginia Fasoulaki
Vassiliki Kapoulea

MSc students:

Margarita Masoura
Nikolaos Vlachopoulos
Maria Kolonia

Research assistant:

Margarita Stapountzi

Undergraduate thesis students:

Alexandros Horomidis
(2020)
Anastasia Meligounaki
Eleni Aligianni
Myrto Mittleton
Florentia Romanou
(2020)
Katerina Stefanaki
(2019)
Christina Thomou
(2020)

Summary

Animal cells coordinate their activity within the organism by responding to signals from other cells and intersecting them with intrinsic developmental programs and external environmental influences. How does this complex set of inputs converge on gene expression? Transcriptional enhancers are a central integrating device by recruiting ensembles of transcription factors (TFs), which are regulated by intrinsic or extrinsic cues. Chromatin states are influenced by and reciprocally exert influence upon enhancer activity, resulting in the fine-tuning of gene expression. An important signal in the animal kingdom is called Notch and uses cell surface proteins, the Notch receptor and its DSL ligands, Delta and Serrate, to enable cells to perform contact-mediated transactions with their neighbours.

Current Aims

We use *Drosophila* as a model system to address a number of questions on the integration of signals and epigenetic states with gene regulation.

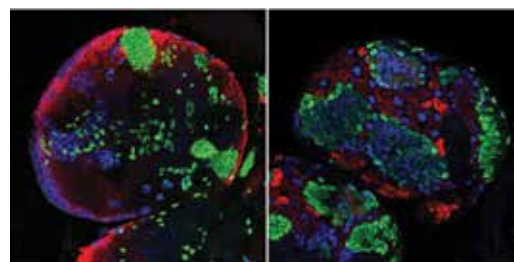
1. Deciphering the mechanism via which aberrant persistence of the Notch signal in the juvenile nervous system leads to malignant tumours. We use “usual suspect” as well as unbiased approaches.
2. A group of TFs central in promoting neural cell fates across phyla are the bHLH proneural factors. We are revisiting their involvement in early embryonic central neurogenesis using genomic approaches.
3. Neuralized is a ubiquitin ligase, which is needed for sending the Delta signal to Notch. We are studying its interaction with the Delta intracellular domain.
4. The adult *Drosophila* intestine relies on a population of stem cells (ISCs) to renew its damaged cells. We are investigating the role of epigenetic modification complexes in ISC activity.

Progress in 2019-2020

We have studied the genome-wide binding of proneural TFs and have started characterizing their target enhancers.

During our study of enhancers active during central and peripheral neurogenesis, we encountered a phenomenon of transvection, the ability of an enhancer to elicit transcription from a promoter on the homologous chromosome. We found that this trans activity is contingent upon the presence of insulators near our reporter transgenes. It is therefore conceivable that insulators may not be strictly negative elements, but more actively participate in enhancer-promoter communication.

We showed that neural stem cell (NSC) hyperplasias caused by aberrantly high Notch signalling in the larval CNS are malignant, by transplanting to adult hosts and observing uncontrollable proliferation and premature death. Using genome-wide approaches, we found that these tumours repress many differentiation factors. It is possible that tipping the balance between stemness and differentiation is sufficient to turn cells malignant upon allografting.



Examples of two brain lobes showing local Notch induced hyperplasias, marked green. Blue is a NSC marker; red is a neuronal marker. In the left brain the affected areas also express *zfh1*, a differentiation gene normally repressed by Notch, and this eliminates the NSC marker and shrinks the hyperplasia.

Other activities

Supervision of the IMBB confocal and Flyroom facilities. Coordination of the Graduate Programme in Molecular Biology and Biomedicine, a joint venture of our Institute with the Departments of Biology and Medicine of the University of Crete. Presentations on developmental genetics to high school students and newspaper articles.

Web page

<http://www.imbb.forth.gr/delidakis>

Publications

Magadi SS, et al. (2020) Dissecting Hes-centred transcriptional networks in neural stem cell maintenance and tumorigenesis in *Drosophila*. **Development** 147 doi:10.1242/dev.191544

Piwko P, et al. (2019) The role of insulators in transgene transvection in *Drosophila*. **Genetics** 212: 489-508 doi: 10.1534/genetics.119.302165

GENOME (IN)STABILITY AND MAMMALIAN PHYSIOLOGY



George A. Garinis

Professor - Collaborating
Faculty Member

GROUP MEMBERS

Postdoctoral scientists:

Georgina Hatzinikolaou
(started in 2012)

Callina Stratigi
(started in 2016)

Evi Goulielmaki
(started in 2016)

Katerina Gkirtzimanaki
(started in 2018)

PhD students:

Ourania Chatzidoukaki

Alexia Akalestou

Athanasios Siametis

Vivian Kalamara

Giorgos Niotis

MSc students:

Edisona Tsakani

Ioanna Stavgiannoudaki

Ermioni Arvanitaki

Paraskevi Apalaki

Lab manager:

Kavadina Katrakili

Summary

We are using a series of genetically engineered mouse models that carry inborn DNA repair defects or tagged proteins involved in DNA repair or transcription to dissect the impact of DNA damage on mammalian physiology during aging. This approach allows us to understand how mammalian cells exploit their natural defense strategies to counteract DNA damage-driven pathologies and prolong healthspan.

Current Aims

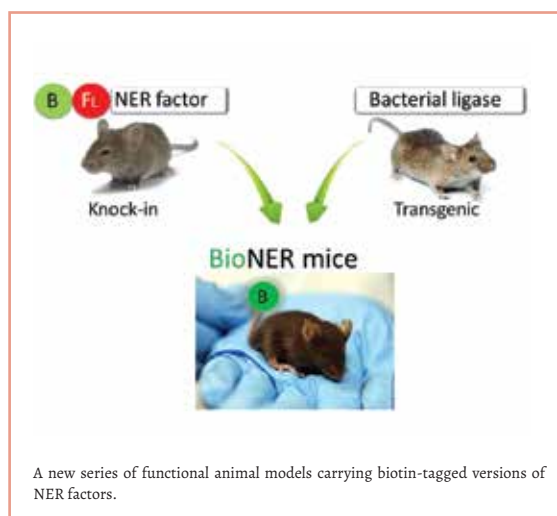
Current work in the lab is focused on:

- Elucidating the impact of DNA damage on immunometabolism:** Besides genome maintenance pathways, multicellular organisms also employ adaptive and innate immune mechanisms to guard themselves against bacteria or viruses. Recent evidence points to reciprocal interactions between DNA repair, DNA damage responses and aspects of immunity; both self-maintenance and defense responses share a battery of common players and signaling pathways aimed at safeguarding our bodily functions over time. Using the mouse, we are investigating the beneficial and unrewarding outcomes of DNA damage-driven inflammation in the context of tissue-specific pathology, metabolism and disease progression.
- Dissecting the functional role of Nucleotide Excision Repair in mammalian development:** How DNA damage triggers the onset of tissue-specific pathology in NER patients and accompanying mouse models remains an intriguing question arguing for tissue-specific responses against deleterious threats. Using an *in vivo* biotinylation tagging approach in mice and a series of cell type-specific knockout animal models, we try to understand how distinct DNA repair mechanisms are functionally linked to developmental gene expression programs in mammals and how chromatin organizers respond to DNA damage during development or with disease onset.
- Delineating the functional links between nucleotide excision repair and transcription:** Recent work has revealed that proteins in nucleotide excision repair (NER) play distinct roles, including some that go well beyond

DNA repair (3). NER factors are components of protein complexes known to be involved in nucleosome remodeling and histone ubiquitination (1), as well as transcriptional activation (2) of genes involved in nuclear receptor signaling, stem cell reprogramming, and postnatal mammalian growth. Using a series of functional genomics, mammalian genetics and biochemical approaches, we are investigating these new mechanisms in relation to the developmental abnormalities and premature disease onset observed in NER syndromes.

Progress in 2019-2020

In 2020, Evi Goulielmaki showed that macrophages secrete exosomes that trigger metabolic reprogramming upon DNA damage (Nature Comms 2020). In the same year, Georgina Chatzinikolaou and Callina Stratigi revealed that the structure-specific endonuclease ERCC1-XPF has a functional role in the repair of transcription-associated DNA damage (preprint; bioRxiv 2020). Most recently, Evi Goulielmaki and Maria Tsekrekou revealed an unexpected link between the Splicing factor XAB2, DNA damage and R-loop processing (preprint; bioRxiv 2020).



Other activities

2020:

- EMBL Council appointment (George A. Garinis)
- ELIDEK postdoctoral grant award (Evi Goulielmaki)
- ERC Proof of Concept grant
- Organization of EMBO workshop on "Developmental Circuits in Aging" May 2020

2019:

- ELIDEK postdoctoral grant award (Georgina Chatzinikolaou)
- Elected EMBO membership (George A. Garinis)
- Friedrich Wilhelm Bessel Research Award (George A. Garinis)
- Coordination of two European networks on DNA damage (aDDress) and Ageing (HealthAge).

Web page

www.garinislab.gr

Publications

(<https://www.garinislab.gr/publications/all-publications>)

Alissafi T, et al. (2020) Mitochondrial oxidative damage dictates regulatory T cell defects in autoimmunity. *Cell Metabolism* 22:S1550-4131(20)30359-4

Goulielmaki E, et al. (2020) Tissue-infiltrating macrophages mediate an exosome-based metabolic reprogramming upon DNA damage. *Nature Commun.* 11(1):42

Chatzidoukaki O, et al. (2020) The DNA Damage Response and Metabolic Reprogramming in Health and Disease. *Trends Genet.* 16:S0168-9525(20)30166-9

DEVELOPMENTAL MORPHOGENESIS LAB



Anastasios Pavlopoulos
Assistant Researcher (C)

GROUP MEMBERS

Lab Manager:

Marina Ioannou

Postdoctoral Researcher:

Evangelia Stamataki

PhD Student:

John Rallis

MSc Student:

Gentjan Kapaj

Diploma Student:

Themistoklis Archontidis

Eirini Daskalaki

Georgia Chalkia

Visiting Student:

Myrto Ziogas

Summary

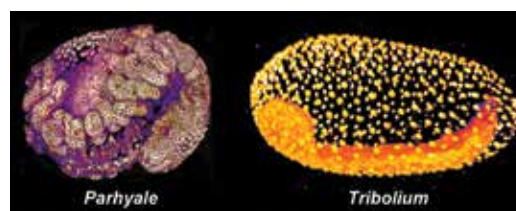
During morphogenesis of multicellular organisms, cells assemble into tissues that are extensively remodeled to yield the characteristic size and shape of organs. Our Developmental Morphogenesis lab studies the patterned cell activities and the underlying physico-chemical mechanisms that orchestrate the emergence of biological form. We have introduced two genetically and optically tractable arthropod species, the shrimp-like crustacean *Parhyale hawaiiensis* and the beetle *Tribolium castaneum*, as powerful and attractive model systems to study the molecular, cellular and mechanical basis of tissue and organ morphogenesis during animal development and evolution. Our research benefits from longstanding and enjoyable collaborative interactions with the Tomancak group at MPI-CBG (Dresden, Germany), the Keller group at HHMI Janelia Research Campus (Ashburn, USA), the Averof group at IGFL (Lyon, France) and others.

Current Aims

While the information about organismal form is encoded in the genome, the sculpting of living structures in 3D space over time is ultimately a physical process. We continue to develop functional genetic and imaging tools to meet the challenges of capturing and measuring the behaviors of thousands of cells, subcellular structures and gene expression dynamics in the normal context of *Parhyale* and *Tribolium* development. In addition to more traditional imaging methods such as widefield and confocal microscopy, we have embraced multi-view light-sheet microscopy to image fluorescently labeled specimens in their entirety, over long periods of time, at high spatial and temporal resolution, and with minimal photo-bleaching and photo-toxicity. We use these multidisciplinary approaches to study the allometric growth of serially homologous limbs, the conservation and divergence in limb patterning mechanisms, and tissue mechanics driving early insect embryogenesis.

Progress in 2019-2020

Quantification of cell behaviors in imaged wild-type or genetically and mechanically perturbed embryos has offered a bottom-up perspective of various morphogenetic processes in *Parhyale* and *Tribolium*. In the case of *Parhyale*, comprehensive reconstructions of fate maps using new open-source software available as a Fiji/ImageJ plugin has provided insights into the cell lineage restrictions and differential cell behaviors contributing to animal limb bud formation and outgrowth. In the case of *Tribolium*, combining imaging of nuclear, membrane, cytoskeletal actin and myosin dynamics with physical modeling has provided insights into the cell and tissue interactions and the forces contributing to widely conserved epithelial movements during animal gastrulation. Comparisons between *Parhyale*, *Tribolium* and other classic model systems in developmental biology, like flies, fish and the mouse, have started shedding light on the conservation and divergence of morphogenetic mechanisms by which animal tissues take shape during development.



Light-sheet microscopy reconstructions of transgenic *Parhyale* and *Tribolium* embryos with fluorescently labeled nuclei. Lateral views, anterior to the left.

Other activities

1. In 2019, the Pavlopoulos lab relocated from HHMI Janelia Research Campus in USA to IMBB-FORTH in Greece.
2. Together with Dr. Giannis Zacharakis at IESL-FORTH, we were awarded a FORTH Synergy grant in 2020 to build a modular light-sheet fluorescence microscope and study the cellular mechanisms driving animal limb diversification.
3. Gentjan Kapaj and John Rallis in the lab were awarded studentships by Fondation Santé and the Bodossaki Foundation for their graduate studies.

Web page

<https://www.imbb.forth.gr/imbb-people/en/publications-pavlopoulos>

Publications

Jain A, et al. (2020) Regionalized tissue fluidization by an actomyosin cable is required for epithelial gap closure during insect gastrulation. **Nature Communications** 11:5604

Pavlopoulos A and Wolff C (2020) Crustacean limb morphogenesis during normal development and regeneration. **The Natural History of the Crustacea** Volume VII - Chapter 2:45-78

Münster S, et al. (2019) Attachment of the blastoderm to the vitelline envelope affects gastrulation of insects. **Nature** 568:395-399

NEUROGENETICS AND AGEING



Nektarios Tavernarakis
Professor - Collaborating
Faculty Member

GROUP MEMBERS

Principal Staff Scientist:
Maria Markaki

Postdoctoral researchers:
Nikolaos Charmpilas
(Left in 2020)

Persefoni Fragiadaki
(Started in 2019)

Georgios Konstantinidis
(Started in 2019)

Eirini Lionaki

Thanos Metaxakis

Konstantinos Palikaras

Andrea Princz
(Left in 2020)

PhD students:

Ioanna Daskalaki

Ilias Gkikas

Kostas Kounakis

Margarita Papandreou

Dionysia Petratou

Christina Ploumi

Aggeliki Sotiriou

Dikaia Tsagkari
(Started in 2020)

MSc Student:

Martha Gjokolaj
(Started in 2019)

Visiting scientist:

Guofeng Lou

Visiting students:

Maria Chountoules
(Left in 2019)

Myrto Ziogas
(Left in 2019)

Research Assistant:

Angela Pasparakis

Summary

Although age-related deterioration of the nervous system is a universal phenomenon, its cellular and molecular underpinnings remain obscure. What mechanisms are responsible for the detrimental effects of ageing on neuronal function? We use the nematode *Caenorhabditis elegans*, a highly malleable genetic model which offers a precisely defined nervous system to monitor and dissect neuron quality control mechanisms during ageing, *in vivo*. Given the pronounced bioenergetic demands and exceptional endurance of neuronal cells throughout the lifespan of an organism, we focus on mitochondrial maintenance and turnover. Maintenance of mitochondrial function and energy homeostasis requires both generation of newly synthesized and elimination of dysfunctional mitochondria. We have recently shown that mitophagy, a selective type of autophagy targeting mitochondria for degradation, interfaces with mitochondrial biogenesis to regulate mitochondrial content and longevity in *C. elegans*. Recent studies have also revealed that defects in mitophagy accelerate the ageing process and are implicated in many pathological conditions, including cancer, metabolic disease, cardiovascular diseases and neuro-degeneration disorders.

Current Aims

Our current work aims at dissecting the contribution of mitophagy in long-term neuron survival. In this context, we also assess how ageing modulates mitophagy to inflict neuronal damage in nematode models of human neurodegenerative disorders. To achieve this goal, we implement a multidisciplinary approach that combines nematode genetics and genomics, molecular biology and biochemical methodologies with state-of-the-art *in vivo* neuronal imaging technology to dissect the involvement of mitophagy in neuronal physiology and neurodegeneration during ageing.

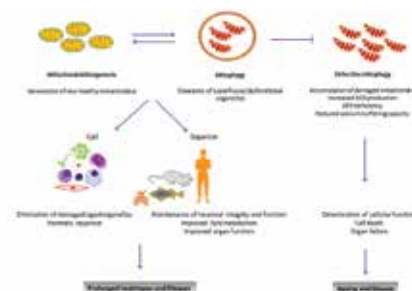
Progress in 2019-2020

We provided critical insights into the role of mitophagy in age-related diseases. In this regard, a recent collaborative

study established the contribution of defective mitophagy to Alzheimer's disease (AD) onset and progression in a manner that is conserved from *C. elegans* and mice to humans. Conversely, mitophagy induction through supplementation of NAD⁺, urolithin A, and actinonin was able to reverse cognitive deficits in both A β and tau nematode models of AD.

Moreover, we revealed a novel link between mitochondria maturation and germ cell differentiation. The findings of this study integrate mitochondria and their homeostasis in the developmental modules which shape the *C. elegans* germline. Furthermore, establish enhanced mitochondrial maturation as a prerequisite for stem cell differentiation in diverse model organisms, and further implicate augmented mitochondrial activity in tumorigenesis.

We contributed to the elucidation of a novel pathogenetic mechanism for Autosomal Dominant Optic Atrophy (ADOA), an incurable, visual loss disorder. It was demonstrated that excess neuronal autophagy depletes mitochondria in axons and triggers neurodegeneration in both nematodes and mouse. Genetic and pharmacological inhibition of autophagy restores mitochondrial content in neuronal processes and reverses vision loss in ADOA mouse model.



Balanced mitochondrial turnover regulates energy homeostasis and ageing.

Coordination of mitochondrial biogenesis and mitophagy is required for maintaining mitochondrial function and metabolic homeostasis at both the cellular and whole-organism level. Conversely, defective mitophagy leads to accumulation of dysfunctional mitochondria, impaired ATP production, increased ROS production and disturbed calcium homeostasis, adversely affecting cellular and organismal viability.

Other activities

- Professor Tavernarakis was elected Vice President of the European Research Council for the Life Sciences domain. He was also elected Member of the European Institute of Innovation and Technology (EIT) Governing Board and Executive Committee, of the American Association for the Advancement of Science (AAAS), of the German National Academy of Sciences "Leopoldina", of the European Academy of Sciences and Arts, of Academia Europaea, Fellow of the Freiburg Research

Collaboration Programme and Corresponding Member of the Academy of Athens.

- Co-organizer of several international scientific conferences.
- Keynote and invited speaker in several international conferences.
- Lab Members were awarded with 3 short-term fellowships.
- International collaborations with researchers in the USA and Europe, as well as with industry.

Web page

<http://www.elegans.gr/>

Publications

<http://www.tavernarakislab.gr/publications/publications.html>

Tavernarakis N (2020) Inflammation brakes mitochondrial metabolism in obesity. *Nature Immunology* 21: 1143-1145

Charmpilas N and Tavernarakis N (2020) Mitochondrial maturation drives germline stem cell differentiation in *Caenorhabditis elegans*. *Cell Death and Differentiation* 27: 601-617

Tavernarakis N (2019) Ageing: Neural excitation moderates lifespan (Moderation of neural excitation promotes longevity). *Nature* 574: 338-340



GENE REGULATION & GENOMICS

METALLOREGULATED TRANSCRIPTION IN *SACCHAROMYCES CEREVISIAE*



Despina Alexandraki

Professor Emerita

(since Nov 2019)-

Collaborating Faculty
Member

GROUP MEMBERS

Senior postdoc:

George S.
Fragiadakis

PhD students:

Dimitra Dialynaki
Kostas Barsakis

Undergraduate thesis
student:

Athanasia
Stavropoulou

Summary

S. cerevisiae provides the power of genetics, a complete genome sequence, a variety of tools and procedures for genome/proteome-wide analyses, and many genes and processes conserved even in mammals. Our group was for a long time involved in the yeast *Saccharomyces cerevisiae* genome sequencing and functional analyses of newly identified gene projects. We have thereof been involved in the molecular analysis of specific gene regulatory pathways in response to environmental changes in yeast. Our reference system is the metalloregulated transcription, a major aspect of metal homeostasis-vital to all organisms. Specific DNA binding transcriptional regulators are functionally modulated by metal availability-Mac1 by copper and Aft1 by iron-by post-translational modifications and by specific protein interactions. Analysis of binary and multiprotein interactions with these factors revealed new protein roles and facts concerning the mechanisms of chromatin organization, transcription per se, and new functional connections between metal-dependent transcription and other cellular signaling pathways such as DNA damage, oxidative stress, metabolism, etc.

Progress in 2019-2020

1. Genome-wide occupancies of Aft1 and Mac1 under various conditions revealed potential interdependencies and additional functions of these proteins concerning the whole chromatin, aside from their known specific gene regulation function.
2. The importance of multitask activities of these proteins in genomic integrity was also revealed by their involvement in non-induced, physiological DNA damage response cellular signaling through their interaction with specific DDR protein factors. Additionally, we (for Mac1) and others (for Aft1) obtained evidence for their involvement in centromeric roles during meiosis and mitosis.

3. We have identified a specific link between the copper/iron homeostasis and the widely used as radiomimetic, DNA damaging drug Zeocin, also used as anticancer and antibiotic agent. Transcriptomic analyses revealed specific interference of the drug with Mac1 function resulting from down regulation of the TORC1 signaling pathway. We have further verified various aspects of the resulting metabolic deregulation (such as autophagy and mitochondrial function) by specific functional assays. This is a new aspect of the cellular responses to Zeocin and of the Mac1 functional regulation.
4. We have examined aspects of the distinct roles of the glutamate dehydrogenase isoforms Gdh1 and Gdh3 in the different growth phases of *S. cerevisiae* at different growth conditions in combination with different relevant yeast mutants.

Current aims

We are currently focusing on further dissecting and understanding of the specific molecular target of the drug Zeocin and the mechanism leading to metabolic deregulation /reprogramming in the presence of the drug.



Other activities

- Chairperson of the inter-departmental Master's Program in "Molecular Biology and Biomedicine" of the Departments of Biology and Medicine at the University of Crete. Renewal of this program was established, to become inter-Institutional including IMBB as a legally equal partner (2018 and 2019).
- Teaching of undergraduate courses and selected topics in graduate courses: Developmental biology, topics on yeast molecular genetics and genomics.
- Scientific Editor (*and translator of a few chapters*) for the translation in Greek of the book: *Developmental Biology*, Eleventh Edition, SINAUER, by Scott F. Gilbert and Michael J. F. Barresi <http://11e.devbio.com/> (University Press of Crete (<http://www.cup.gr>) (2019).
- Since November 2019, Emeritus Professor of the Biology Department at the University of Crete.

Web page

<https://www.imbb.forth.gr/en/research-en/gene-regulation-epigenetics/item/82-despina-alexandraki>

Publications

Voutsina A, et. al (2019) Synergy of Hir1, Ssn6 and Snf2 global regulators is the functional determinant of a Mac1 transcriptional switch in *S. cerevisiae* copper homeostasis. *Curr Genet.* 65:799-816.

Gkouskou K, et al (2019) Distinct associations of the *Saccharomyces cerevisiae* Rad9 protein link Mac1-regulated transcription to DNA repair. *Curr Genet.* 66:531-548.

UNDERSTANDING THE GENETIC BASIS AND THE MOLECULAR MECHANISMS OF ATHEROSCLEROTIC CARDIOVASCULAR DISEASE



Dimitris Kardassis, PhD
Professor - Collaborating
Faculty Member

GROUP MEMBERS

Senior research assistant:

Paraskevi Papakosta

Postdoctoral researcher:

Efstathia Thymiakou

PhD students:

Dimitris Nasias

Dimitris Theofilatos

Katerina Dalakoura-

Karagkouni

Isidoros Axiotis

Summary

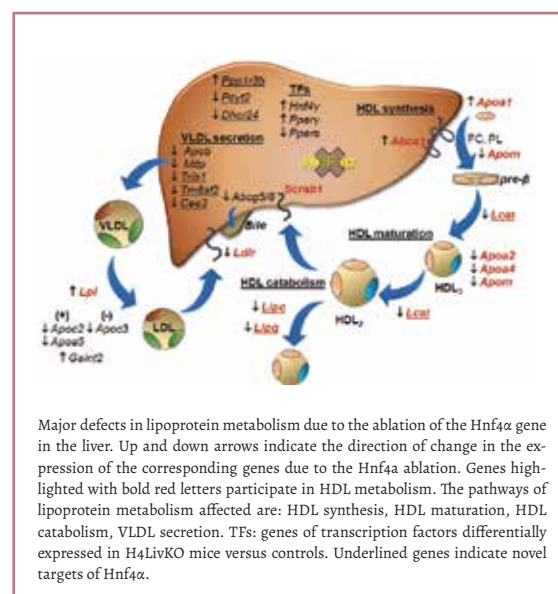
Atherosclerotic Cardiovascular Disease (ASCVD) is the leading cause of death worldwide. Several risk factors including obesity, type II diabetes, non-alcoholic fatty liver disease and dyslipidemia predispose to ASCVD by mechanisms that are not fully understood. Reliable genetic or non-genetic biomarkers are also needed in order to increase the value of current risk prediction algorithms. In our lab we are studying the genetic determinants of atherosclerosis using animal models combined with virus-mediated gene transfer and omics technologies with the goal to understand better the pathogenesis of atherosclerosis and to identify novel biomarkers and drug targets for ASCVD.

Current aims

One major aim of our current research is the understanding of the molecular mechanisms by which high density lipoproteins (HDL) protect from atherosclerosis and how this athero-protection is compromised by mutations in HDL genes or in chronic inflammatory diseases such as rheumatoid arthritis. A second aim is to identify key genes and gene networks in metabolic organs, such as the liver and adipose tissue, which contribute to the pathogenesis of metabolic diseases such as non-alcoholic fatty liver disease (NAFLD) and the metabolic syndrome (MetS). To achieve our goals we are using genetically engineered mouse models (transgenic, knock out) combined with AV- or AAV-mediated gene transfer and transcriptomics (expression arrays, RNA-seq) technologies in close collaboration with the mouse station and the genomics facility of IMBB.

Progress in 2019-2020

During 2019-2020 we identified changes in hepatic and adipose transcriptome as well as in circulating miRNAs that are associated with the pathogenesis of MetS in a humanized mouse model of MetS (apoE3L.CETP mice). In collaboration with the group of T. Lutz in Zurich we showed that acute weight loss or RYGB surgery are equally efficient in restoring MetS pathology and the liver transcriptome in apoE3L.CETP mice with diet-induced MetS. These studies identified PPAR γ as a key upstream regulator of several genes that are implicated in MetS pathogenesis including PPAR γ itself. We showed that liver-specific ablation of Hepatocyte Nuclear Factor 4A in mice is associated with defects in HDL metabolism, hepatic steatosis and premature death. Finally we identified and characterized exonic single nucleotide polymorphisms (SNPs) in genes of HDL metabolism in a Greek cohort with extreme HDL cholesterol levels.



Other activities

- Member of the board of the Hellenic Society of Biochemistry and Molecular Biology (HSBMB) (2017-2019)
- Head of the working group on the Pathophysiology of Atherosclerosis of the Hellenic Atherosclerosis Society (HAS) (2019-2021)
- Member of the Management Committee of COST Action CA17129 (CardioRNA) and invited speaker in the first WG meeting in Lisbon (February 2019).

Web page

<https://www.imbb.forth.gr/en/research-en/gene-regulation-epigenetics/item/83-dimitris-kardassis>

Publications

Nasias D, et al. (2020) Transcriptome analysis of the adipose tissue in a mouse model of metabolic syndrome identifies gene signatures related to disease pathogenesis. **Genomics** 50888-7543(19)31032-8

Thymiakou E, et al. (2020) Defects in High Density Lipoprotein metabolism and hepatic steatosis in mice with liver-specific ablation of Hepatocyte Nuclear Factor 4A. **Metabolism** 110:154307

Nasias D, et al. (2019) Significant changes in hepatic transcriptome and circulating miRNAs are associated with diet-induced metabolic syndrome in apoE3L.CETP mice. **J Cell Physiol.** 234(11):20485-20500

STEM CELL FATE REGULATION AND THE ROLE OF PROMYELOCYTIC LEUKEMIA (PML) PROTEIN



Androniki Kretsovali
Principal Researcher (B)



Joseph Papamatheakis
Professor Emeritus-
Collaborating Faculty
Member

GROUP MEMBERS

Research Technician:
Takis Makatounakis

PhD students:
Amalia Vogiatzoglou
Gesthimani Theodosi

Summary

Our lab interests are focused on the mechanisms by which transcriptional, epigenetic and signal transduction factors regulate normal and cancer cell fate. In the recent years we have centered our studies on the diverse and contrasting roles of PML-the main constituent of multitasking sub-nuclear structures (PML bodies) -in different cell types and processes. Our lab has shown that although mainly known as a tumor suppressor, PML promotes cell cycle and self-renewal in pluripotent stem cells and is able to assist reprogramming into pluripotency of somatic cells. We are extending these studies by examining the functions of PML in the regulation of Neural Stem Cells (NSC) fate. We also study PML-dependent molecular signatures associated with tumor –suppressing or promoting effects in breast cancer and glioblastoma.

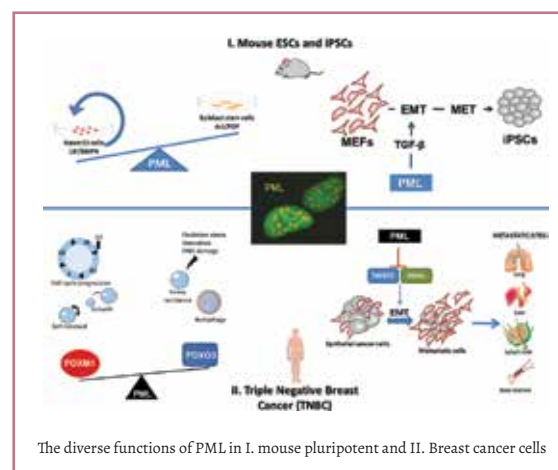
Current Aims

1. **Neurogenesis and neurodegeneration:** We are currently studying the effects of synthetic Micro Neuro Trophins (MNTs) in collaboration with the laboratories of Th. Kalogeropoulou (NRI) and A. Gravanis and I. Charalambopoulos (Medical School, IMBB) on the self-renewal and stress response of NSC and neurons. The goal is to develop an experimental system for modeling neurodegenerative diseases. Moreover we will examine the role of PML in NSC functions and neurodegeneration. For this purpose we will employ both NSC that do not express PML (PML KD) and mice derived from crossing of the 5XFAD mouse (an experimental model of familial AD) with the PML KO mouse.
2. **Breast cancer and glioblastoma (GB):** We are currently using PML knock down (KD) in MDA MB 231 and MCF7 breast cancer cell lines to study the in vitro physiology (proliferation, invasion, autophagy) and in vivo behavior (tumor growth and metastasis) and correlate those to altered gene expression profiles.
In the GB area, we have developed patient's derived glioblastomas to study drug responses, in vitro and by xeno-

grafts as well as clonal evolution. In collaboration with G. Zacharakis (IESL) and V. Sakalis (ICs) we integrate GB-cell physiology and imaging with mathematical modeling to further dissect GB invasiveness.

Progress in 2019-2020

1. We have used mouse ES cell-derived NSC and human neuroblastomas and shown that ablation of PML alleviates cells' ability to respond to stress. Specifically more ROS and especially Hydrogen Peroxide radicals are accumulating in PML deficient cells. Knock down (KD) of PML in ES cells inhibits the neuroectodermal differentiation.
2. We have studied in vitro physiology (migration/invasion, proliferation, autophagy) in vivo xenografts (local tumor growth, metastasis) and performed RNA seq analysis of parental and PML –KD MDA MB 231 and MCF7 cells. Results show that PML loss leads to increased stemness and aggression of MDA MB 231 cells accompanied by a dramatic increase of lung- and to a lesser degree other organ-metastasis.



The diverse functions of PML in I. mouse pluripotent and II. Breast cancer cells

Other activities

The group is welcoming the ELIDEK Post-doctoral Researcher Dr. Fabien Moretto who will develop a new research project on how non-coding transcription can regulate gene expression and control cell-fate decisions.

Web page

<http://www.imbb.forth.gr/kretsovali>
<http://www.imbb.forth.gr/papamatheakis>

Publications

- Kokotidou C, et al. (2019) Designer Amyloid Cell-Penetrating Peptides for Potential Use as Gene Transfer Vehicles. **Biomolecules** 10(1):7 doi: 10.3390/biom10010007
- Sachini N, et al. (2019) Promyelocytic leukemia protein (PML) controls breast cancer cell proliferation by modulating Forkhead transcription factors. **Mol Oncol.** 13(6):1369-1387 doi: 10.1002/1878-0261.12486
- Oraipoulou ME, et al. (2019) A 3D tumor spheroid model for the T98G Glioblastoma cell line phenotypic characterization. **Tissue Cell** 59:39-43 doi: 10.1016/j.tice.2019.05.007

ETS FAMILY TRANSCRIPTION FACTORS



George Mavrothalassitis

Professor - Collaborating
Faculty Member

GROUP MEMBERS

PhD Student:

Angeliki Vogiatzi

Masters Student:

Aikaterini
Kandianaki

Technical supports:

Aikaterini
Dalakoura-
Karagkouni

Bioinformatics:

Ismini Batsavia

Diploma students:

Maria Kokkori
Ioanna Stefani
Athanasios Katsalifis
Aikaterini
Kandianaki

Summary

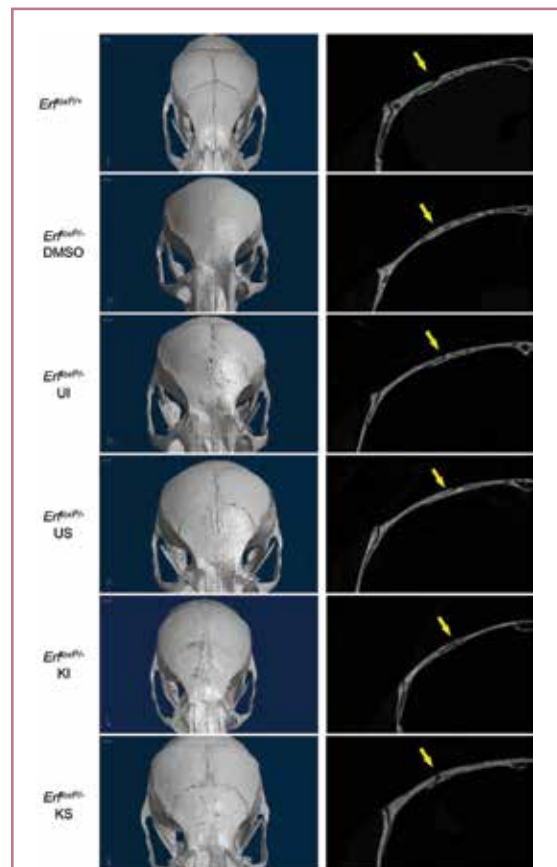
We are interested in the transcriptional regulation along the RTK – MAPK - ETS, pathway exploring the regulation, control, mechanism of action and developmental contribution of the transcriptional repressor ERF. We posit that signaling pathways can also quantitatively signal their inactive state through repressors. Thus loss of function of a repressor may recapitulate abnormal activation of the pathway. Interestingly, when an inhibitor/repressor is functionally inactivated rather than eliminated as in the case of ERF, it may be reactivated and ameliorate pathologies associated with pathway activation.

Current Aims

Our goal is to decipher the contribution of the ubiquitously expressed, Mapk-regulated transcriptional repressor ERF in development and disease and explore its potential as a therapeutic target. Erf defects have been shown to lethally affect placenta and hematopoietic embryo development, and lead to syndromic craniosynostosis, Chitayat syndrome and prostate cancer. Utilizing cellular and animal models we aim to explore additional Erf-mediated phenotypes, recapitulate the associated diseases and mechanisms of action and implement Erf-targeting pharmacological therapeutic approaches.

Progress in 2019-2020

We continued the studies on Erf, developing mesenchymal stem cells from cranial suture and analyzing the mechanism of Erf contribution in their differentiation. In parallel we evaluated the ability of pharmacological compounds to enhance Erf function and ameliorate craniosynostosis. We developed an animal model for Erf-induced prostate cancer in mice and currently analyze its similarities with the human disease regarding PCa onset and metastasis. Finally, we are evaluating the role of Erf in T-cell and neuronal development, processes known to be effected by Mapk signaling.



Pharmacological compounds enhancing Erf function can ameliorate craniosynostosis. Dorsal anterior volume renderings (images on the left) and transverse section image midway the right coronal suture (image on the right) derived from microCT scans of Erf-competent (*Erf^{flxP/+}*) and Erf-insufficient (*Erf^{flxP/-}*) animals at P65. Littermates were treated on alternate days from P5 to P65 with 5 mg/kg intraperitoneally (UI, KI) or 0.5 mg/kg subcutaneously (US, KS) over the skull with the compounds or with the inhibitor solvent only (DMSO). All animals in a litter received the same treatment. Yellow arrows point at the coronal suture position.

Other activities

Chair of the Graduate Studies Oversight Committee at Medical School of the University of Crete. Supervision of the University of Crete confocal facility.

Web page

<http://www.imbb.forth.gr/mavrothalassitis>

Publications

Vogiatzi A and Mavrothalassitis G (2019) Craniofacial, orofacial and dental disorders: the role of the RAS/ERK pathway. *Expert Rev Mol Med* 21: e2

GENE REGULATION IN THE IMMUNE SYSTEM



Charalampos Spilianakis

Associate Professor -
Collaborating Faculty
Member

GROUP MEMBERS

Special Research Assistants:
Manuela Kapsetaki
Deppie Tsoukatou

PhD students:

Tomas Zelenka
George Papadogkonas
Dionysios-Alexandros Papamattheakis
Thodoris Savvidis
(left in 2019)

MSc students:

Antonis Klonizakis
(jointly w/ C. Nikolaou)

Diploma Thesis Students:

Alexandros Minakakis
Joana Troka
Elena Stoka
Ioannis-Rafail Tzonevrakis
Nikos Arapitsas
Thanassis Fileles
George Takousiis
Lito Parapera
Papantoniou

Summary

We investigate the mechanisms, based upon protein factors and lncRNAs, that have an impact on the three-dimensional organization of chromatin and act as an epigenetic determinant regulating the development and cell lineage specification in the adaptive and innate murine immune system.

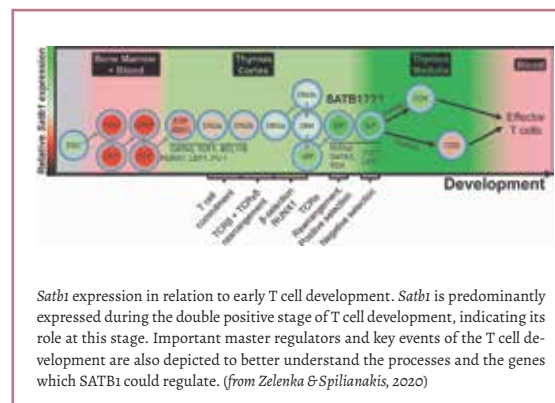
Current Aims

We use a combinatorial approach of genomics approaches such as RNAseq, ChIPseq, ATACseq, RRBS, HiChIP, HiC and CHART, among others, to study how chromatin structure is shaped in cell types of the murine immune system. We want to identify main players in genome organization which we then target in genetically modified mouse models in order to determine the effect on physiology, such as the development and function of the immune system. Utilizing holistic approaches combined with molecular modeling we try to identify the hierarchy of gene regulatory networks responsible, upon their deregulation, for the cause of disease. In parallel we work on the identification of molecular links between the mode of action of lncRNAs in shaping the chromatin structure and regulating metabolic pathways.

Progress in 2019-2020

An outstanding question for understanding how the immune system functions is how chromatin is organized in three dimensions, what factors are responsible for generating or maintaining this loopscape structure and how gene regulatory pathways are formed to support the (patho)physiological onset of several processes. To address these questions, we have now utilized either wild type or mutated cells harboring a

tissue specific deletion of the SATB1 genome organizer specifically in T cells. We have performed HiC and HiChIP experiments for both genotypes for SATB1, CTCF and H3K27ac. We have developed novel pipelines for the analysis of the data and proceeded to molecular modeling to predict novel genomic interactions between important players of T cell development and cell lineage specification. SATB1 deletion in T cells leads to a severe autoimmune phenotype in mice with gender specific differences. To this end, we have identified SATB1-dependent molecular mechanisms defining X-chromosome inactivation and therefore shedding light to the gender-specific differences identified in autoimmune diseases. Finally, in collaboration with physicists we have uncovered the special biophysical features of SATB1 protein, responsible for driving phase separation in the eucaryotic cell nucleus and thus forming quite diverse multifunctional molecular machines with discrete physiological effects.



Other activities

Invited speaker at 2019 EMBO Workshop "The genome in three dimensions" and the "2019 Fellows Symposium" of FONDATION SANTE.

Web page

<http://www.spilianakislabs.gr/>

Publications

<https://pubmed.ncbi.nlm.nih.gov/?term=spilianakis&sort=date>

Zelenka T and Spilianakis CG (2020) SATB1-mediated chromatin landscape in T cells. **Nucleus** 11(1): 117-131

Salataj E, et al. (2019) Developmental Conservation of microRNA Gene Localization at the Nuclear Periphery. **PLoS One** 14(11): e0223759. PMID: 31682635

Patsalos A, et al. (2019) The BACH1-HMOX1 Regulatory Axis Is Indispensable for Proper Macrophage Subtype Specification and Skeletal Muscle Regeneration. **J Immunol.** 203(6):1532-1547 PMID: 31405954

MOLECULAR ERYTHROPOIESIS



John Strouboulis

Research Director (A)*

*Currently Professor at King's College London

GROUP MEMBERS

Post-doctoral Researchers:

Elena Karkoulia

Grigoris Tsaknakis

PhD Students:

Ioannis-Marios

Roussis

Alexandra Vatikioti

Research Assistant:

Marina Ioannou

MSc project student:

Dimitra Mavroeidi

Summary

Our lab is studying the molecular pathways of red blood cell differentiation in physiological hematopoiesis and in hematological disease. We are focusing on the GATA1 “master” erythroid transcription factor, its close interacting co-factor FOG-1 and the maintenance DNMT1 DNA methyltransferase. We are using proteomic, genomic, computational, and functional genetic and genomic approaches in characterizing the protein complexes of these factors and the molecular pathways they regulate in physiological erythropoiesis.

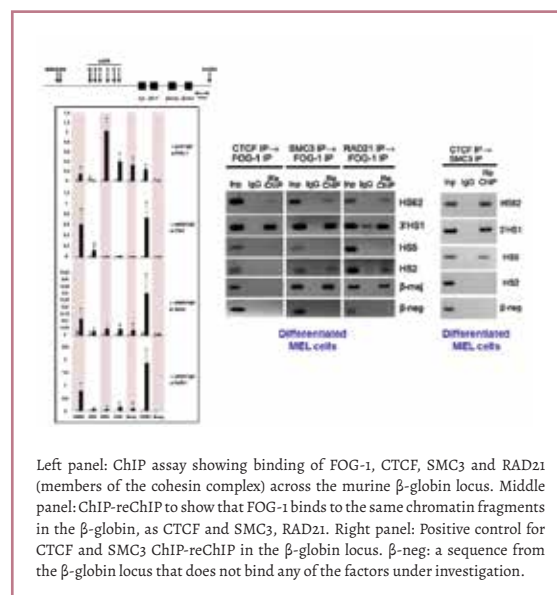
Current aims

Work in our lab is focusing on: (i) the differential characterization of GATA1 and GATA1short protein interactomes in murine fetal erythropoiesis. GATA1short is an N-terminally truncated GATA1 isoform associated with hematological disease, such as Diamond-Blackfan anemia; (ii) the characterization of FOG-1 function in gene regulation at the level of 3-dimensional chromatin domains of gene activity (DNA looping). FOG-1 is required for GATA1-mediated DNA looping during erythroid differentiation, however little is known about the molecular basis of this function; (iii) characterization of DNMT1 functions in erythropoiesis and in β -globin gene regulation. DNMT1 is implicated in γ -globin repression in adult erythroid cells, making it a potentially druggable target in epigenetically reactivating γ -globin in treating β -hemoglobinopathies.

Progress in 2019-2020

- We have generated knock-in mouse models expressing biotin-tagged full length GATA1 or GATA1short, resulting in the efficient *in vivo* biotinylation tagging of both endogenously expressed GATA1 isoforms. Phenotypic analysis revealed that the biotin-GATA1short mice present with a transient anemia in early (E12.5) fetal liver erythropoiesis. This developmental window coincides with a transient wave of definitive hematopoietic progenitors colonizing the fetal liver, suggesting that these progenitors are particularly sensitive to GATA1 dysfunction. To gain further insight, we are characterizing the differential protein interactomes of GATA1 and GATA1short in murine fetal liver erythropoiesis by mass spectrometry (MS), in collaboration with Dr. Jeroen Krijgsvelde DKFZ, Heidelberg.

- We characterized FOG-1 protein complexes in erythroid cells to identify interactions with CTCF and the cohesin complex, known to be involved in the delineation of chromosomal topological (TADs) and in the communication of distal gene regulatory elements by DNA looping. We showed FOG-1 to be required for the enhanced recruitment of CTCF and cohesins to the murine β -globin locus with erythroid differentiation, which coincides with DNA looping between the Locus Control Region with the β -globin promoter. We propose that FOG-1 serves as a critical factor nucleating interactions of proteins required for DNA looping in the β -globin locus, including GATA1, LDB1, CTCF and the cohesin complex.
- We knocked out DNMT1 by gene editing in murine erythroid cells. We found that cell cycle arrest and terminal erythroid differentiation are incomplete in the DNMT1 knockout cells. We also saw de-repression of the murine β H1-globin gene, an orthologue of the human γ -globin gene, but not of the embryonic ϵ -globin gene, thus validating DNMT1 as a potential target for the therapeutic γ -globin reactivation in treating β -hemoglobinopathies.



Other activities

Alexandra Vatikioti was awarded a prestigious Stavros Niarchos Foundation-FORTH predoctoral fellowship. The PI was the IMBB representative to the EPIC-XS European Proteomics Infrastructure Consortium funded by the EU. The PI was the main organizer of the 2019 FEBS Workshop on Chromatin Proteomics. The PI edited a Special Issue on GATA Transcription Factors in IUBMB Life.

Web page

<https://www.imbb.forth.gr/imbb-people/en/strouboulis-home>

Publications

Fugazza C, et al. (2020) The Coup-TFII orphan nuclear receptor is an activator of the γ -globin gene. **Haematologica** 2020 doi: 10.3324/haematol.2019.241224 [Epub ahead of print]

Juban G, et al. (2020) Oncogenic Gata1 causes stage-specific megakaryocyte differentiation delay. **Haematologica** 2020 doi: 10.3324/haematol.2019.244541 [Epub ahead of print]

Vatikioti A, et al. (2019) Translational regulation and deregulation in erythropoiesis. **Exp Hematol**. 75:11-20

EPIGENETICS & TRANSCRIPTION



Ioannis Talianidis

Research Director (A)

GROUP MEMBERS

Post-doctoral Researchers:

Haroula Kontaki

Marina Koukaki

Ioanna Peraki

PhD Students:

Ourania

Galanopoulou

Evaggelia

Evaggela
Tachmatzidi

Maria Vassilarou

Diploma Students:

Kostas Kydonakis

Summary

Normal biological functions in a multicellular organism rely on intricate orchestration of the basic cellular features preset by genetic constitution and a sophisticated network of cellular gene expression patterns, which are governed by epigenetic regulation. Epigenetic phenomena refer to the establishment of heritable changes in gene expression without alterations in primary DNA sequence. They impact on many physiological and pathological processes, including aging, metabolism and carcinogenesis. Our research focuses on epigenetic mechanisms regulating liver development, hepatic metabolism and liver cancer pathogenesis.

Current aims

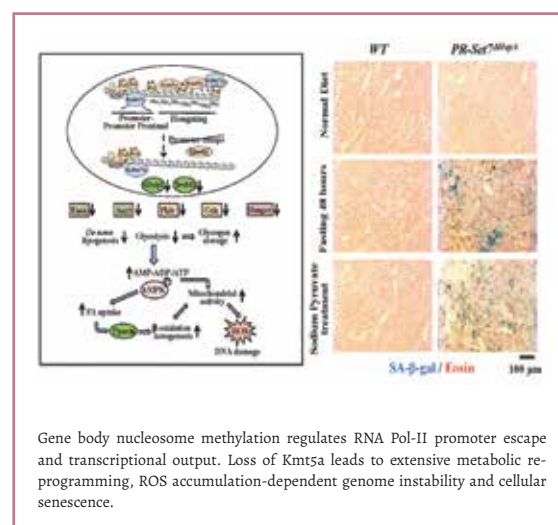
Recent studies from our lab and other investigators raised the notion that cellular de-differentiation may play central role in liver regenerative processes following injury and during the early stages of cancer development. Understanding the role of epigenetic regulatory mechanisms in the stemness characteristics of existing adult progenitor cells and those generated via de-differentiation of adult hepatocytes, is at the center of our current research interest. In particular, we aim at gaining deeper insights into the mechanism of transcription-coupled processes that regulate cellular differentiation and de-differentiation during metabolic stress, cancer initiation and tissue regeneration.

Progress in 2019-2020

Our work on transcription factor binding dynamics during liver development revealed that stable gene expression patterns are the result of combinatorial activity of multiple transcription factors, which mark regulatory regions long before activation and promote progressive broadening of active chromatin domains. Both temporally stable and dynamic, short-lived binding events contribute to the developmental maturation of active promoter configurations. Further work on the developmental bookmarking function of master regulators illuminated remarkable parallels between the principles employed for gene activation during hepatocyte differentiation, during evolution, and upon mitotic exit.

Studying the histone methylases Smdy3 and Setd8, we identified two novel regulatory layers of cancer-inducing cellular senescence and de-differentiation mechanisms. Both enzymes regulate cancer initiation by influencing the emergence and expansion of cancer stem cells, a cell population that has been presumed to resist chemotherapy and responsible for tumor re-emergence after treatment. Our preclinical studies have demonstrated that pharmacological inhibition of these enzymes offer unprecedented specificity and efficiency for treating hepatocellular carcinoma.

In collaboration with Celia Martinez-Jimenez lab at Hemholtz Centrum, Munich, we have initiated a comprehensive study to obtain molecular level understanding of heterochromatin regulators in the differentiation potential of adult progenitor cells. Our initial scRNA-seq data suggest that multiple progenitor cell populations exist in the intestinal epithelium and the liver that can be distinguished from progenitors generated via de-differentiation of fully differentiated cells. The current challenge is to correlate the high level plasticity of epigenomes and 3D genomic landscapes with the inter-conversion potential of progenitor and differentiated cell types.



Gene body nucleosome methylation regulates RNA Pol-II promoter escape and transcriptional output. Loss of Kmt5a leads to extensive metabolic reprogramming, ROS accumulation-dependent genome instability and cellular senescence.

Other activities

Members of the lab contributed to the development and testing new experimental approaches in IMBB facilities.

Hosted undergraduate and rotation students for training.

The PI served as a national representative in the preparation of the EU Mission in Cancer program.

Presentations in AXA Research Fund events and contribution to the organization of Onassis Lectures in Biology.

The lab hosted Cov-2 testing program for FORTH employees.

Web page

<https://www.imbb.forth.gr/imbb-people/en/talianidis-overview>

Publications

Karagianni P, et al. (2020) Bookmarking by Non-Pioneer Transcription Factors during Liver Development Establishes Competence for Future Gene Activation. *Cell Rep.* 30:1319-1328

Patathanasiou M, et al. (2020) Identification of a dynamic gene regulatory network required for pluripotency factor-induced reprogramming of mouse fibroblasts and hepatocytes. **EMBO J** 102236 doi: 10.15252/embj.2019102236

**PLANT
MOLECULAR
BIOLOGY**

RNA BIOLOGY- PLANT DEVELOPMENTAL BIOLOGY

**Kriton Kalantidis**Professor - Collaborating
Faculty Member**GROUP MEMBERS**

Senior Research Assistants:

Konstantina

Katsarou

Christos Andronis

Postdoctoral Research

Scientists:

Anthony James

Nikoleta

Kryovrysanaki

PhD students:

Irimi Bardani

Paraskevi Kallemi

Emily Grypioti

(completed 2019)

Research Assistants:

Mary Providaki

Anastasia

Nikoloudi

Masters Students:

Nora Papadima

(completed 2019)

Myrsini Fragiadaki

Martha Tselika

Undergraduate Students:

Emmanuel Kokkas

(completed 2020)

Nikh Gkoura

(completed 2020)

Nikoleta Pateraki

Summary

Higher eukaryotes have developed a mechanism of sequence-specific RNA degradation known as 'RNA silencing'. RNA silencing involves the generation of short interfering RNAs (siRNAs) from double-stranded RNA through double-strand-specific RNases, called Dicers. The siRNAs are incorporated into the RNA-induced silencing complex (RISC) resulting in sequence-specific cleavage of target RNAs. RNA silencing is the major defense pathway plants have against viruses.

Our interests include mechanistic aspects of RNA silencing, such as understanding the roles of individual proteins, investigating the cross-talk between RNA silencing pathways and RNA pathogens (eg. RNA viruses, satellite RNAs and viroids) and evolutionary aspects of silencing, such as the conservation of silencing pathways across evolutionarily distant clades.

Current Aims

The overall aims are to decipher mechanistic and evolutionary aspects of RNA silencing, primarily, but not exclusively in plants.

Our current research aims include:

1. Elucidate the non-DICER RNaseIII proteins in *Nicotiana benthamiana*
Plants have two groups of RNaseIII endonucleases, including the well-studied DICER proteins and another set of RNaseIII-type nucleases that remain understudied. We have initiated a project to decipher their roles in tobacco (*N. benthamiana*) and to investigate their potential involvement in viroid replication.
2. Understand the interplay between satellite RNAs of viruses and RNA silencing pathways
Satellite RNAs are small RNA molecules that require a helper virus to infect plants. We are studying the interaction of satellite RNAs with plant RNA silencing pathways.
3. Understand the role of SERRATE in plant antiviral responses
SERRATE is a Zinc-finger protein, which is conserved in eukaryotes and is a key factor in several RNA processes, including miRNA biogenesis and pre-mRNA alternative splicing. Very little is known about its role in biotic stress responses.
4. Generate mutants of Dicer genes in tobacco through gene editing

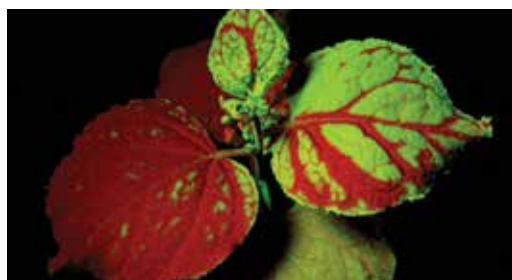
DICER proteins are key components in miRNA and siRNA biogenesis, participating in developmental regulation, epigenetic modifications, stress responses and viral defense.

5. Elucidate the role of DICERs in Diatoms

Diatoms are single cell algae contributing 20% of global carbon fixation, have a central role in the aquatic food chain and produce high value pharmaceutical, cosmetic and industrial compounds. Despite their importance, various molecular mechanisms remain understudied.

Progress in 2019-2020

1. Five RTL proteins (RTLA to E) were identified in *N. benthamiana*. We cloned four of these and initiated a reverse genetics analysis for their respective genes.
2. Using tomato bushy stunt virus and two satellite RNAs we have studied the plant response in a collection of DICER-suppressed plant lines. The results show an alteration of both virus and satellite RNAs levels, proving a combined effect of the silencing machinery against these pathogens.
3. SERRATE knock-down plants were infected with viroids and viruses revealing a significant effect of this gene on the biology of these pathogens.
4. Generated *dcl2&3* mutants in *N. benthamiana* using CRISPR/Cas9 gene editing
5. *In silico* analysis unveiled a diverse DCR/AGO/RDR repertoire in diatoms. DCR knockouts were generated and their mRNA/siRNA transcriptomes characterized through sequencing, showing a drastic reduction in sRNAs mapping to transposable elements and a concomitant transcriptional activation of TEs.



Systemic silencing of GFP in tobacco.

Other activities

Sequenced the genome of two Greek grapevine varieties (Athiri and Aidani).

Analysis of the virome of Cretan vineyards.

Selected Impact Activities:

Attendance at "Researchers Night" to interact with public via hands-on experiments with plants.

Secondary school visits to discuss Biotechnological research in plants.

Participation in Grapevine and Olive initiatives to help producers evaluate the significance of virus infections.

Web page

<http://www.imbb.forth.gr/kalantidis>

Publications

<https://www.imbb.forth.gr/imbb-people/en/publications-kalantidis>

Katsarou K, et al. (2019) Viral Detection: Past, Present, and Future. **Bioessays** doi:10.1002/bies.201900049

Kryovrysanaki N, et al. (2019) SERRATE, a miRNA biogenesis factor, affects viroid infection in *Nicotiana benthamiana* and *Nicotiana tabacum*. **Virology** 528:164-175 doi:10.1016/j.virol.2018.12.011

MOLECULAR PLANT PHYSIOLOGY-BIOTECHNOLOGY



Panagiotis Moschou

Associate Professor -
Collaborating Faculty Member

GROUP MEMBERS

Postdoctoral researchers:

Alexandra Deli

Artemis Perraki

Androniki Bibi

Chen Liu

(SLU lab)

PhD students:

Leda Tympa

Andriani

Mentzelopoulou

Ioannis

Chatzianestis

Anna Tornkvist

(SLU lab)

Master students:

Vassiliki Katidou

Anna Wulgarakis

(Starting in 2021)

Undergraduate students:

Eirini Karampidaki

Rafail Gkritzas

Stefanos Mastis

Summary

Work in our group focuses on the roles of protein and RNA homeostasis in the development and perception of the environment. We are particularly interested in how catabolism and processing of molecules such as proteins and RNA dictate the basic biological processes of stem cell fate regulation, asymmetric cell division, cell polarity and regeneration. In our research, we mainly use the model plant *Arabidopsis* but we also use crop models such as rapeseed and tomato.

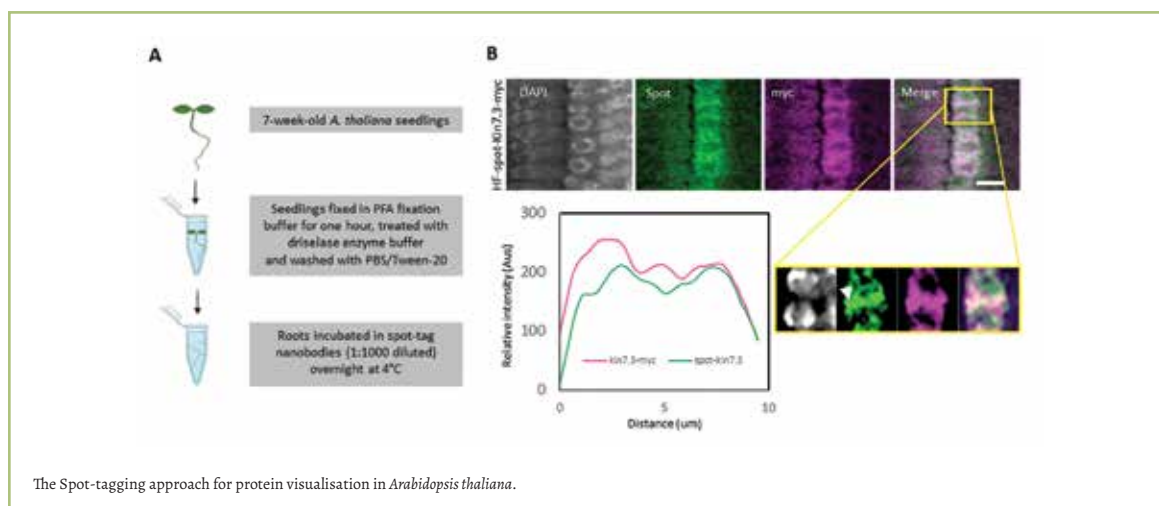
Current Aims

We study modules of proteolysis and RNA homeostasis controlling proprioception, cell fate and plant development and how these processes impinge on endocytosis, exocytosis and alternative splicing. The modules we investigate are highly adaptable and regulated by the environment. We recently succeeded in the development of *in vitro* reconstitution systems that will allow us to study these processes in minimalistic models at single-molecule resolution. We aim also to further establish the role of the cell shape, geometric edges and mitotic peptides in these processes. We exploit advanced imaging approaches, biophysics, single-molecule dynamics and structural data (main focus on prion-forming proteins) to build holistic models on the functioning of our modules. Furthermore, the development of applications based on the aforementioned processes in crop improvements is on the way.

Progress in 2020 (the group was not active in 2019)

We established a proximity biotinylation system by which signalling complexes can be identified in plant cells with unprecedented resolution (Arora et al., 2020). This system allowed us to identify the composition of novel phase-separated compartments in plants, showing that they regulate RNA metabolism and vesicle trafficking. This approach can be coupled with the most recent development of *in situ* protein probing we have developed combined with advanced microscopy to study spatiotemporal regulation of signal transduction (Mentzelopoulou et al., 2020); see figure). Applications of our microscopy approaches were used to understand the dynamics of plant immune receptors (Duxbury et al., 2020). Overall, our findings challenged the notion of discrete membrane organelles and unify processes seemingly distinct, such as endocytosis and RNA turnover.

We introduced novel concepts in the field of hormonal regulation highlighting that although hormones converge on transcriptional repressors that can be degraded by the proteasome to initiate a response, the process of a less-explored proteolytic branch called 'limited proteolysis' seems to regulate hormonal signalling (Liu et al., 2020). How the hormonal pathways are finely tuned in a cell-autonomous level is not known, while modes of regulation of timing in plant cells of regulation remain obscure. Our new results provide examples of modular protein networks with the ability to steer hormonal pathways. In a publication in preparation, we plan to provide a paradigmatic shift in how hormonal regulation is conducted in plants.



Other activities

We have an active role in the discussions about the usage and applications of CRISPR in plant biotechnology. Organization of EMBO conference in Crete, 2021 bridging gaps between plant and animal cell death.

Web page

<https://www.imbb.forth.gr/el/research-el/plant-molecular-biology-el/item/4122-panagiotis-n-moschou>

Publications

Arora D, et al. (2020) Establishment of Proximity-dependent Biotinylation Approaches in Different Plant Model Systems. **The Plant Cell** 32: 3388-3407

Duxbury Z, et al. (2020) Induced proximity of a TIR signaling domain on a plant-mammalian NLR chimera activates defense in plants. **Proc Natl Acad Sci U S A** 117: 18832-18839

Minina EA, et al. (2020) Classification and Nomenclature of Metacaspases and Paracaspases: No More Confusion with Caspases. **Mol Cell** 77: 927-929

MICROBIOLOGY AND BIOTECHNOLOGY



Panagiotis F. Sarris

Assistant Professor -
Collaborating Faculty
Member

GROUP MEMBERS

Postdoctoral researchers:

Glykeria Mermigka
Christos Christakis

PhD students:

Vassiliki
Michalopoulou
Kontantinos
Kotsaridis
Dimitra Tsakiri

Savvas
Paragkamian

(co-supervision with Dr. E.
Pafilis – HCMR).

Visiting students:

Giulio-Flavio Rizzo
Univ. of Catania, Italy
(left in 2019)

Summary

I am passionate about Molecular Host-Pathogens Interactions (MHPIs). In my group we use plants as host model-systems to study MHPIs.

We investigate the fundamental mechanisms to understand both microbial pathogenicity (in susceptible hosts) and the role of Innate Immunity (in resistant hosts).

Current Aims

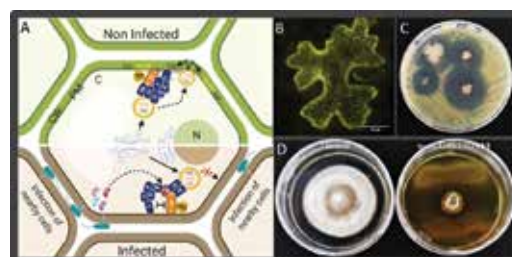
To understand animal and plant defense, the most important challenge is to characterize, at the molecular level, the interactions and signaling of the host immune-system, as well as, pathogens' virulence mechanisms.

Pathogens use common strategies to colonize their animal and plant hosts. Their pathogenicity largely depends on the delivering/translocation into host-cells of virulence proteins, known as "effectors". The exact role(s) and the subcellular targets of these virulence components remain an important open question in MHPI field, which has only partially been elucidated. Plant and animal carry intracellular NLR immune-receptors that detect pathogens' presence. NLR activation in both systems leads to a localized Programmed Cell Death (PCD). Recently we -and collaborators- discovered that certain plant NLRs include additional domains to their canonical structure. These integrated protein-domains (IDs) act as "decoys" for pathogens. We found the origin of these IDs through the duplication of the effectors' original virulence targets. We try to answer: **1)** what are the effectors' targets (in susceptible host-cell) and **2)** how their perception by NLR-IDs activates defence, is a crucial step in our understanding of host defence, as well as, the microbial virulence.

Furthermore, we investigate the endophytic microbiome of plants that live in extreme environments. We isolate new and known bacterial species that reveal interesting features regarding their ability to produce antibiotics and anti-fungal compounds (against pathogens of clinical and agricultural interest) etc.

Progress in 2019-2020

- We reviewed, upon invitation, how plants NLR-receptors act by forming inflammasome-like structures, known as "Resistosome" (Mermigka and Sarris, *TrendsImmunol.* 2019; Mermigka et. al., *TrendsPlantSci.* 2019).
- We identified and reported the potential mechanism by which the NLR-IDs are evolved in plants (Andersen, et. al., *Front. Genet.* 2020).
- Using a number of IDs from various NLRs, as baits, we identified novel pathogenicity host-targets of bacterial and viral pathogens (Michalopoulou, et. al., *BioRxiv*, 2020; Mermigka, et. al., 2020 in preparation).
- We characterized and reported the culturable microbiomes of halophytic plants, uncovering the role of these microbes in pathogens' growth inhibition and in salt tolerance. We generated a bacterial strains collection that consists of more than 600 isolates (Christakis, et. al., *BioRxiv*, 2020).
- We have identified a number of potential virulence components from the economically important quarantine-pathogen *Xylella fastidiosa* (Sertedakis, et. al., 2020, in preparation).



A & B: Schematic representation and confocal image of a microbial effector inhibiting host-cell exocyst complex. C & D: Anti-bacterial and anti-fungal components production by new bacterial species isolated and identified by our group.

Other activities

- Our research (Sarris et.al., *Cell*, 2015) was highlighted as one of the most important discoveries in Biology [*Science* (2016), *Signaling*, 9(409)].
- Project coordination: ~1mil euro to study the halophytic microbiomes and bioactive plant components for their potential use as bioinoculants in a sustainable food production.
- Trained a number of undergraduate and postgraduate students (Dept. of Biology, UoC).
- Member of the *Sectoral Scientific Council* (SSC) in "Agricultural Production, Agro-Biotechnology" of the Greek National Council for Research and Innovation (Apr 2018-2020; FEK 200/10.04).

Web page

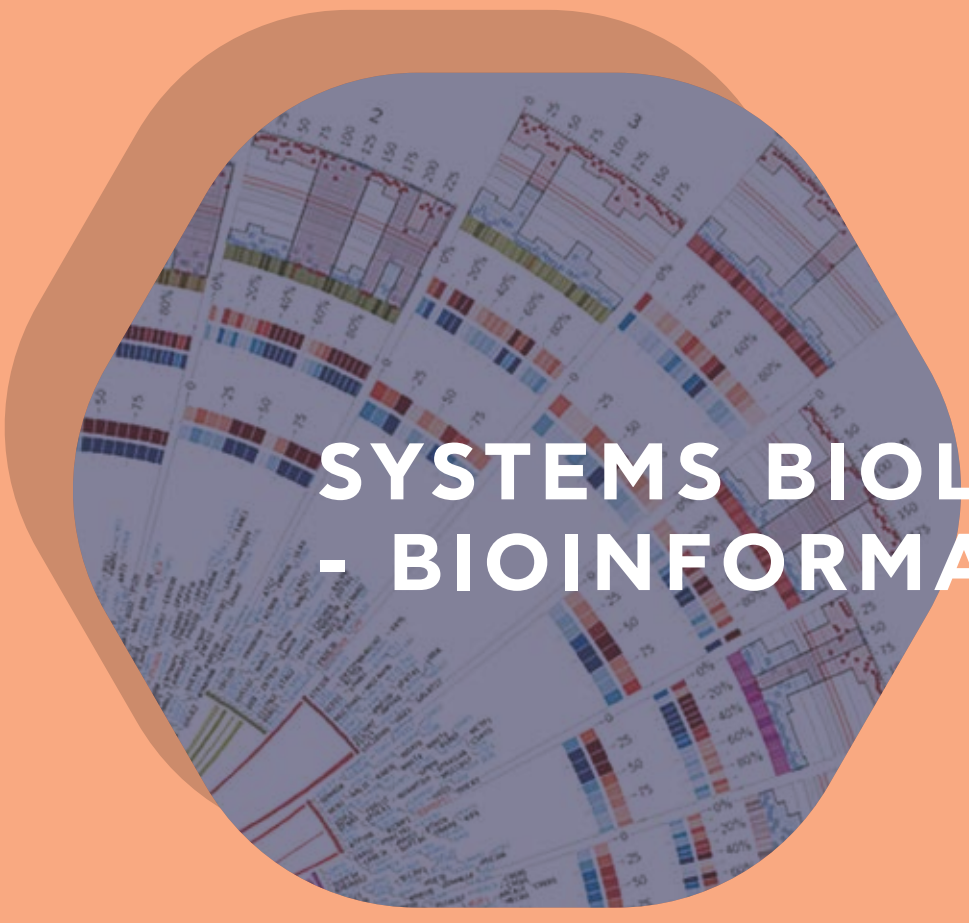
<https://www.imbb.forth.gr/imbb-people/el/sarris-home;>

Publications

Mermigka G and Sarris PF (2019) The Rice of plant Resistosomes. **Trends in Immunology** 40(8) pp:670-673

Mermigka G, et al. (2020) Plant and Animal Innate Immunity Complexes: Fighting Different Enemies with Similar Weapons. **Trends Plant Science** 25(1) pp: 80-91

Kioulis A, et al. (2020) Intraspecific diversification of the crop wild relative *Brassica cretica* Lam. using demographic model selection. **BMC Genomics** 14;21(1):48



SYSTEMS BIOLOGY - BIOINFORMATICS

COMPUTATIONAL GENOMICS GROUP



Christoforos Nikolaou

Assistant Professor -
Collaborating Faculty
Member*

*Currently Researcher (B) at
BSRC "Alexander Fleming",
Athens

GROUP MEMBERS

PhD Students:

Stylianios
Mavropoulos
Papoudas

MSc Students:

Lydia Kalaitzaki
Antonis Klonizakis
(jointly with Spilianakis lab)
Eleni Lianoudaki
Vasilis Ntasis
Sofia Papanikolaou
Iliia Varamogianni
Mamatsi

Summary

Our group's long standing interest has been the study of chromatin and genome structure in relationship to gene regulation. We particularly focus on the role of genome organization in the modulation of expression programs during cell differentiation and in the development of complex inflammatory diseases. Towards this end, we are employing computational analysis of multi-omics data, with particular focus on RNASeq, ChIPSeq, and Chromosome Conformation assays.

Current Aims

Recent developments at the interphase of molecular, cellular biology and biophysics are converging to an enhanced view of the eukaryotic nucleus. Its main characteristic is a functional compartmentalization, which is coupled with gene expression through the creation of structural entities such as topologically-associated domains, chromosomal compartments and macromolecular condensates. Our main working hypothesis is that underlying spatial constraints in the architecture of coding and regulatory elements will be reflected on all aspects of genome organization.

We test this hypothesis by employing positional constraint analyses in transcriptomic datasets, to reveal strong spatial preferences in the organization of transcription and gene co-expression, preferences that are, in addition, reflecting the dynamics of cellular processes during development and disease.

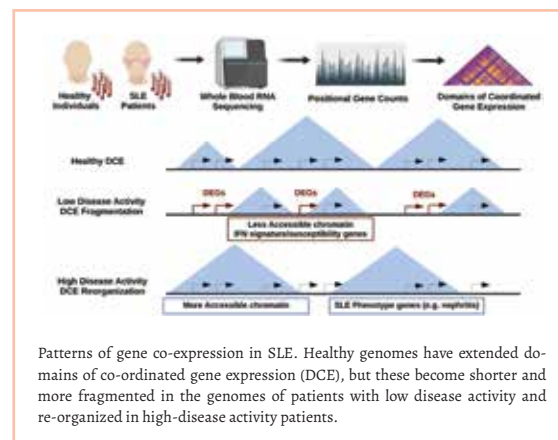
Progress in 2019-2020

We have been able to describe topological elements of eukaryotic genome architecture that underlie the dynamics of gene expression under an inflammatory stimulus. In a work by Stylianios Mavropoulos Papoudas (2020), we showed that prolonged activation of mouse fibroblasts by TNF leads to extensive gene expression changes that are organized in particular chromosomal domains. Through the introduction of bipartite functional-positional genomic networks, we showed that the patterns of these domains undergo two major transi-

tions in time; an early one (at 1h), accompanied by a generalized activation of inflammatory processes and a late (post 24h) that is associated with a functional switch towards differentiation pathways.

In a similar line of work by Vasilis Ntasis (2020) we studied the spatial patterns of gene coexpression in a large cohort of Systemic Lupus Erythematosus (SLE) patients. In this, first ever, topological transcriptomic analysis in a complex disease, we found that gene coexpression domains are significantly reorganized in SLE with more fragmented patterns being observed in patients of even low disease activity. This work is being followed up by experimental work with the aim of identifying disrupted associations between disease-associated genes and their cognate enhancer elements, that would enable a mechanistic interpretation of disease progression.

In a number of collaborative works with other IMBB groups we were also able to contribute transcriptomic analyses in the study of cell differentiation and tumorigenesis in humans (Sachini et al, 2020, with the Papamatheakis lab) and Drosophila (Magadi et al, 2020 with the Delidakis lab). In a collaboration with the Spilianakis lab (Salataj et al, 2019) we showed how miRNA genes are also spatially constrained in a way that is conserved across species.



Other activities

Teaching

Introduction to Data Analysis, Biology Department, Undergraduate
Computational Biology, Biology Department, Undergraduate
Introduction to Bioinformatics, Graduate Program in "Molecular Biology and Biomedicine", Biology Department-IM-BB-Medical School
Algorithms for Bioinformatics, Graduate Program in "Bioinformatics", Medical School-IMBB

Conferences

Co-organization of the 14th Annual Meeting of the Hellenic Society for Computational Biology and Bioinformatics (Patras, Greece 2019)

Web page

<https://www.biology.uoc.gr/el/personnel/1024>

Publications

Papoudas S, et al. (2020) Monitoring the prolonged TNF stimulation in space and time with topological-functional networks. **Computational and structural biotechnology journal** 18, 220-229

Ntasis V, et al. (2020) Extensive fragmentation and re-organization of transcription in Systemic Lupus Erythematosus. **Scientific Reports** 10 (1), 16648

Magadi S, et al. (2020) Dissecting Hes-centred transcriptional networks in neural stem cell maintenance and tumorigenesis in Drosophila. **Development** 147, 22

BIOINFORMATICS DATA ANALYSIS



Pantelis Topalis

Senior Staff Scientist (A)

GROUP MEMBERS

Special Research assistant:

George
Papagiannakis

MSc students:

Alexia Akalestou
(left June 2019)

Ippokratis
Messaritakis, MD

Summary

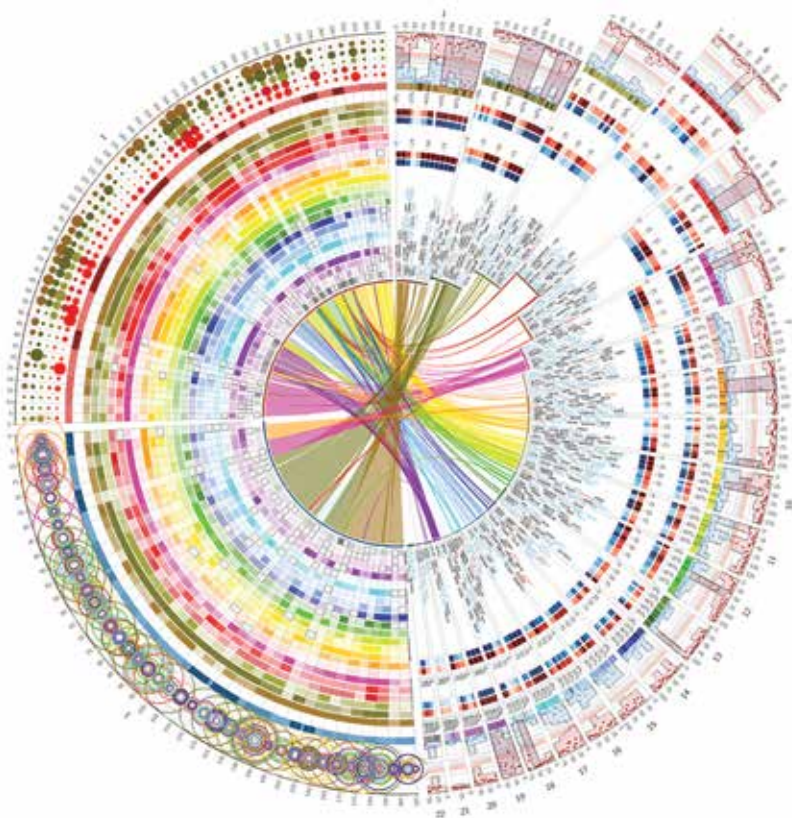
In an era where production of large datasets is getting more affordable the need to analyse them using computers is becoming more critical. New technologies allowing single cells to be studied provide new challenges to data analysis science. The Bioinformatics group collaborates with IMBB research groups in Heraklion and Ioannina to facilitate the various analyses of biological data (genomic, transcriptomic or combinatorial). We provide guidance in data analysis and we have installed and maintained Chipster platform that allows biologists without coding experience to run their own workflows. Training on Chipster is available for IMBB members upon request.

Capabilities

- Chipster platform and custom scripts are available upon request depending the nature of data to be analysed.
- Experience in the processing, management and analysis of large biological data sets.

Progress in 2019-2020

In the last 2 years Bioinformatics group expanded its collaboration analysing datasets in the frame of InfaVEC-2 program with several groups in Europe (Italy, Switzerland) analysing transcriptomic dataset of several mosquitoes. Also we expanded our toolsets to support technologies such as DamID-Seq, DSBs (double stranded breaks) data, microRNAs.



Other activities

- Analysis of datasets related to DNA damage: A) Datasets showing that macrophages mediate metabolic reprogramming upon DNA damage and B) Datasets showing Ercc1-XPF interacts with Topoisomerase II.
- We have participated in the analysis of the genome of *Nezara viridula*.
- P. Topalis supervised the creation of PEMA a tool for environmental DNA metabarcoding analysis.
- Analysis of datasets related to the role of Haspin in gametogenesis in collaboration with IMBB-BR

Web page

<https://www.imbb.forth.gr/imbb-people/el/bioinformatics-support-group-members/item/2437-pantelis-topalis>

Publications

Goulielmaki E, et al. (2020) Tissue-infiltrating macrophages mediate an exosome-based metabolic reprogramming upon DNA damage. **Nat Commun.** 11(1):42. PMID: 31896748

Denecke S, et al. (2020) A transcriptomic and proteomic atlas of expression in the *Nezara viridula* (Heteroptera: Pentatomidae) midgut suggests the compartmentalization of xenobiotic metabolism and nutrient digestion. **BMC Genomics.** 21(1):129

Zafeiropoulos H, et al. (2020) PEMA: a flexible Pipeline for Environmental DNA Metabarcoding Analysis of the 16S/18S ribosomal RNA, ITS, and COI marker genes. **Gigascience** 9(3): giaa022 PMID: 32161947



FACILITIES

HERAKLION

ANCIENT DNA AND POPULATION GENOMICS



HEAD OF FACILITY

Dimitris Kafetzopoulos
Despoina Vassou
Ioannis Talianidis

GROUP MEMBERS

Post-Doctoral Researcher:
 Nikolaos Psonis

Research assistant:
 Eugenia Tabakaki

Summary

The Ancient DNA Lab was established in 2016 as a FORTH core facility, utilizing more than 30 years of IMBB experience in genomics and the power of novel technologies in DNA analysis. The lab was built with high standards, under the auspices of the Prefecture of Crete that immediately identified the perspectives and the importance of the lab on supporting the archaeological research and promoting the cultural heritage of the country. It was established by the late Dr. Dimitris Kafetzopoulos, founder and leader of the lab until the beginning of 2020.

Focused on analyzing DNA from archaeological biological residues in order to answer questions of the modern archaeological research, the scientific goals of the aDNA lab mainly include ancient population genetics, genetic identification and phenotype determination of individuals of the past, tracing of ancient pathogens, investigation of diet habits of the past, reconstruction of the evolutionary history of animal species, and species molecular identification.

The scientific activities of the lab are funded by competitive National and European scientific programs. The lab is considered a national infrastructure in the context of the Hellas-CH (Cultural Heritage) program of the National Roadmap for Research Infrastructures.

The lab provides support for researchers working with ancient, historical, and other sensitive samples. Researchers across Greece, the Balkans and east Mediterranean are encouraged through workshops, network exchanges, and collaborations to make use of the facility. The aDNA Lab team constantly places new aims to innovative interdisciplinary projects. Furthermore, the lab trains students and early stage scientists in aDNA methodologies.

Current Aims

The current aims of the aDNA lab include:

- i) The exploitation of the (bio)archaeological wealth of Greece and the expansion of its collaborations.
- ii) The continuous update, development and establishment of question-driven, research-specific experimental and computational methodologies, following the latest scientific advancements in the field.
- iii) The study of the 2nd Greek Colonization in Greece, focusing on the genetic interaction between the migrating populations of the Metropolis with the local communities.

- iv) The investigation of the evolutionary history of extinct large mammals such as the Cypriot dwarf hippo.
- v) The exploration of the genetic relationships among medieval and post medieval individuals from the island of Crete and other Mainland individuals from the same period.
- vi) The genetic study of individuals from diverse prehistoric archaeological Greek sites who appear culturally differentiated (e.g. Neolithic – Bronze Age populations).
- vii) The analysis of ancient microbiomes and pathogens in order to identify causes of ancient epidemics, trace extinct microbial lineages and explore the evolutionary history of microorganisms relevant to public health.
- viii) The molecular species identification of organisms found in sediments (e.g. within amphorae) or from isolated parts such as biological human-made artifacts (e.g. elephant ivory).

Progress in 2019-2020

- Upgrade of the ancient DNA lab facility and establishment of optimized experimental and computational protocols for ancient DNA analysis in order to support new applications. Financial support by the actions HELLAS-CH and POLITEIA-II (NSRF 2014-2020), the European Research Infrastructure for Heritage Science Preparatory Phase (E-RIHS-PP, H2020-INFRADEV-02-2016) and the Advanced Research Infrastructure for Archaeological Data Networking in Europe - plus' — (ARIADNEplus, H2020-INFRAIA-2018-2020).
- Implementation of a research project in collaboration with the University of Lisbon and the Natural History Museum of Crete, regarding molecular identification of an elephant finding from southwestern Portugal.
- Achieved funding for the competitive project "APOIKIA, Ancient DNA analysis in novel multidisciplinary approach of ancient Corinthian colonization. Ancient Amvrakia and Ancient Tenea as demonstration examples" (EPAnEK, NSRF 2014-2020).



The study of the 2nd Greek Colonization events in the geographic area of Greece. Focus on the Corinthian Colonization of Ancient Amvrakia.

Other activities

Organization of the Webinar "Archaeogenetics in the context of the Research Infrastructure HELLAS-CH.gr" 24 September 2020, with two presentations.

Presentation in the Workshop OPTO-CH 2019 meets POLITEIA II. Foundation for Research and Technology, Heraklion, Crete, Greece, 6 June 2019.

Poster presentation in Conference "Reconstructing the Human Past - Using Ancient and Modern Genomics. EMBL Heidelberg, Germany, 31 Mar - 3 Apr 2019

Web page

<https://ancient-dna.gr/index.php/el/>

Publications

Psonis N, et al. (2020) Molecular identification and geographic origin of a post-Medieval elephant finding from southwestern Portugal using high-throughput sequencing. *Sci Rep.* 10(1):19252. doi: 10.1038/s41598-020-75323-y PMID: 33159124

ANIMAL (MOUSE) & GENOME EDITING CORE FACILITY



HEAD OF FACILITY

Theodoros Kosteas

Senior Staff Scientist (A)

FACILITY MEMBERS

Veterinarians:

Costas Kourouniotis

Eleni Moltsanidou

Debbie Tsoukatou

(part time)

Animal Technologist:

Hara Roumpaki

Animal Technicians:

Eleni Ntagiasi

Androniki

Vardoulaki

Stella Chalkiadaki

Sonia Kantali

Konstantina Sarri

Support Technician:

Ektoras Xenakis

Summary

The use of mice for scientific and research purposes is a practice that has substantially contributed to the promotion of biomedical science. The Animal (mouse) & Genome Editing Core Facility at the IMBB – FORTH have significant experience in animal welfare, the production of transgenic/genetically modified mice as well as in housing, supplying and breeding them for basic and translational research. Our unit provides high-standard services towards 25 biomedical research groups, executed by a team of 11 highly skilled and experienced personnel in a modern up-to-date facility. Our infrastructure consists an independent mouse facility of 1,500m², the largest in Greece, consisting of 4 units (SPF, experimental, behavioral & quarantine) and one of the two available transgenic/genome editing facilities nationwide. Our facility operates in accordance with the National (Presidential Decree 56/30.04.2013), the European Directive (2010/63/EU) and the Guidelines issued by the Federation of European Laboratory Animal Science Associations (FELASA). It operates under licenses issued from the Veterinary Service Office of the Prefecture of Crete: EL91-BIObr-01 and EL91-BIOexp-02, for the establishment, breeding and providing mice for scientific purposes.

Current Aims

Our facility is a partner in the EU funded INFRAFRONTIER GR/Phenotypos program and was recently accepted as an associate member in the EU-LIFE network of Core Facilities as a result, we now have the available infrastructure, experience we will soon be able to implement the latest CRISPR/Cas9 methodologies for the rapid generation of conditional

knock-out mouse models, cryopreservation and archiving of, our mouse lines. These skills and qualities are central towards achieving the best performance and competitiveness for our institute and our collaborators.

These methodologies will have an important impact on our facility and will allow us to further:

1. Accelerate our services in a cost-effective time frame required for the generation of new mouse models.
2. Help reduce the number of mice being utilized in the procedure.
3. Provide available funding which will be directed towards our facility/institute rather than to commercial suppliers.
4. Enhance, improve and promote the competitiveness and collaboration of the services offered from our facility to the IMBB and the University of Crete.
5. Cryo-preserve and deposit custom-engineered conditional knockout animal models to the European Mouse Mutant Archive (EMMA).

Progress in 2019-2020

Through available funding provided by the INFRAFRONTIER and the IMBB, our main task was to upgrade, introduce and provide new services towards our research groups by: i) recruiting new personnel ii) relocating/upgrading our SPF facility iii) installing an air shower and cage/bottle washer iv) expanding our neurobehavioral lab and v) upgrading our transgenic/genome editing facility through the purchase of new instruments such as: a) an Eppendorf micro-manipulator unit b) a NEPA21 electroporator and c) a Zeiss inverted microscope.



i) Upgraded Genome Editing Lab ii) Purchased new cage/bottle washer iii) Installed new air shower unit

Other activities

- Members from our facility participated in various meetings, courses and received training fellowships:
- RSPCA Meeting on Severe Suffering. Athens, Greece.
- 12th FORTH Scientific Retreat. Patras, Greece.
- 2nd Meeting of the Greek Laboratory Animal Welfare Committee. Thessaloniki, Greece.
- EMBO Practical Course: Humanized Mice in Biomedicine. Heidelberg, Germany.
- Surgical Techniques & Cryopreservation demonstration, University of Florence, Italy.
- Our veterinarian, Eleni Moltsanidou was awarded a CARE training grant from the Federation of European Neuroscience Societies (FENS).
- Our Scientific Director, Theodoros Kosteas was awarded an EMBO Core Facility Fellowship.

Links

<https://www.imbb.forth.gr/imbb-people/en/animal-house>

<https://www.infrafrontier.gr/phenotypos>

<http://www.felasa.eu/working-groups/guidelines/felasa-guidelines-and-recommendations/>

<https://eu-life.eu/research-excellence/working-groups-task-forces/core-facilities>

<https://www.imbb.forth.gr/imbb-people/en/animal-house-publications>

CELL CULTURE ROOMS



HEAD OF FACILITY

George Vrentzos
Androniki
Kretsovali

Summary

Since 1985, IMBB maintains a self-contained Cell Culture Facility that houses all equipment needed for the growth, maintenance, and analysis of animal cells. The facility provides basic support to investigators: preparation, filtration and testing of numerous cell culture media and is a source of culture supplies and sterile reagents. The facility provides technical support for **a)** growing a range of different cell lines and hybridomas including mammalian and insect cell lines as well as primary cells **b)** large scale cell cultures **c)** transfection of cells and expansion of different clones. The main facility is located on the 1st floor (Room A208) of the IMBB building and has three separate rooms equipped with HEPA filtered air flow and UV lamps. A second part of the facility is housed in the basement of the Institute and accommodates insect cell cultures, the parasite transgenesis laboratory and tissue engineering activities. The cell culture facility complies with all the EU regulations regarding biohazard material handling and disposal.

Equipment

The cell culture facility contains:

- Ten laminar flow hoods, biosafety level II for the sterile handling of cells,
- Ten CO₂, 37°C incubators for growing mammalian cells
- Five 25°C incubators for insect cell lines
- Four refrigerated centrifuges

Microscopes:

1. OLYMPUS IX-70 Inverted fluorescence microscope + HAM-AMATSU CCD Camera
2. Leica DM-IL Inverted fluorescence microscope + Leica DFC-310FX Camera
3. ZEISS PrimoVent Inverted microscope + ZEISS Axio Cam Erc5s
4. Euromex FE 2955 Inverted microscope
5. Nikon ECLIPSE TE 2000-U Inverted fluorescence microscope
6. OLYMPUS IMT-2 Inverted microscope

Electroporators:

1. Lonza amaxa biosystems Nucleofector II
2. BTX Harvard Apparatus Electro Square Porator + SAFETY STAND 630 B
3. BIO-RAD Gene Pulser
- A flow cytometer

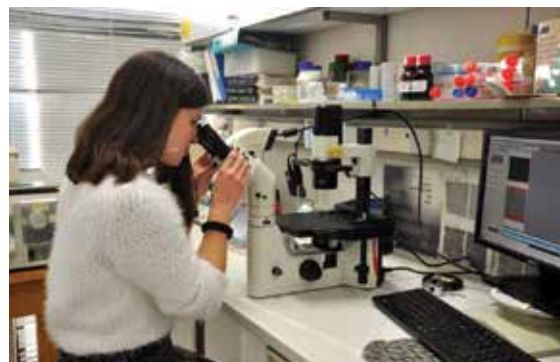
Services

Specialized training and advice for:

Media preparation, filtration and testing
Cell culturing and preservation
Cell Banking in liquid nitrogen
Mycoplasma detection
Cell cloning
Hybridoma production
DNA transfection
Toxicity testing
Mass cell culture using microcarriers, suspension, roller or hollow fiber procedures

Progress and impact in 2019 and 2020

Facility staff has provided support for basic aspects of animal cell culture and specialized training for more than twenty groups from the IMBB, the University of Crete and the broader Greek Biomedical community.



CONFOCAL IMAGING FACILITY



HEAD OF FACILITY

Christos Delidakis

FACILITY MEMBERS

Postdoctoral scientist:

Margarita Stapountzi

Lab Technician:

Alexandros Babaratsas

Summary

Analysis of complex biological systems relies greatly on high quality optical imaging of both fixed and live specimens. Fluorescence confocal microscopy is the platform of choice for such observation and is widely used by most IMBB groups for a great variety of assays.

Capabilities

Our main workstation is an inverted Leica SP8 scanning confocal microscope. A second Biorad μ Radiance 2000 fitted to an upright Zeiss microscope is used if the main microscope is oversubscribed. The SP8 is equipped with two scanners, a regular one and a fast resonant scanner, and allows for a selection among eight different laser wavelengths (405-633 nm) for excitation of fluorophores throughout the light spectrum. The microscope is connected to an environmental chamber with controllable temperature and CO₂ for live cell/tissue imaging. Additional modules, such as FRAP and Live Data Mode, can be used for complex experiments and subsequent analysis. Individual users are trained by the main facility scientist, Margarita Stapountzi. They are allowed to use the platforms only after completing at least 15 hours of theoretical and hands-on training. At the end of 2020 we had 88 accredited users from 24 research groups. If slots are available, we train and assist scientists from nearby institutions (Departments of Biology and Medicine of the University of Crete) for their imaging needs. A large variety of fixed and live samples are imaged in our facility, including cells (cell culture, animal/patient samples, cells on polymer scaffolds), dissected tissues (fruit flies, insects, crustaceans, mice, plants) or whole organisms (fruit fly embryos, nematode worms).

Progress in 2019-2020

We made all necessary repairs to ensure the system is in top working order, e.g. installed a new xy-stage, installed a new Argon-laser, repaired the scanner module.



Other activities

Examples of recent activities supported by the facility are:

- Imaging of neuronal and mesenchymal cells on 2D and 3D scaffolds for tissue engineering applications
- Imaging of organs of the olive fly associated with symbiotic bacteria with the aim to discover new targets for pest control
- Studying SATB1 phase transitions in vivo, utilising the light-inducible CRY2-mCherry optoDroplet system
- Live cell imaging of exosome uptake by recipient cells upon DNA damage
- imaging of Drosophila mosaic CNSs that contain stem cell tumours

Publications

The confocal Facility is acknowledged in a large number of the Institute's publications.

ELECTROPHYSIOLOGY UNIT



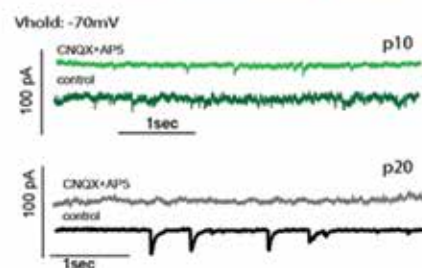
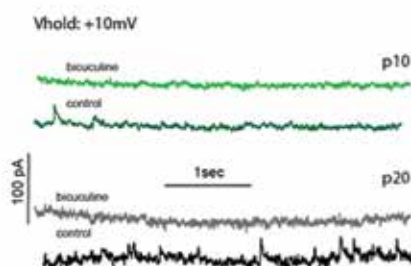
HEAD OF THE UNIT

Kyriaki
Sidiropoulou

Description

The electrophysiology unit contains all the necessary equipment for performing both field and patch-clamp recordings from brain slices and/or cell cultures. The services provided include the experimental design, the actualization of the experiments and data analysis on the following:

- short-term and long-term plasticity experiments from brain slices of various cortical areas, the hippocampus or amygdala
- current-clamp recordings to study neuronal excitability, spontaneous or evoked synaptic responses from brain slices with pharmacological applications if necessary.
- recordings can also be performed from YFP or GFP- labeled cells, either from brain slices or neuronal cultures
- voltage-clamp recordings to study spontaneous excitatory and/or inhibitory activity from brain slice or cell cultures



Web page

<https://www.imbb.forth.gr/imbb-people/en/electrophysiology-unit-home>

Publications

Kalemaki K, et al. (2020) Developmental changes of early postnatal inhibitory circuits in the mouse medial prefrontal and primary somatosensory cortex. **BiorXiv** doi: 10.1101/ 2020.01.21. 913913

Kourgiantaki A, et al. (2020) Neural Stem Cell Delivery via Porous Collagen Scaffolds Promotes Neuronal Differentiation and Locomotion Recovery in Spinal Cord Injury. **Nature Regenerative Medicine** 15; 5:1

Chalkiadaki K, et al. (2019) Development of the MAM model of schizophrenia in mice: Sex similarities and differences in hippocampal and prefrontal cortical function, **Neuropharmacology** 144:193-207 doi: 10.1016/j.neuropharm.2018.10.026

INSECT FACILITY



HEAD OF FACILITY

Christos Delidakis

FACILITY MEMBERS

Research assistants:

Ioannis Livadaras
(Head Technician, microinjection specialist)

Alexandros Babaratsas
(*Drosophila* stock maintenance)
Lefteris Spanos
(mosquito rearing)

Fly kitchen personnel:

Melina Karyotaki
Athina Livadara
Georgia Nistikaki

Summary

The Insect facility provides the infrastructure and the technical support for rearing and experimentation on different insects used in programs of basic and applied research. It is a housing facility for a number of insects: fruit flies, medfly, olive fly, cotton bollworm, and other agricultural pests as well as insects of medical importance (mosquitoes). Furthermore, the facility equipment supports genetic experiments performed by the members of several groups as well as the production of transgenic lines for the majority of the above insects. Five research groups are associated with the facility and use the premises in an everyday basis for fly/insect work. Occasionally researchers from other parts of Greece or abroad are hosted and their research is supported by the facility through collaborative research programs.

Capabilities

- The main Insect rearing space of the facility is made up of three walk-in incubators with controlled humidity, temperature and light-dark cycle which surround the central Fly-room space. An additional walk-in incubator is used for olive fly rearing and a two-room isolated space is dedicated to mosquitoes' rearing and handling.
- The central Fly-room space is a large lab with 5 work stations equipped with stereo-microscopes, light sources and anesthetization stages connected with a centralized CO₂-delivery system. Two dark chambers for fluorescence stereo-microscopy complete the setup.
- Highly experienced technicians are responsible for the maintenance of insect stocks and provide technical assistance to researchers and training of the newcomers.
- The facility also includes a separate lab space dedicated to insect embryo microinjections which is equipped with a state-of-the-art system for embryo injections. Ioannis Livadaras, our microinjection technician, is very experienced not only in routine *Drosophila melanogaster* transgenesis but also in producing transgenic lines for other insects (i.e. medfly, olive fly, flour beetles, cotton bollworm). The microinjection facility is also used for other purposes, such as transplanting tumours and inoculating pathogens into insect hosts.

Progress in 2019-2020

A new LED fluorescent light source was fitted onto one of our fluorescence stereo-microscopes enabling easier observation of whole insects.

Projects that have made extensive use of the facility:

- A pipeline for generating neural stem cell tumours in *Drosophila* larvae and transplanting / propagating them in adult hosts has been developed.
- Several CRISPR projects have been carried out in *Drosophila* and non-model insect pests, such as in olive fruit fly, *Tetranychus urticae* and *Helicoverpa armifera*, aiming to characterize insecticide resistance mechanisms and/or validate the discovery of novel insecticide targets for pest control.
- Development of biotechnology –based innovative approaches for olive fruit fly control, via targeting its symbionts (dysbiosis)
- *Drosophila* models of infectious diseases have been established in order to genetically dissect evolutionarily conserved pathways in hemocytes immunity (macrophage-like cells).



Photos of A) central Fly-room space B) dark chamber for fluorescence stereo-microscopy

Other activities

- The facility was represented in the Researchers Night and Science Festival annual events held at FORTH in 2019 and 2020. We presented aspects of the biology of the insects reared in the facility to students of primary and secondary schools as well as to the broader public who visited our stand, highlighting the impact of the related research programs on medical and agricultural issues.
- The facility hosted visits and tours from high school students as part of school programs to promote careers in science.

Web page

www.imbb.forth.gr/imbb-people/en/flyroom-insectarium-home

Publications

Siden-Kiamos I, et al. (2020) A method for purification of *Plasmodium* oocysts from mosquito midguts. **Sci Rep.** 10(1):7262 doi: 10.1038/s41598-020-64121-1
Koidou V, et al. (2020) Efficient genome editing in the olive fruit fly, *Bactrocera oleae*. **Insect Mol Biol.** 29(4):363-372 doi: 10.1111/imb.12640 PMID: 32141659
Piwko P, et al. (2019) The role of insulators in transgene transvection in *Drosophila*. **Genetics** 212: 489-508 doi: 10.1534/genetics.119.302165

FACS - SORTING FACILITY



HEAD OF FACILITY

Zaharenia Vlata

Takis
Makatounakis

Summary

A special unit for analysis and sorting of cells with Flow Cytometry has been installed and operates at the IMBB facilities. Flow cytometry is a technique that detects and measures different cell populations based on their optical properties (fluorescence). The above technology uses special instruments called flow cytometers, such as FACS (Fluorescence Activated Cell Sorting) and FACS - SORTER.

The **FACS** function allows the measurement of individual biological particles such as cells, nuclei, chromosomes, etc. An extension of this technology is the sorting of flow cells using the **FACS - SORTER**, which, in addition to cellular analysis, further isolates cells, bacteria and other particles of a similar size, in living form. The various isolated cells can then be investigated using microscopy, biochemistry and functional experiments.

The application of this methodology is available to all research groups of FORTH and the University of Crete, providing high quality cell analysis and sorting, technical support and advice for experimental design and specific sorting protocols.

Equipment

The Flow Cytometry unit operates a cell sorter (Moflo Cell - SORTER) and a cell analyzer (FACSCalibur).

i) Moflo Cell – SORTER.

The unit is equipped with a DakoCytomation (now Beckman - Coulter) MoFlo High-Performance Cell Sorter and the special software program SUMMIT™ (<http://www.coulterflow.com/bcflow/instrumentsot.php>). MoFlo - Sorter utilizes three lasers: 1) **UV - 355nm**, 2) **Blue - 488nm** and 3) **Red Diode - 643nm**, which can measure up to 9 cellular markers at a rate approaching 70,000 cells per second. Several common fluorochromes (FITC, R-PE, PerCp-Cy5.5, PE-Cy7, APC, APC - Cy5.5) and fluorescent GFP protein have been successfully used. The unique laser configuration allows the simultaneous separation of 4 different populations with over 99% purity and high recovery rates."

ii) FACSCalibur.

FACSCalibur is a top-of-the-line cell analyzer with two lasers:

- 1) **488 nm Ion Laser Blue-Green Argon**, 2) **633 nm Red Helium Neon laser**.

FlowJo software is installed on a computer near to the FACSCalibur to analyze flow cytometry data.

Three fluorescence channels and two scatter channels are available from the 488 nm laser (FSC, SSC, FL1, FL2, FL3). One fluorescence channel is available from the 633 nm laser (FL4).

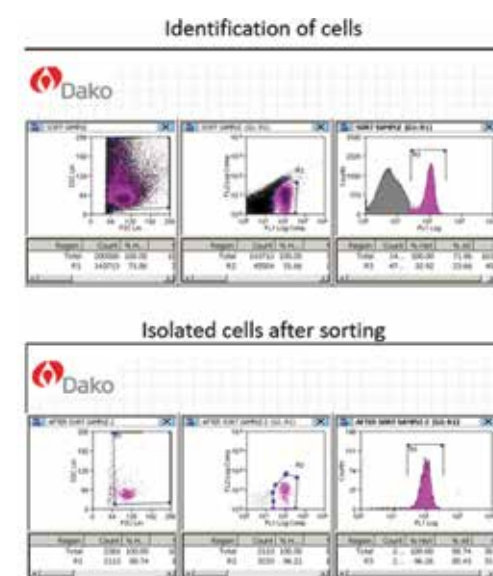
Progress in 2019 -2020

Moflo Cell - SORTER operated for 22 days a month and provided cell sorting services in 15 laboratories of FORTH and in 12 at the University of Crete.

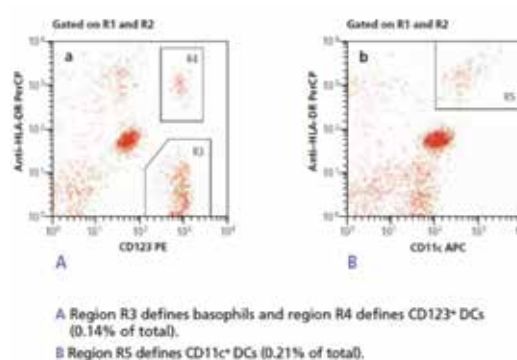
FACSCalibur continued to provide technical assistance to FORTH researchers by conducting flow cytometry-based analysis.



1. Moflo Cell – SORTER



2. FACSCalibur



GENOMICS FACILITY



HEAD OF FACILITY

**Vasiliki
Theodorou
Ioannis Talianidis**

FACILITY MEMBERS

Research assistants:

Eirini Stratidaki

Niki Gounalaki

Data management and
analysis:

Emmanouil Dialinas

Summary

For the past 20 years, Next Generation Sequencing (NGS) technologies have been revolutionizing genetic and genomic research, providing unprecedented depth and detail in chromatin functions in health and disease. Numerous NGS applications are now routinely used in biomedical research. The IMBB Genomics Facility offers experimental consulting, quality control, library preparation and sequencing services to IMBB researchers and external users.

We assist scientists to advance their research by generating high quality sequencing data. We offer a personalized experimental design, proposing the best suited experimental approach, tailored to the projects needs and available resources. We also offer basic bioinformatics analysis and data interpretation on an everyday basis.

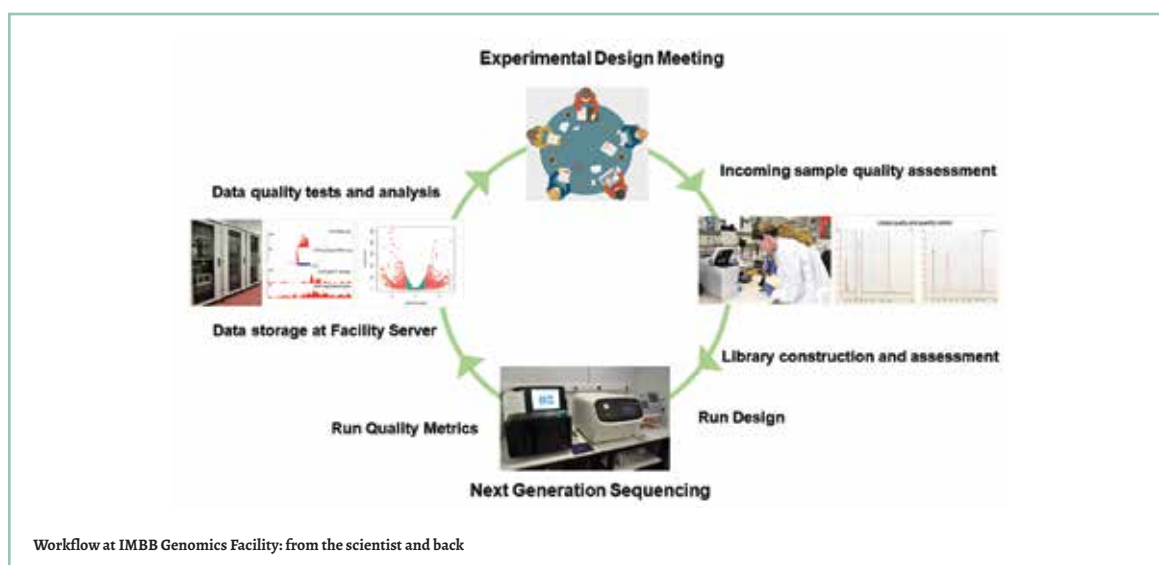
Capabilities

The facility uses the NextSeq 500 instrument routinely. Sequencing for specialized applications is performed on Ion Torrent S5 System. The facility has several years' experience in the following services:

1. RNA-sequencing
2. ChIP-sequencing
3. Whole Exome Sequencing

Progress in 2019-2020

- Established the 3'-quant-seq protocol, as a more cost- and data effective alternative to full RNAseq service.
- Established the 16S rRNA sequencing service for microbiota analysis.
- Set-up new RNA-seq protocols for degraded material.
- Established SARS-Cov2 virome sequencing service using amplicon sequencing approach.
- Refurbished the Genomics Server (32-core, 128GB RAM, 20TB storage), raw data archiving and storing until publication, implemented pipelines for sequencing and data quality assessments and delivering to the user standard bioinformatics analysis results in soft files as well as available tools for user driven meta-analysis for data interpretation. 2-3 weeks from sample to data delivery.
- Minimized sequencing costs for users, making NGS for affordable, cost-effective but at the same time providing high quality sequencing data.



Other activities

Participated in the annual Researchers Night and Science Festival, and in the ITE-FORTH retreat presenting the available technologies and highlighting the contribution of NGS applications in advancing scientific knowledge to the broader public that visit our stand.

Web page

<https://www.imbb.forth.gr/imbb-people/en/genomics-facility-home>

Publications

Magadi SS, et al. (2020) Dissecting Hes-centred transcriptional networks in neural stem cell maintenance and tumorigenesis in *Drosophila*. **Development** 147(22) doi: 10.1242/dev.191544. PMID: 33229432

Psonis N, et al. (2020) Molecular identification and geographic origin of a post-Medieval elephant finding from southwestern Portugal using high-throughput sequencing. **Sci Rep.** 10(1):19252 doi: 10.1038/s41598-020-75323-y

Chroni A, et al. (2020) Identification and characterization of a rare variant in apolipoprotein A-IV, p.(V336M), and evaluation of HDL functionality in a Greek cohort with extreme HDL cholesterol levels. **Arch Biochem Biophys.** 696:108655. doi: 10.1016/j.abb.2020.108655 Epub ahead of print, PMID: 33130088

HISTOLOGY UNIT



HEAD OF UNIT

Marina Koukaki

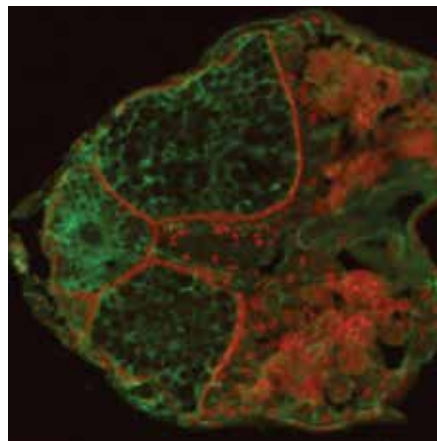
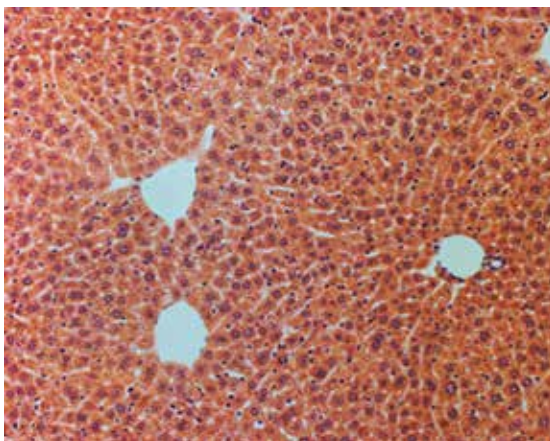
The Histology Lab is a Support Unit of IMBB equipped with *Automatic Tissue Processor (Leica TP1020; Paraffin Embedding Station (Leica EG1150H)-Cold Plate (Leica EG1150C); Rotary Microtome (Leica RM2125) and (RM2255) - Water bath for Paraffin Sections (Leica HI1210) - Flattening Table for Clinical Histopathology; Cryostat with UVC Disinfection (Leica CM1850UV); and Stereo Microscope (Leica M125).*

The Unit provides technical support and Training to perform histology and Immunocytochemistry experiments on frozen sections or paraffin embedded material.

Progress and impact in 2019 and 2020

The lab provided assistance for fifteen Research Groups of IMBB and the wider Biomedical Research Community of Herakleion.

Twenty five scientists were trained in histological analysis protocols.



HIGH CONTENT SCREENING MICROSCOPY UNIT (HCSM)



HEAD OF UNIT

Elena Deligianni

Summary

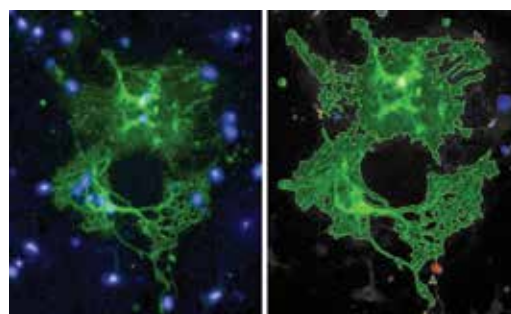
The HCSM provides biomaging services as required by research projects within IMBB/FORTH. The facility is equipped with an Operetta High Content Screening system (Perkin Elmer). A typical HCS assay is capable of generating automatically thousands of images, which is a major bottleneck for processing and analyzing microscopy data. Using assay-specific pipelines we can image samples, analyse data, assess outcomes and report findings. Imaging is available for 3D, fixed or live samples. Analysis is based on the cell morphology as it assessed by fluorescence markers. HCSM is the ideal tool for drug screening and discovery, as well as observing morphological changes during assays. Users are encouraged to participate throughout the process, namely in the design of the analysis pipeline and the analysis of the results. Typical applications where HCSM could be incorporated include Measurement of Neurite outgrowth, Cell migration, Cell growth, proliferation and differentiation, Cell death, Apoptosis, Autophagy, Assessment/Monitoring of the cell cycle and DNA damage, Cell stress, Cell differentiation, Cell metabolism, Ca^{+2} signaling and Cell tracking.

The HCSM module Operetta™ by Perkin Elmer is equipped with:

Automated epi-fluorescence imager with widefield and confocal scanning (spinning disk)
Transmitted light and digital-phase contrast imaging.
Independent excitation and emission filter wheels.
Environmental heating chamber for live imaging and adjustable CO₂ supply
Kinetics capabilities for carrying out time course experiments
2X, 10X 20X and 40X lenses

List of recent projects (2019 onwards)

- Measurements of cell proliferation over a period of 120h, in order to assess drug effect on cancer cell lines compared to untreated samples.
- Calculation of the percentage of differentiation in progenitor cells and assessment of the gene expression in those differentiated cells
- Monitoring of single-cell (LIF-cranial suture mesenchymal progenitor cells) proliferation during osteogenic differentiation and comparison between genotypes
- Live Imaging on tumor cells injected on *Drosophila melanogaster*, in order to measure the rate and the symmetry of cell division
- Growth Monitoring of neuron cells on 3D collagen matrix for potential drug screening
- Drug screening for Neuroprotective agents on Neuron Stem Cells carrying the Amyotrophic Lateral Sclerosis SOD1 gene mutation
- Drug Screening of nanoparticles-conjugated drugs on *in vitro Plasmodium berghei* cultures
- Screening of the essential's oil effect on *in vitro Plasmodium berghei* cultures
- Monitoring Neuroepithelial cell differentiation and interactions with specific protein markers.
- Monitoring mitochondrial apoptosis on lymphoma cell lines
- Monitoring the rate and the percentage of egress of fungal cells from macrophages on live cultures over a 24h- period in the presence of drugs
- Monitoring the effect of gene mutations on cell egress, from thymus glands, on live imaging
- Measuring microglia activation in the presence of the bacterial endotoxin lipopolysaccharide
- Measuring the expression of GFP-tagged genes in whole brain sections.
- Measuring the development of axons in Dorsal root ganglia plants
- Assessing potential insecticide inhibition of the efflux transporter P-glycoprotein in insect cell lines
- Assessing Cell Penetrating Peptides (CPP) entrance in insect cell lines and their use in pesticide delivery
- Viability tests of cell lines after gene deletions
- Quantification of BNN20 neuroprotective effect by comparing cell death of primary oligodendrocytes treated with LPC (lysophosphatidylcholine) and BNN20 for 24 hours against primary oligodendrocytes treated only with LPC.



left: An oligodendrocyte stained for myelin. right: Segmentation of the image to estimate oligodendrocyte area.

Other activities

Since 2019, the IMBB-HCSMU joined the EATRIS-GR research infrastructure, and in 2020 the Unit is included in the of the EU-LIFE research consortium under the IMBB/FORTH membership.

MINOTECH BIOTECHNOLOGY



HEAD OF FACILITY

Dimitris Koutsoulis

(up to 2019)



Ioannis Talianidis

FACILITY MEMBERS

Research assistants:

Dimitris Dialektakis

Xrysoula Vamvakia

Xara Giata

Summary

MINOTECH biotechnology (Mb) is the in-house biotechnology production facility of IMBB-FORTH and has over 30 years of experience specialized in production of bacterial-derived proteins. Mb produce a wide array of high purity and superior quality Restriction Endonucleases, DNA Modifying Enzymes and Molecular Weight Markers that aim to meet the needs of scientists engaged in Research, the Biotechnology Industry and the Clinical Laboratory. Mb products are cited in more than 200 peer-reviewed publications and are supplied to customers directly or under OEM agreements through major European, Asian and North American distributors.

Mb is active in the production of Molecular Biology tools and is highlighted as one of the 16 international suppliers of Restriction Enzymes (REs) in the largest database on REs (REBASE, rebase.neb.com).

Capabilities

MB facility offer:

- Large scale (mg to gram) protein purification of tagged or untagged proteins. Mb team members are highly-experienced in downstream processing steps and can help optimize target protein purification scheme. A series of different types of chromatography can be tested (size exclusion, Ion exchange, affinity, hydrophobic interaction). FPLC equipments are capable of up to 100ml/min flow rates.
- Controlled production of biomass (mainly microbial) over a wide range of controlled conditions (culture media, temperature, pH, aeration, stirring, etc). The Fermentation facility houses all equipment needed for the maintenance, growth, collection and storage of microorganisms (bacteria, fungi, etc). Cell mass is produced according to strict quality criteria and is accompanied with Specifications and Technical data sheet that includes all relevant information on growth conditions.

Progress in 2019-2020

- Negotiations with Venture Capital Funds (Equifund) resulted to the establishment of a spin off company, EnzyQuest.
- Commercial agreement with EnzyQuest for the exploitation of products and scientific results.
- Training of young scientists: 2 BSc thesis and 1 MSc thesis.
- Identification and characterization of an extremophilic DNA modifying enzyme (collaboration with IMBB Genomics Facility and M. Kokinidis Laboratory)
- Participate in FET-OPEN grant entitled "Towards an instrument-free future for molecular diagnostics at the point-of-care (Acronym: Free@POC) coordinated by Prof Electra Gizeli.



Minotech biotechnology infrastructure includes equipment needed for expression and down-stream processing steps of bacterially derived protein

Other activities

Participated in the annual Researchers Night and Science Festival, and the ITE-FORTH retreat presenting the available technologies

Web page

<https://www.minotech.gr>

PROTEIN PURIFICATION FACILITY



HEAD OF FACILITY

**Charalampos
Pozidis**

Summary

The development of techniques and methods for protein purification have been an essential pre-requisite for many of the advancements made in biology. The key to successful and efficient protein purification is to select the most appropriate techniques, optimize their performance to suit the requirements and combine them in a logical way to maximize yield and minimize the number of steps required.

The facility purifies proteins from *E. coli*, insect, mammalian cells and sera using a variety of chromatographic methods and techniques while at the same time develops or evaluate new techniques and advanced protocols.

Furthermore, we also provide biophysical characterization of purified proteins such as, molecular mass determination or molecular weight, molar mass distributions of heterogeneous samples, accurate aggregation and oligomeric states of proteins, stoichiometry of tightly bound protein heterocomplexes, determination of mass-averaged root mean square radius etc.

Progress in 2019-2020

During these two years the facility continued the well-established collaboration with many of the institute research groups on a range of projects involving either protein purifications or biophysical characterization of purified proteins.



PROTEOMICS FACILITY (PROFI)



HEAD OF FACILITY*

Michalis Aivaliotis

*Currently Associate Professor
of Biochemistry at Aristotle
University of Thessaloniki

GROUP MEMBERS

Technician:

Nikos Kountourakis

Postdoctoral Researchers:

Konstantina Psatha

Georgia

Orfanoudaki

Summary

Proteomics facility at IMBB (ProFI) is equipped with state-of-the-art mass spectrometry-based proteomics and bioinformatics tools, in combination to metabolomics, applying various approaches and developing new methodologies for the identification and characterization particularly of proteins and of metabolites. ProFI's highly trained and experienced staff provide excellent research and work as service facility to researchers within IMBB, but also to external academic and industrial laboratories.

Capabilities

- A high-resolution tandem mass spectrometer (LTQ-Orbitrap XL with ETD) coupled to an Easy nLC (nano Liquid Chromatography; Thermo Scientific) is situated within the facility.
- Application of established and development of new bottom-up and top-down shotgun proteomic and metabolomics analyses, including:
 - Biomolecule mass determination by mass spectrometry
 - Protein identification and quantitation for many biological systems and questions,
 - Study of protein post-translational modifications and interactions
 - Accurate mass determination by mass spectrometry flag
 - Metabolite identification and quantitation for many biological systems and questions.
- Help and advice on any aspect of the application of proteomics, metabolomics, and bioinformatics to the exploration of biological problems.

Progress in 2019-2020

During 2019-2020, ProFI has collaborated with many of the Institute's research groups and external labs, evidenced by 8 publications and many conference proceedings. For example, collaborators with our contribution i) have shown that DNA damage in tissue-infiltrating macrophages mediates an exosome-based metabolic reprogramming, ii) have developed computational methods for the identification of biologically meaningful associations between known and novel proteins and iii) have investigated the proteins involved in mosquito resistance to insecticides. Our collaborative work with Dr. Spyrou G. and his research group at CING has applied untargeted metabolomics on stage-specific Cypriot Huntington's disease (HD) patients, exploring putative neurodegenerative biomarkers for HD. ProFI has, also, participated in 4 National and International founded research projects and continued to build on method development.



ProFI is equipped with an LTQ-Orbitrap XL – ETD mass Spectrometer coupled to a nLC system with auto-sampler.

Other activities

- Conference organizing Committee (Co-chair): HB-12 2019, IMBB-FORTH
- Member of EU Project "EPIC-XS Consortium".
- Two Postdoc Scholarships, two international and one national conference awards were awarded in members of our lab.
- "Multiplex ELISA development for the clinical diagnosis and prognosis of patients with chronic kidney disease: method assessment and optimization utilizing mass spectroscopy", Funding Source: GSRT-Infrastructure.
- Participated in running 1 International workshop (HB-12 2019) and presented at COST CliniMARK Training School (2019).
- Trained 2 PhD students from Cyprus and 3 undergraduate students from UoC.

Publications

<https://www.imbb.forth.gr/imbb-people/en/profi-publications/refereed-papers>

Goulielmaki E, et al. (2020) Tissue-infiltrating macrophages mediate an exosome-based metabolic reprogramming upon DNA damage. **Nat Commun.** 11:42

Balabanidou V, et al. (2019) Mosquitoes cloak their legs to resist insecticides. **Proceedings of the Royal Society B** 286:1907 doi: 10.1098/rspb.2019.1091

Theodosiou T, et al. (2020) UniProt-Related Documents (UniReD): assisting wet lab biologists in their quest on finding novel counterparts in a protein network. **NAR Genom and Bioinform** 2(1):lqaa005



ROLE OF BINARY SWITCHES IN DEVELOPMENTAL TRANSITIONS



Spyros Georgatos

Professor - Collaborating
Faculty Member

GROUP MEMBERS

Post-doctoral researchers:

Anastasia Christogianni
Eleftheria Karanika
Fani Kiosse
Diana-Maria Potsi
Eleftheria Hatzadonaki
(former)

Summary

We investigate histone modifications occurring during developmental and cell cycle transitions. In the past, we have studied tandem methyl-phos marks, such as H3T3ph-K4me3-R8me2-K9me3-S10ph, in connection with the corresponding histone code readers (HP1, LBR) or writers (Haspin). Work with mouse embryonic stem cells and their partially differentiated derivatives (embryoid bodies) has led us to consider another model system, namely the male germline. The formation of male gametes from undifferentiated spermatogonia recapitulates early embryonic events. Similar to embryonic stem cells, the chromatin of spermatogenic precursors is studded with binary histone modifications and undergoes major state transitions as the cells mature. However, in this case, the differentiation process is repeated continuously throughout the adult life, which implies the existence of a robust program that is implemented with high precision in each "wave" of gamete maturation.

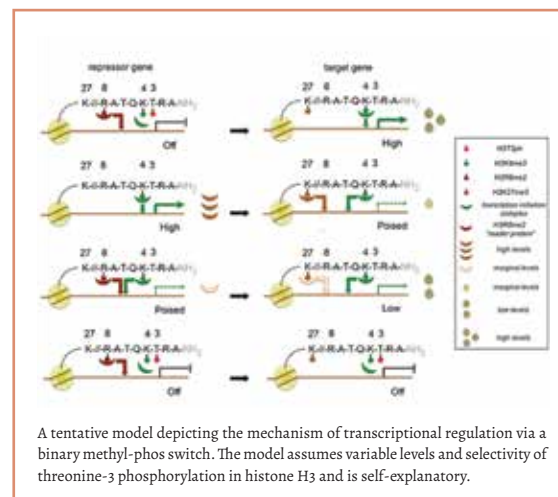
Current Aims

The formation of male gametes involves at least three synchronized transitions: from undifferentiated, diploid spermatogonia to meiotic spermatocytes; from spermatocytes to haploid spermatids; and from spermatids to mature spermatozoa. Each developmental stage is characterized by a unique gene expression program that unfolds in space and time. In the mouse, differentiating cells move in a spiraling fashion along and across the seminiferous tubule, from the periphery to the lumen. This process is repeated in cycles of 34.5 days. Our aim is to map the epigenetic landscape during this period and correlate changes in histone modifications with expression pattern. To this end, we are moving in two directions: (i) we employ peptide inhibition assays at the level of indirect immunofluorescence, to decipher when individual methyl-phos

marks are established during spermatogenesis and spermiogenesis; and (ii) we are trying to characterize histone code readers that recognize these modifications individually or in combination. When the conditions allow, we also plan to exploit haspin-knockout mice, to identify the exact mechanisms of repression or de-repression of spermatogenesis and spermiogenesis-specific genes (see Figure).

Progress in 2019-2020

The last two years we have completed our profiling project with embryonic stem cells, characterizing cell lines that lack or overexpress the kinase haspin and the nuclear envelope protein LBR. In parallel, we have examined in detail the occurrence of the H3T3ph-K4me3-R8me2 motif (PMM) in mouse testis. The results show that this composite motif is assembled and erased in distinct steps at the transition between early round and late round/elongating spermatids.



Other activities

We organized the purchase and installation of a new light sheet microscope in the Campus and consolidated all imaging facilities, from confocal to electron microscopy, under the same umbrella, through a Memorandum of Cooperation signed by IBE-ITE and the University of Ioannina.

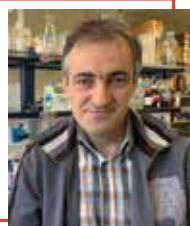
Web page

<http://www.imbb.forth.gr/georgatos>

Publications

Karanika E, et al. (2020): Haspin-dependent and independent effects of the kinase inhibitor 5-Iodotubercidin on self-renewal and differentiation. **Scientific Reports** 10: 232

INTERPLAY BETWEEN ENDOCYTOSIS, SIGNALING AND EXOCYTOSIS IN ENDOTHELIAL CELLS: MOLECULAR MECHANISMS AND ROLE IN BLOOD VESSEL PHYSIOLOGY



Savvas Christoforidis
Professor - Collaborating
Faculty Member

GROUP MEMBERS

Postdoctoral researchers:
Evangelia Goula
Despoina Gkeka

PhD students:
Alexandra Papafotika
Valia Karamani

Master's student:
Victoria Koloi

Summary

The inner wall of blood vessels is covered by endothelial cells, which play critical roles in the majority of fatal illnesses, including the cardiovascular diseases, inflammatory disorders and neo-angiogenesis in cancer. Key endothelial molecules that play important role in these pathophysiological processes are stored in specialized organelles, called Weibel Palade bodies. These organelles secrete their cargo molecules in the blood circulation upon activation of endothelial cells by extracellular ligands, e.g. by binding of VEGF to VEGFR2. Ligand/receptor complex formation results also in receptor endocytosis, which controls the output of receptor signalling. Our work aims to shed light into the spatio-temporal orchestration and the molecular interconnections between receptor endocytosis, signalling and exocytosis in endothelial cells and to understand their implications in vascular physiology and in serious vascular diseases.

Current Aims

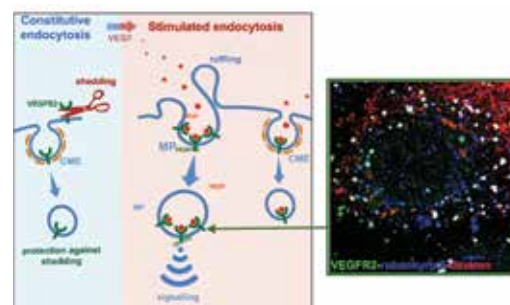
We previously found that constitutive endocytosis of VEGFR2 protects the receptor against shedding (Basagiannis D and Christoforidis S, *J Biol Chem*, 2016, 291, 16892-16903), while addition of VEGF reroutes VEGFR2 towards macropinocytosis, a critical event for VEGF-induced signalling *in vitro* and for angiogenesis *in vivo* (Basagiannis D et al., *J Cell Sci*, 2016, 129, 4091-4104; Basagiannis D. et al., *Sci. Rep.*, 2017, Mar 22;7:45035). Following these findings, we are currently aiming in elucidating the mechanism by which macropinocytosis controls VEGFR2 signalling and how the signals are conveyed to Weibel Palade bodies, for triggering exocytosis.

Besides, as VEGFR2 is critical in hESCs differentiation towards the vascular lineage, we aim to map the trafficking routes of VEGFR2 along cellular differentiation and address their impact in stem cell self-renewal and on their differentiation towards the vascular lineages. Ultimately, the findings of our studies will contribute to the design of more effective therapeutic approaches in vascular diseases.

Progress in 2019-2020

We found that, following activation of endothelial cells by VEGF, a number of signalling molecules are recruited on macropinosomes, where they colocalize with VEGFR2. Besides, we identified contact sites between endosomes and Weibel Palade bodies, serving as a mechanism for cargo exchange (Gkeka D, et al. manuscript in preparation). Ongoing studies aim to address whether these contacts deliver signalling messages that trigger Weibel Palade body exocytosis.

Given the importance of VEGFR2 in hESC differentiation towards the vascular lineage, we have mapped the trafficking routes of VEGFR2 throughout the differentiation steps of hESCs towards endothelial cells and studied their importance in differentiation. This study revealed that trafficking and signalling pathways of plasma membrane receptors are re-programmed as pluripotent cells differentiate towards specific cell types, and that macropinocytosis could play critical for differentiation towards the endothelial commitment (Zografou S, et al., to be submitted in Jan 2021).



Left scheme, in blue background: Constitutive endocytosis via the clathrin route protects VEGFR2 against shedding (CME: clathrin-mediated endocytosis). Left scheme, in pink background: VEGF switches internalization of VEGFR2 towards macropinosomes (MP), which are imaged on the right via confocal microscopy.

Other activities

- Poster award to PhD student K. Galanopoulou, 70th HSBMB Annual Conference, "The cytoplasmic Acetoacetyl CoA Thiolase (ACAT2), a novel Rab5 effector, regulates endocytic membrane transport" 29.11-01.12, 2019, Athens, Greece
- Participation in the EMBO Conference "The physics and chemistry of endocytosis at multiple scales" 1 – 6 September 2019, Ischia, Italy
- PCT patent application in 2020 on "Method of preparation and use of phosphoinositide 3-kinase inhibitors for cancerous diseases".

Web page

<https://www.imbb.forth.gr/imbb-people/index.php/en/christoforidis-laboratory>

Publications

(not applicable for 2019/2020)

NEUROBIOLOGY AND BIOCHEMISTRY



Christos G. Gkogkas

Principal Researcher (B)

GROUP MEMBERS

Postdoctoral Researchers:

Kleanthi
Chalkiadaki
Gilliard Lach

PhD student:

Elpida Statoulla

Summary

Proteins catalyse most of the reactions in the cell on which life depends. Translational control is defined as a change in protein production per mRNA per unit of time, and it is a powerful means to alter protein abundance. Our lab is particularly interested in understanding the molecular, cellular and signalling mechanisms of translational control in the brain and how they control complex brain functions and behaviours, such as learning, memory, social interactions, anxiety and fear and how they affect brain health (e.g. in neurodegeneration). We focus on elucidating the contribution of 2 key signalling pathways in brain translational control: the mammalian/mechanistic target of rapamycin (mTOR) and the mitogen activated protein kinase (MAPK) pathways and use that knowledge to design novel therapeutics for brain disorders such as Autism Spectrum Disorders (ASD).

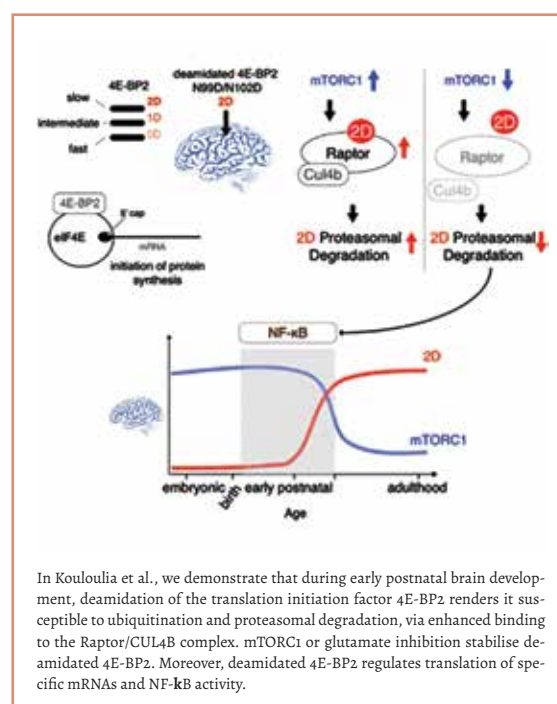
Current Aims

Our current work is aimed at:

1. Understanding the link between translational control and metabolic regulation in ASD.
2. Studying key signalling pathways upstream of translational control (mTOR, MAPK).
3. Elucidate the role of translational control in sensory neurons linked to tactile hypersensitivity in ASD.
4. Develop new techniques for studying translational control genome-wide in brain.

Progress in 2019-2020

We have discovered a new neuron-specific mechanism linking translational control to NF- κ B and metabolic regulation via deamidation of the translation factor 4E-BP2. This mechanism is crucial for early postnatal brain development and can go awry in neurodevelopmental disorders such as ASD (1), (2). We have revealed the contribution of translational control pathways, genome-wide, in rodent Pavlovian fear conditioning, revealing for the first time the contribution of the conditioned stimulus in gene-expression changes (2). Finally, we have developed a new technique for translational profiling in synaptic fractions (synaptosomes) (4).



In Kouloulia et al., we demonstrate that during early postnatal brain development, deamidation of the translation initiation factor 4E-BP2 renders it susceptible to ubiquitination and proteasomal degradation, via enhanced binding to the Raptor/CUL4B complex. mTORC1 or glutamate inhibition stabilise deamidated 4E-BP2. Moreover, deamidated 4E-BP2 regulates translation of specific mRNAs and NF- κ B activity.

Other activities

To expand our research in human models, we have established 2D (excitatory neurons) and 3D (forebrain organoids) models derived from induced pluripotent stem cells (2) in collaboration with the University of Edinburgh, UK.

We have guest edited a special issue on Translational Control for Cellular Signalling.

We have hosted 2 undergraduate student trainees from the Biology and Technology Department, Ioannina, Greece.

Web page

<https://www.gkogkaslab.com/>
<https://www.imbb.forth.gr/en/research-en/biomedical-research/item/5035-christos-g-gkogkas>

Publications

- Simbriger K, et al. (2020) Uncovering memory-related gene expression in contextual fear conditioning using ribosome profiling. **Prog Neurobiol.** 101903 doi:10.1016/j.pneurobio.2020.101903
- Simbriger K, et al. (2020) Monitoring translation in synaptic fractions using a ribosome profiling strategy. **J Neurosci Methods** 329, 108456 doi:10.1016/j.jneumeth.2019.108456
- Kouloulia S, et al. (2019) Raptor-Mediated Proteasomal Degradation of Deamidated 4E-BP2 Regulates Postnatal Neuronal Translation and NF- κ B Activity. **Cell Rep** 29, 3620–3635 e3627 doi:10.1016/j.celrep.2019.11.023

LABORATORY OF EPIGENETICS AND CHROMOSOME BIOLOGY



Frank Fackelmayer
Research Director (A)

GROUP MEMBERS

Senior scientist:

Carmen Eckerich

PhD student:

Evangelia Athanailidou

Diploma students:

Georgios Sigalas
(2020)

Anjeza Gjokai
(2019)

Summary

Research in our laboratory aims to shed light on the regulation of essential processes in the cell nucleus. The overarching question is how different layers of epigenetic regulation cross-communicate. Our projects range from low-level modification of chromatin proteins by the human family of Protein Arginine Methyltransferases (PRMTs), to investigating properties of nuclear scaffolding proteins and the global nuclear architecture as the highest level of epigenetic regulation. Presently, we particularly focus on the protein SAF-A (Scaffold Attachment Factor A), which interacts with a multitude of other nuclear factors and components, including PRMTs, long non-coding RNAs (lncRNA) and nuclear actin. We aim to understand how SAF-A sets up “functional neighborhoods” in the cell nucleus, and how these contribute to genome stability and chromosome territory integrity.

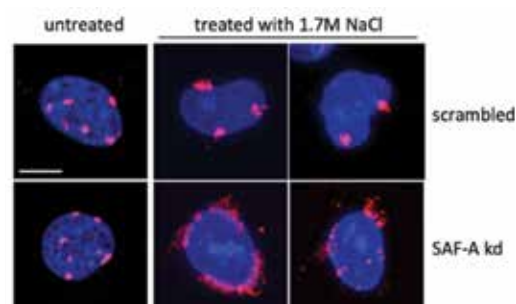
Current Aims

We investigate how Protein Arginine Methyltransferases are regulated to provide enzymatic activity - which is a key component in epigenetic control of gene expression - at the right time and at the right place. We and others have found that arginine methylation via PRMTs plays a role in a plethora of human diseases, such as cancer and neurological disorders, and now try to understand how the balance of splicing isoforms and regulation of local enzyme availability through liquid-liquid phase separation and recruitment to nuclear scaffolding structures contributes to medical conditions. Our current research tries to link epigenetic modification of chromatin proteins, through PRMTs and other enzymes, and expression of lncRNA with the abundant architectural protein SAF-A. This protein is, among other functions, an essential factor in the epigenetic silencing of the second X chromosome in female mammals, and is essential for the stability of chromosome territories in live cells. Understanding the complex interplay between different layers of epigenetic regulation holds promise in future application to more precisely control cell fate decisions.

Progress in 2019-2020

In order to address these questions, we establish and develop methods and experimental models that have great potential to reveal the interplay of molecular hierarchies involved in the functional architecture of the cell nucleus in health and disease.

For example, we have established BioID, a method of proximity labeling of proteins in close neighborhood (within less than 5nm) with a protein of interest, and have first results from MALDI mass spectrometry that identified around 800 proteins in close proximity of SAF-A. Gene ontology analysis revealed that the majority of these proteins are involved in RNA metabolism, epigenetic regulation of chromatin structure, and DNA replication. Also, we have established a novel method for the targeted degradation of RNA in the vicinity of a protein of interest, and have shown that applying this method to SAF-A leads to a collapse of global chromatin structure. Since March 2020, we pursue a new project funded by the Hellenic Foundation for Research and Technology (ELIIDEK), in which we establish a method for CRISPR-directed tagging of endogenous human genes with either fluorescent tags, or an auxin-inducible degron for rapid knockdown of a protein of interest. We have already successfully applied this state-of-the-art tagging technology to SAF-A, and have started to investigate the functional consequences of the depletion of nuclear SAF-A. In a complementary approach, we have fluorescently tagged the human gene for CDK9 to visualize active transcription foci in living cells, which we expect to be affected by altering the level of nuclear SAF-A.



Knockdown of SAF-A affects the stability of chromosome territories. Chromosome territories were labeled by replicative incorporation of Bromodeoxyuridine (BrdU) over night, and were allowed to segregate for 5 days afterwards. Then, chromosome territories were immediately visualized by anti-BrdU immunofluorescence (red), or cells were first extracted with high salt. Note that territories, although identical in appearance in untreated cells, are highly disrupted in cells in which SAF-A was knocked down by RNA interference (SAF-A kd), but resist extraction in control cells (scrambled). Bulk DNA was counterstained with To- Pro3 (blue).

Other activities

Frank Fackelmayer acts a National representative of Greece in past and ongoing EU COST Actions as follows:

since 5/2019	Greek National Representative (National contact point) and Member of the Management Committee of COST Action CA18127 “International Nucleome Consortium”; Coordinator for Short Term Scientific Missions (STSM) of the COST Action
since 3/2019	Greek National Representative (National contact point) and Member of the Management Committee of COST Action CA16120 “European Epitranscriptomics Network”
3/2015-3/2019	Substitute Greek National Representative and Member of the Management Committee of COST Action CM1406 “Epigenetic Chemical Biology”; Dissemination Coordinator of the COST Action

Web page <http://www.imbb.forth.gr/fackelmayer>

Publications (not applicable for 2019/2020)

NEUROBIOLOGY & BIOCHEMISTRY



Michaela Filiou

Assistant Professor-
Collaborating Faculty
Member

GROUP MEMBERS

Laboratory Teaching Staff:
Konstantinos
Konidaris

Postdoctoral Researchers:
Chrysoula Komini
Angeliki-Maria
Vlaikou

Research Technician:
Markus
Nussbaumer

Diploma thesis Students:
Marianthi Firoglani-
Moschi
Alexandros Tsiouris

Summary

Our lab studies the molecular mechanisms of neuropsychiatric disorders. Our goal is to:

- understand how mitochondria, organelles that fuel cells with energy, shape stress- and anxiety- related behaviors
- identify molecular signatures and candidate biomarkers for neuropsychiatric phenotypes and treatment responses
- explore the potential of mitochondria as therapeutic targets for neuropsychiatric disorders

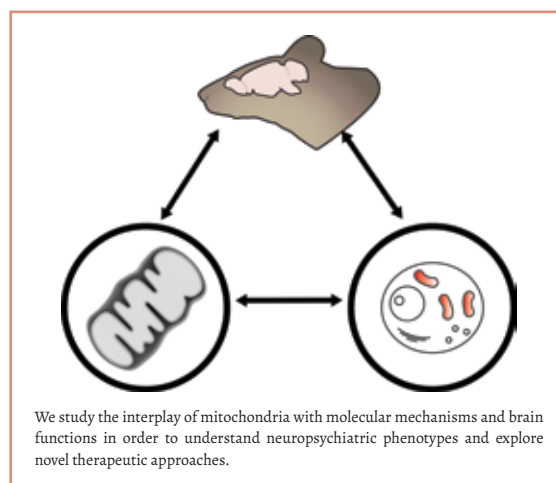
To do so, we work with mouse models, patient samples and cell culture, using behavioral biology, protein biochemistry, proteomics/metabolomics approaches and pharmacology. Our vision is to create a collaborative environment, fostering scientific growth and communication.

Current Aims

- We are investigating how mitochondria shape stress responses in mouse models that undergo chronic or acute psychological stress
- We are studying whether interventions such as enriched environment confer stress resilience and how mitochondria mediate these stress-relieving effects
- We are identifying altered metabolomics signatures upon stress exposure in brain regions and peripheral material
- We are exploring whether administration of substances that selectively target mitochondrial changes exert anxiolytic effects and improve stress-coping behavior

Progress in 2019-2020

Our lab joined the BR-IMBB in February 2020 and the University of Ioannina in April 2018 (relocation of PI and lab member from the Max Planck Institute of Psychiatry in Munich). The lab has secured funding from HFRI (2018-2021), NSRF (2020-2021) and Fondation Santé (2020-2021). During the period 2019-2020, we have published 6 papers and submitted 3 more. We have also established the breeding of the High and Normal Anxiety-related Behavior (HAB/NAB) mouse model in Ioannina (brought from the Max Planck Institute of Psychiatry).



Other activities

- We actively participate in dissemination activities to different target groups, including [PharmaCon](#), the [12th Panhellenic Meeting of Bioscientists](#) and [Ioannina Science Festival](#)
- We organize 'Neurotalks' sessions together with two other research groups at the University of Ioannina, open to the Neuroscience community, to discuss our research activities and offer soft skill training to early career scientists
- We have trained an internship undergraduate student and are currently training two diploma thesis students
- The lab has now a Facebook page [@BiochemLabBET](#)

Web page

<https://www.imbb.forth.gr/en/research-en/biomedical-research/item/5337-michaela-filiou>

Publications

Chousidis I, et al. (2020) Cannabinol in the spotlight: Toxicometabolomic study and behavioral analysis of zebrafish embryos exposed to the unknown cannabinoid. **Chemosphere** 252:126417

Filiou MD and Sandi C (2019) Anxiety and brain mitochondria: A bidirectional crosstalk. **Trends Neurosci** 42:573-88, (Journal Cover)

Papadopoulou Z, et al. (2019) Unraveling the serum metabolomic profile of post-partum depression. **Front Neurosci** 2019 13:833

MULTI-SCALE MODELLING OF BIOLOGICAL TISSUES AND SYSTEMS



Dimitrios I. Fotiadis

Professor - Collaborating
Faculty Member

GROUP MEMBERS

Collaborating Faculty member
Yorgos Goletsis
Associate Professor

Senior Postdoctoral researchers
Nikolaos Tachos
Vassilis Tsakanikas
Antonios Sakellarios
Konstantina Kourou
Eugenia Mylona
Kostas Tsiouris
George Rigas
Evanthia Tripoliti
Petros Toumpaniaris

Senior Researchers:
Georgia Karanasiou

Research fellows:
Savvas Kiriakidis
Thanasis Pardalis
Christos Androutsos
Marina Georgoula
Dimitris Pleouras
Vassilis Karaiskos
(left 2020)

PhD students:
Gianna Karanasiou
Ioannis Andrikos
(left 2019)
Penelope Ioannidou
Michalis Sarafidis

Coordination assistants:
Vangelis Fotiou
Ioanna Kastani
(left 2020)

Summary

The Unit of Medical Technology and Intelligent Information Systems (MedLab) is a highly innovative and self-contained research unit strongly activated in the fields of Biomedical Engineering and development of Intelligent Information systems. It has an internationally acknowledged excellence in conducting high quality scientific research and developing innovative Information Technology (IT) applications, products and services. Our activities are mainly based on international collaborations in the framework of European and Nationally funded projects (FP5, FP6, FP7, and Horizon 2020).

Current Aims

MedLab's research activities cover a variety of subjects through and they are classified into the following domains: Multi-scale Modeling of Biological Tissues and Systems, Bio-material tissue/drug interaction and in silico clinical trials, Biomedical & Genetic Data Analysis, Wearable Systems for monitoring and treatment of patients, Big Data Platforms, Big Data Analytics / Machine learning.

Progress in 2019-2020

Multi-scale Modelling of Biological Tissues and Systems

A unique expertise in multiscale modelling of biological tissues and systems. The scope of research is wide, dealing with numerical modelling of: a) Numerical modelling of cardiovascular disease, b) Computational modelling of bone pathologies, c) glycaemic control in diabetic patients, and d) Modelling of hearing function in middle and inner ear.

Biomaterial, tissue/drug interaction and in silico clinical trials

Development of in silico digital solutions for the design, development and evaluation of medical devices and drugs, before market release. The in silico solutions rely on the integration of multi-disciplinary and multi-scale models which are able to predict the short, medium-, and long-term devices performance, considering the interaction with the arterial tissue.

Big data platforms

Development of big data platforms where the patient data are

shared and stored under secure federated cloud databases. Big data curation workflows are applied on the data to enhance their quality by removing incompatible and inconsistent fields. Data harmonization workflows based on lexical and semantic analysis are applied on the federated data to overcome the ensuing structural and conceptual heterogeneity among the complex data structures.

Wearable systems for monitoring and treatment of patients

Wearable sensor technology allows for monitoring vital signs and movement in an individual's native environment. The technology of these sensors is advancing rapidly while they are becoming increasingly widespread in daily living products. Exploiting this technology along with the development of machine learning and artificial intelligence models to modernize health monitoring and management in the elderly and other patient populations.

Biomedical and genetic data analysis

Analysis of biomedical and genetic datasets for the development of computational methods towards predicting the disease status and outcomes. Our long-term objective is to create artificially intelligent and machine learning predictive models of cancer and other diseases for the translation of biomedical data to precision diagnosis and treatment.



The research activities, personnel, research projects, funding and startups of the Unit of Medical Technology and Intelligent Information Systems

Other activities

International collaboration in research projects, funded by EU and other bodies and establishment of two startups.

Web page

<http://medlab.cc.uoi.gr/>

Publications

Pezoulas VC, et al. (2020) Overcoming the barriers that obscure the interlinking and analysis of clinical data through harmonization and incremental learning. *IEEE Open Journal of Engineering in Medicine and Biology* 1 3-90 doi: 10.1109/OJEMB.2020.2981258

Kigka VI, et al. (2019) A three-dimensional quantification of calcified and non-calcified plaques in coronary arteries based on computed tomography coronary angiography images: comparison with expert's annotations and virtual histology intravascular ultrasound. *Computers in Biology and Medicine* 113 doi: 10.1016/j.compbiomed.2019.103409

Tripoliti E, et al. (2019) HEARTEN KMS - A knowledge management system targeting the management of patients with heart failure. *Journal of Biomedical Informatics* 94 doi: 10.1016/j.jbi.2019.103203

MOLECULAR MECHANISMS OF ANGIOGENESIS AND CROSSTALK TO METABOLISM



Theodore Fotsis

Prof. Emeritus-
Collaborating Faculty
Member

GROUP MEMBERS

Senior Research Scientist:

Sofia Bellou

Postdoctoral researcher:

Ioannis
Angelopoulos
(until 31.08.2020)

PhD Students:

Evangelos
Drougkas*

Maria Markou*
(*co-supervised with C.
Murphy)

MSc Students:

Emannouel
Iakovidis
(2020)

Eftychia Kosmeri
(2019)

Visiting scientist:

Maria
Chatziathanasiadou

Summary

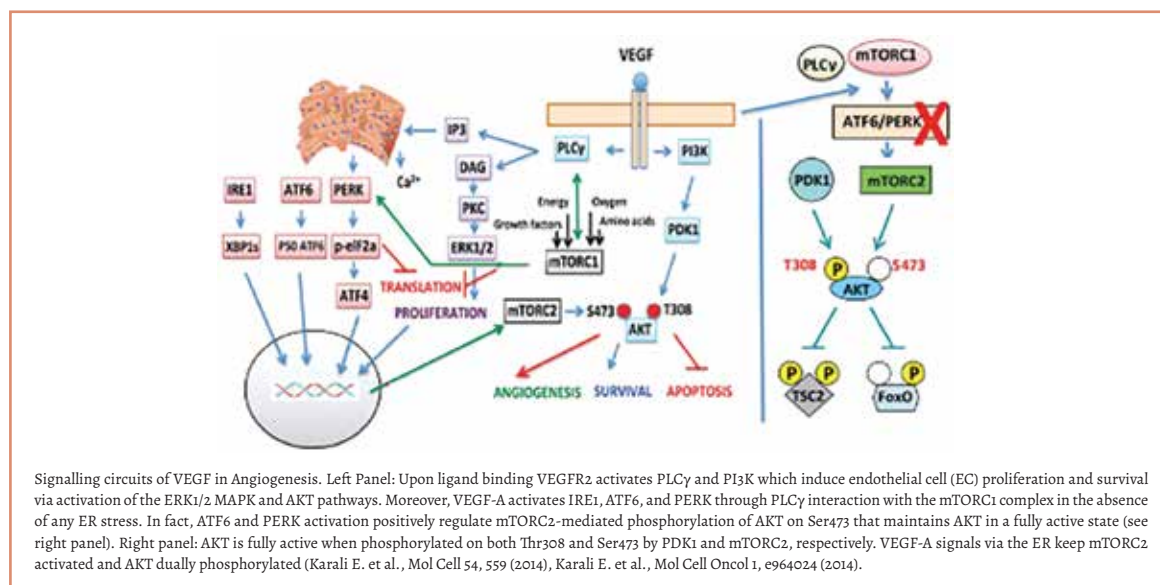
In vessel morphogenesis via **vasculogenesis**, mesodermal progenitors differentiate into endothelial cells and form a primary vascular plexus. Subsequent recruitment of pericytes (PC) or smooth muscle cells (SMCs) results in maturation and stabilisation of the nascent plexus (**vascular myogenesis**). **Angiogenesis** involves the formation of new vessels from pre-existing ones, mainly by sprouting and **lymphangiogenesis** refers to the growth of the lymphatic vessels. **Vascular Endothelial Growth Factors (VEGFs)** are the most important regulators of vessel morphogenesis in health and disease.

Current Aims

1. To continue work on endothelial cell (EC) signalling during angiogenesis and the influence of receptor trafficking on the signalling output.
2. To understand the purpose of switching ATP production from oxidative phosphorylation to lactic fermentation aerobically in ECs [aerobic glycolysis (AG) - Warburg effect] and, in particular, the consequences on regulation of angiogenesis. To elucidate the molecular mechanism of the switch.
3. To investigate the crosstalk between regulatory circuits of angiogenesis and metabolism.

Progress in 2019-2020

We have shown that VEGF-induced PLC γ interacts with mTORC1 activating the ER sensors IRE1, ATF6 and PERK linking EC survival to angiogenesis, thereby associating them to EC metabolism (energy and nutrient levels, growth factor milieu, oxygen tension) (see figure). The utilisation of AG by ECs further enhances this association. Work has been completed on vessel engineering (Markou, M. et al. 2020), regulatory circuits (Papadopoulos, A. et al. 2020) and the effect of receptor trafficking on signalling output (Kostopoulou, N, Bellou, S. et al. 2021 submitted).



Other activities

E. Iakovidis carried out his MSc dissertation on the anti-mitotic and anti-angiogenic effects of Mediterranean diet on cancer prevention.

Pension from the University of Ioannina (31.08.2019)

Honorary member of FORTH (13.10.2019)

Professor Emeritus of the University of Ioannina (19.12.2019)

MSc dissertation presentation by E. Iakovidis on the protective effect of Mediterranean diet on cancer (27.10.2020)

Web page

<http://www.imbb.forth.gr/fotsis>

Publications

Markou M, et al. (2020) Tissue Engineering Using Vascular Organoids From Human Pluripotent Stem Cell Derived Mural Cell Phenotypes. **Front Bioeng Biotechnol.** 8:278

Papadopoulos A, et al. (2020) Combined transcriptomic and phosphoproteomic analysis of BMP4 signaling in human embryonic stem cells. **Stem Cell Res** 50:102133

NF- κ B SIGNALLING PATHWAYS IN SENESCENCE AND CANCER



Evangelos Kolettas

Associate Professor-
Collaborating Faculty member

GROUP MEMBERS

Postdoctoral research
scientists:

Georgios Markopoulos
(Left in February 2020)

PhD students:

Nektaria-Maria Leli
(Completed May 2019)

Eugenia Roupakia
(Completed December 2020)

Panagiotis Ntellas

MSc students:

Alexandros Sarras

Undergraduate students:

Evridiki-Christina Paniopoulou

Summary

NF- κ B transcription factors (TFs) are critical regulators of pro-inflammatory/stress-like responses, and their immediate upstream signaling components are aberrantly expressed and/or activated in pulmonary diseases, including non-small cell lung cancer (NSCLC), and have been implicated in the unfavourable prognosis for patient survival.

NF- κ B TFs bind to DNA as heterodimers or homodimers of five subunits: RelA/p65, c-Rel, RelB, p50 and p52. These TFs are activated by two upstream activating serine/threonine kinases IKK α and IKK β . Activation of NF- κ B is achieved by two main signalling pathways: An IKK β -mediated canonical NF- κ B pathway and an IKK α -mediated non-canonical NF- κ B pathway. In the former, c-Rel/p50 and RelA(p65)/p50 heterodimers are restrained in the cytoplasm of most cells not experiencing a pro-inflammatory/stress-like response by NF- κ B inhibitors, the I κ Bs. Activation of canonical NF- κ B pathway is initiated by the phosphorylation of I κ B α causing its ubiquitination and subsequent proteasomal degradation, resulting in c-Rel/p50 or p65/50 heterodimer nuclear translocation and target gene regulation. Activation of the non-canonical NF- κ B pathway involves the NF- κ B Inducing Kinase (NIK)-dependent phosphorylation of IKK α leading to the generation of p52/RelB heterodimers that translocate to the nucleus and regulate a distinct set of non-canonical NF- κ B targets.

IKK β /canonical NF- κ B signalling and non-canonical heterodimers have been implicated in the genesis of NSCLC in the context of oncogene activation. In contrast to the involvement of IKK β /NF- κ B in inflammation-linked NSCLC, the functional roles of IKK α in NSCLC remain unclear. Our lab's interest is to further understand how NF- κ B signalling regulates DNA damage impacting on senescence and cancer.

Current Aims

Because IKK α and IKK β function differently, with IKK α acting as a growth suppressor, and IKK β as a growth promoter, we aim to understand the molecular mechanisms of each IKK on senescence and cancer:

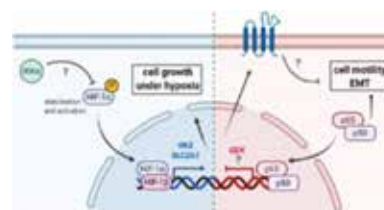
1. *Mechanism of action of canonical IKK β /NF- κ B in NSCLC:* While somatic oncogenic mutations and the chemical carcino-

gen urethane lead to activation of canonical NF- κ B, the mechanism(s) by which it contributes to NSCLC is still under investigation. Hence, we developed human NSCLC models and analysed their gene expression profile to begin to unravel the potential mechanisms involved.

2. *NF- κ B-miRNA network in NSCLC:* We employed Nanostring miRNA analysis to characterise IKK-regulated miRNAs involved in DNA damage responses (DDR) and NSCLC growth.
3. *Mechanism of action of IKK α in NSCLC:* RNA-seq and bioinformatics analysis identified genes including TFs and chromatin remodelers involved in human NSCLC growth. In addition, one of the IKK α -regulated miRNAs appears to affect chromatin remodeling. We aim at elucidating the role of IKK α -TF-miRNA in NSCLC.
4. *Mechanism of action of IKK α in oncogene induced senescence:* Because IKK α acts as a growth suppressor, we aim to define its functional role in *K-Ras* expressing human fibroblast cell cycle progression, DDR and SASP, during OIS, by developing inducible cell systems.

Progress in 2019-2020

- Canonical IKK β /NF- κ B acts as a tumour promoter in NSCLC by affecting a small number of genes (1, 2), and a new mechanism of action has been defined (submitted).
- We showed that IKK α is an evolutionary conserved tumour suppressor both in mouse and human NSCLC models, by influencing hypoxia-inducible pathways required for the enhanced growth of tumours *in vivo* (2, 3).



IKK α acts as tumour suppressor by influencing hypoxia-inducible pathways required for the enhanced tumour growth *in vivo*. IKK β /canonical NF- κ B promotes human NSCLC growth *in vivo* by inhibiting the expression of tumour metastasis cell surface suppressors and promoting cell motility, EMT and invasion

Other activities

- Editorial Work
 - Guest co-editor, special issue of *CELLS* on 'The DNA Damage Response in Cell Physiology and Disease' (https://www.mdpi.com/journal/cells/special_issues/Damage_Response)
 - Guest co-editor for a joint special issue of *CANCERS-CELLS* on 'NF- κ B signalling in cellular responses to threats, cancer development and therapy' (https://www.mdpi.com/journal/cancers/special_issues/nuclear_factor_kappaB; https://www.mdpi.com/journal/cells/special_issues/NF-Kappa_B)
- Co-editor of the translation of *Molecular Cell Biology*, by Lodish *et al* (2016), Freeman & Co (Utopia, 2020).
- *Principles of Development*, by Wolpert L, Tickle C & Arias AM, 2019, 6th e; Oxford University Press; Translation in Greek of Chapter 1 (Broken Hill Publishers Ltd, 2020).

Web page

<https://www.imbb.forth.gr/en/research-en/biomedical-research/item/5510-evangelos-kolettas>

Publications

Ntellas P, *et al.* (2020) Old player-new tricks: Non angiogenic effects of the VEGF/VEGFR pathway in cancer. *Cancers* 12(11): E3145

Markopoulos G, *et al.* (2019) Epigenetic regulation of inflammatory cytokine-induced epithelial-to-mesenchymal cell transition and in the generation of cancer stem cells. *Cells* 8(10) pii: E1143

Chavdoula E, *et al.* (2019). CHUK/IKK α loss in lung epithelial cells enhances non-small cell lung cancer (NSCLC) growth associated with HIF up-regulation. *Life Science Alliance* 2(6): e201900460

VASCULAR ENDOTHELIAL CADHERIN IN CARDIAC DIFFERENTIATION



Panos Kouklis

Assistant Professor-
Collaborating Faculty
member

GROUP MEMBERS

Postdoctoral research
scientists:

Violetta Maltabe

MSc students:

**Dimitrios
Papanikolaou**

Summary

Cadherins comprise a family of transmembrane molecules involved in cell-cell adhesion and signal transduction. Their functional significance is demonstrated in key biological processes like organogenesis, tissue homeostasis, migration, and cancer. The endothelial-specific VE (Vascular Endothelial)-cadherin is expressed in endothelium and is involved in angiogenesis, signaling and endothelial barrier regulation. We found that, during ESCs differentiation VE-cadherin-mediated AJs constitute a major adhesive structure in Isl1⁺/Mef2c⁺ cardiovascular progenitors. Based on VE-cadherin promoter activity, we isolated a novel dual cardiovascular and endothelial progenitor cell population (CEDPs) during ESCs in vitro differentiation. These cells could self-renew and differentiate further to cardiomyocytes and mature endothelial cells in vitro. Moreover, they survive and differentiate to cardiomyocytes after transplantation in the myocardium of adult rats.

Current Aims

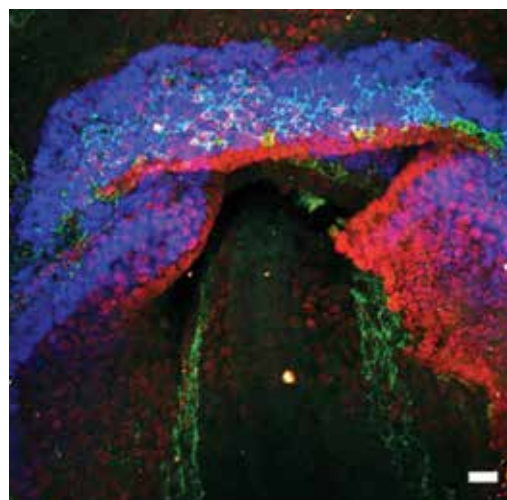
To investigate the functional significance of VE-cadherin expression in cardiac differentiation. Towards this purpose we will use VEC-null ESCs in differentiation studies in vitro. We plan to analyze cardiovascular emerging populations by single-cell transcriptome analysis. Another approach will be to study VE-cadherin⁺ cells during early mouse embryogenesis by lineage tracing.

To test CEDPs as a cell source for cardiac regeneration. We plan to analyze CEDPs in detail by single-cell transcriptome analysis and inject them in the heart of mice after myocardial infarction induction by left coronary artery ligation. Our aim will be to study the effect of CEDPs after infarction in Left Ventricle remodeling.

Progress in 2019-2020

Disruption of CDH5 gene in pluripotent ESCs by CRISPR Expression of VE-cadherin in cardiac progenitors during mouse embryogenesis

We study the expression patterns of N- and VE-cadherin at the onset of cardiac differentiation during early mouse cardiogenesis. VE-cadherin-mediated AJs can be detected at E7.5 and constitute major adhesive structure of cardiovascular progenitors population at E8.5-E9.5.



Mouse embryo at E8.5 stained for VE-cadherin (green), Isl1 (red) and Mef2c (blue).

Web page

<http://www.imbb.forth.gr/kouklis>

Publications

Maltabe V, et al. (2020) Challenges in stem cell-based approaches for myocardial regeneration after myocardial infarction. **Stem Cells: From Hype To Hope** 17 (ISBN 978-3-11-064243-8)

Thrasymoulou S, et al. (2020) VL30 retrotransposition is associated with induced EMT, CSC generation and tumorigenesis in HC11 mouse mammary stem-like epithelial cells **Oncology reports** 44 (1), 126-138

INTRA-TUMOR HETEROGENEITY AND THERAPY RESISTANCE



Angeliki Magklara

Associate Professor-
Collaborating Faculty member

GROUP MEMBERS

Post-doctoral Researchers:

Ioannis Verigos
(left in October 2020)

Anastasia Kougiumtzi

PhD students:

Dimitrios Kordias
Myrto Kastrisiou

Master's students:

Eleftherios Sinanis
Polyxeni Kalisperati

Undergraduate students:

Ioanna Zikopoulou
(left in October 2020)
Nikolaos Iatridis

Summary

Intra-tumor heterogeneity is considered a major driver of therapy resistance; thus, the identification and comprehension of the underlying mechanisms is of utter importance for the development of more effective therapies. Intra-tumor heterogeneity is a consequence of genetic and epigenetic alterations. Liquid biopsy has recently emerged as a powerful, non-invasive technique that can capture tumor evolution in real time and uncover novel genetic alterations that may cause therapy resistance, before the patients' clinical deterioration. Furthermore, liquid biopsy biomarkers have shown great promise for early diagnosis and prognosis of cancer.

Cancer stem cells (CSCs) are defined as a small subset of aggressive tumor cells marked by their capacity for self-renewal and differentiation, as well as, their resistance to conventional therapeutic schemes. Functional and phenotypic differences between CSCs and non-CSCs are attributed predominantly to differential epigenetic mechanisms that distinguish the two subpopulations. Consequently, definition of the epigenetic state of CSCs should give us valuable insights for the development of targeted therapies competent to eliminate this aggressive subpopulation.

Current Aims

The overall goal of our lab is to delineate intra-tumor heterogeneity on the genetic and epigenetic level, in order to gain a better understanding of therapy resistance and improve patient outcome.

Our current work is aimed at:

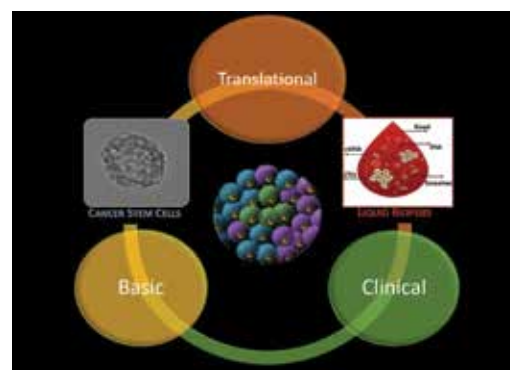
- Unraveling the molecular underpinnings of epigenetic heterogeneity in breast cancer and its contribution to therapy resistance. Our research focuses on cancer stem cells, as they are an ideal system to study the unique epigenetic profile of this treatment-resistant subpopulation and identify its differences from the treatment-sensitive tumor cells.
- Developing novel *in vitro* systems to study intra-tumor epigenetic heterogeneity in breast cancer, such as drug-resistant cancer cell lines and 3D spheroids.
- Developing and applying highly sensitive liquid biopsy

techniques for the detection of circulating tumor DNA (ctDNA) in cancer patients.

- Evaluating ctDNA as a non-invasive biomarker in different types of cancer.

Progress in 2019-2020

- We have identified the histone demethylase LSD1 as a major regulator of cancer stemness and chemoresistance in breast cancer^{1,2}.
- We have developed several breast cancer cell lines resistant to different anti-cancer drugs and we are investigating their epigenetic profile vs. the parental ones.
- In collaboration with the Oncology Department of the University of Ioannina, we are carrying out clinical studies in colorectal and pancreatic cancer patients using a highly sensitive digital PCR technique for the detection of ctDNA.
- We have developed and are currently evaluating a new gene panel to detect ctDNA in colorectal patients using Next-Generation Sequencing⁴.



Our lab investigates intra-tumor heterogeneity carrying out basic, translational and clinical research. Our basic research focuses on the study of breast cancer stem cells; this subpopulation can generate tumorspheres (photo by I.Verigos) that serve as a valuable *in vitro* system. Liquid biopsy techniques allow for the detection of tumor material, such as ctDNA, circulating tumor cells (CTCs), exosomes and RNA, isolated from blood specimens (picture adapted by A. Kougiumtzi). Our translational studies seek to evaluate new ctDNA biomarkers in the early detection of therapy resistance and management of various tumor types, aiming to their application in clinical practice.

Other activities

- Dr. A. Magklara spent 6 months at Dr. Weinberg's laboratory at the Whitehead Institute of Biomedical Research (MIT, Cambridge, USA) as a Fulbright Visiting Scholar.
- Dr. A. Magklara served as the guest academic editor of the Special Issue: "Epigenetic Dysregulation in Cancer: from Mechanism to Therapy" of the journal *Cancers*.
- Members of the group participated in international and national conferences.
- We participated in three successful grant proposals funding our basic and translational research.

Web page

<https://www.imbb.forth.gr/imbb-people/en/magklara-laboratory>

Publications

Verigos J, et al. (2019) The Histone Demethylase LSD1/KDM1A Mediates Chemoresistance in Breast Cancer via Regulation of a Stem Cell Program. *Cancers* (Basel); 11(10)

Karakaidos P, et al. (2019) LSD1/KDM1A, a Gate-Keeper of Cancer Stemness and a Promising Therapeutic Target. *Cancers* (Basel); 11(12)

Kastrisiou M, et al. (2019) Clinical Application of Next-Generation Sequencing as A Liquid Biopsy Technique in Advanced Colorectal Cancer: A Trick or A Treat? *Cancers* (Basel); 11(10)

OOCYTE AND EARLY EMBRYO DEVELOPMENT



Petros Marangos

Associate Professor-
Collaborating Faculty
member

GROUP MEMBERS

Postdoctoral researchers:

Sandra Gonzalez
Malagon

PhD Students:

Konstantina Niaka
Kristy Agapitou

Summary

Cell cycle regulation during gametogenesis and early embryonic development plays an important role in fertility, embryo development and the delivery of healthy offspring. Research on the cell cycle of developmental processes can also provide information on the actions of meiotic and stem cell regulators that are implicated in cell cycle regulation during cancer development. Our laboratory specializes in the examination of cell cycle regulation and DNA damage response during mammalian oocyte and early embryonic development.

Current Aims

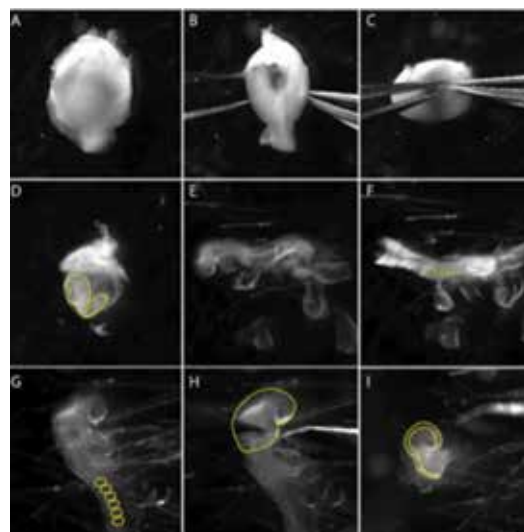
Our lab studies the mammalian cell cycle and DNA damage response of oocytes, eggs and the embryonic stem cells of pre-implantation embryos. In addition, we are examining the relation between maternal age and oocyte aging with chromosomal abnormalities, aneuploidy and embryonic development. We are also determining the role and function of oocyte cell cycle regulators presenting oncogenic expression. Furthermore, we focus on the study of the cell cycle and DNA damage response of neural crest stem cells.

Progress in 2019-2020

In 2020 we published the results of an international collaborative work examining the DNA damage response of human oocytes. It has been known, from groundbreaking work from our lab that mouse oocytes with damaged DNA can resume meiosis and undergo nuclear envelope breakdown. Under conditions of high levels of damage, oocytes arrest in metaphase of meiosis-I in a process involving Spindle Assembly Check-point (SAC) signalling. In our recent publication we report that human oocytes harboring DNA damage progress through meiosis-I and subsequently form a metaphase-II egg, revealing

the absence of a DNA-damage-induced SAC response. Our results suggest that DNA damage accumulated in meiosis I, such as could occur during *in vitro* maturation procedures, does not prevent oocyte cell division and therefore could persist in morphologically normal looking metaphase-II eggs.

In 2019, along with our collaborators at King's College London, we established a well-defined, routine protocol for the culture of primary cranial neural crest cells. Due to our two-dimensional culturing approach, the distinct tissue populations (neural plate versus premigratory and migratory neural crest) can be readily distinguished. Using live imaging approaches, we can then identify changes in neural crest induction, EMT and migratory behaviors.



Isolation of cranial neural crest explants from an e8.5 embryo. Images are stills from a video documenting the micro-dissection technique. (A–C) Dissection of the embryo from the uterus. (D) shows embryo inside the visceral yolk sac (yellow line). Extraction of the embryo from the visceral yolk sac. (E) Lateral views of the embryo at stage 8.5 lateral. (F) Dorsal view of the embryo at stage 8.5. (G) Close up look at the cranial region of the embryo. Remove extraembryonic membranes from the cranial region; somites are marked with a yellow line. (H) Dissections of anterior neural plate are performed under the first branchial arch (yellow line). (I) Lateral view of anterior neural plate dissection. Neural folds, where neural crest cells arise, are marked with a yellow line. Movie was taken using a stereo-microscope with a widefield apochromatic lens at 3.0X zoom. *JoVE* 2019, (152), e60051, doi:10.3791/60051.

Other activities

2 Posters in national and international conferences

The lab was invited to participate in the Microscopy Infrastructure Network set by IMBB/FORTH and the University of Ioannina.

Web page

<https://www.imbb.forth.gr/en/research-en/biomedical-research/item/5537-petros-marangos>

Publications

Rémillard-Labrosse G, et al. (2020) Human oocytes harboring damaged DNA can complete meiosis I. **Fertility and Sterility** 113(5), 1080–1089 e2

Gonzalez Malagon SG, et al. (2019) Dissection, Culture and Analysis of Primary Cranial Neural Crest Cells from Mouse for the Study of Neural Crest Cell Delamination and Migration. **JoVE (J. Vis. Exp.)** (152) e60051, doi:10.3791/60051

MOLECULAR MECHANISMS OF VASCULAR MORPHOGENESIS AND VESSEL ENGINEERING



Carol Murphy

Principal Researcher (B)

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Maria Markou

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(2020)

Gianna Savvou
(2019)

Aikaterini Apostolidi
(2019)

Aristea Pavlou
(2020 visiting)

A major requirement for proper viability and function of an implantable construct is the availability of blood vessels to support its *in vivo* growth. Vascularisation remains a critical obstacle in engineering thicker, metabolically demanding organs, such as the heart muscle, brain and liver. Thus, there is great interest in generating tissue-engineered constructs that are already vascularised before implantation. We have already successfully differentiated hESCs/hiPSCs, via mesoderm, to Vascular Progenitor Cells (VPCs) and subsequently to endothelial cells (ECs) using a modified protocol (Tsolis et al., J. Proteome Res. 2016, 15:1995-2007) (see Figure). Research in our lab also focuses on investigating the role of the endocytic pathway in growth factor signaling and the role of endocytic trafficking during pluripotency and differentiation. In the last few years we initiated a new line of research investigating the role of endocytic trafficking in the differentiation of hESCs/hiPSCs to mature ECs.

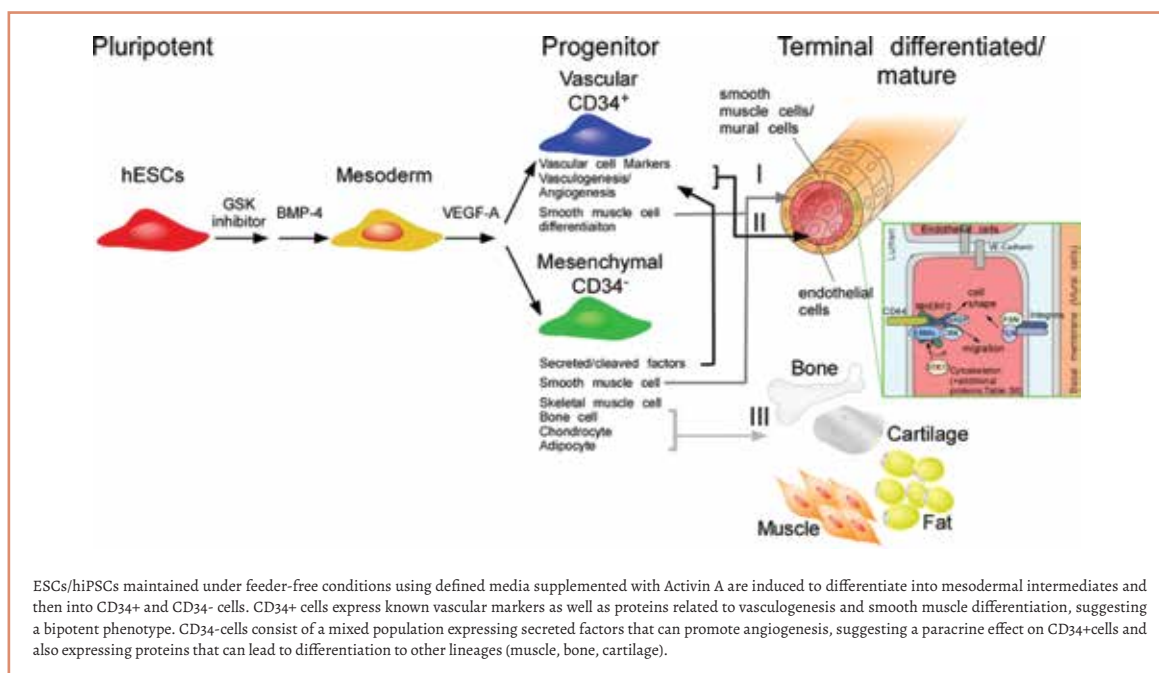
Current Aims

1. To characterise and isolate the specific mesodermal population that responds to VEGF differentiating to VPCs and ECs.

2. To identify the vasculogenic signalling circuits of VEGF by determining the VEGF-induced phosphoproteome and transcriptome in mesodermal progenitors.
3. To establish vessel engineering approaches including the use of vascularised constructs/organoids to support Regenerative Medicine applications.

Progress in 2019-2020

In addition to differentiating hESCs/hiPSCs to VPCs and ECs (Tsolis et al. 2016 and figure), we succeeded in generating human contractile and synthetic smooth muscle cells (SMCs) from hiPSCs (1). Moreover, when such hiPSC-EC-SMC vascular organoids were embedded in hydrogels composed of defined ECM components, more human vasculature emerged with increased anastomoses with mouse vasculature and more perfused blood vessels compared to hiPSC-EC spheroids (1). Concerning identification of the regulatory circuits of VEGF in vasculogenesis, we have carried out a similar combined transcriptomic and phosphoproteomic analysis of BMP4 on hESCs (2). Finally, we have begun to address the role of endocytic trafficking in differentiation and pluripotency (4).



Other activities

We participate in a collaborative consortium that synthesises and tests compounds with useful imaging properties (Vrettos et al.).

Web page

<http://www.imbb.forth.gr/murphy>

Publications

Markou M, et al. (2020) Tissue Engineering Using Vascular Organoids From Human Pluripotent Stem Cell Derived Mural Cell Phenotypes. *Front Bioeng Biotechnol.* 8:278

Papadopoulos A, et al. (2020) Combined transcriptomic and phosphoproteomic analysis of BMP4 signaling in human embryonic stem cells. *Stem Cell Res* 50:102133

Vrettos EI, et al. (2020) Development of programmable gemcitabine-GnRH pro-drugs bearing linker controllable "click" oxime bond tethers and preclinical evaluation against prostate cancer. *Eur J Med Chem* (In Press).

HISTONE CHAPERONES AND THEIR ROLES IN GENE EXPRESSION AND SIGNAL TRANSDUCTION



Thomais Papamarcaki

Associate Professor-
Collaborating Faculty
member

GROUP MEMBERS

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Zoe Karetso

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Outskouni

Summary

The research of our lab is focused on the study of the molecular interactions and structural characteristics of histone chaperones and chromatin remodelling factors. Our long-term goal research is to understand their interplay with chromatin components and their roles in chromatin dynamics, gene expression and signal transduction.

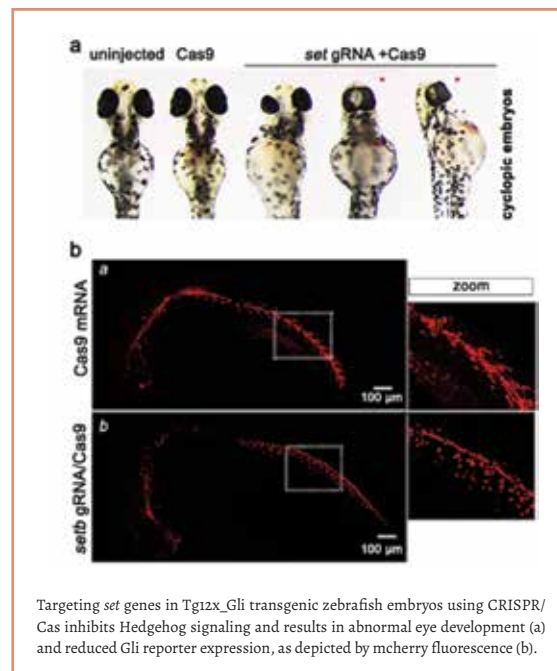
Current Aims

The recent research direction of the lab is the study of the physiological roles of chromatin remodelers in Hedgehog signaling and embryonic development using zebrafish as a model-organism. To accomplish this, we plan to:

1. Target the expression of genes involved in chromatin remodelling in Tg12x_Gli transgenic zebrafish embryos, which express the mCherry reporter under regulation of Gli transcription factors.
2. Study the protein interactions of histone chaperones with key players of the Hedgehog pathway.
3. Establish in vivo assays for the pharmacological inhibition of Hedgehog signalling using the zebrafish model system.

Progress in 2019-2020

Our work has revealed the involvement of the histone chaperone SET/I2PP2A in Gli1-mediated transcription. This work suggests SET/I2PP2A as a promising druggable target for treatment of several tumors arising from abnormal activation of the Hedgehog pathway, pointing to the significance of our findings in clinical research.



Targeting *set* genes in Tg12x_Gli transgenic zebrafish embryos using CRISPR/Cas inhibits Hedgehog signaling and results in abnormal eye development (a) and reduced Gli reporter expression, as depicted by mCherry fluorescence (b).

Other activities

- Teaching Biochemistry to Medical School Students
- Diploma Thesis of undergraduate students in the laboratory.
- Master Thesis of graduate students in the laboratory.

Selected Impact Activities

- Evaluation of new compounds that inhibit Protein Phosphatase 2A (PP2A) activity.

Web page

<http://www.imbb.forth.gr/papamarcaki>

Publications

Serifi I, et al. (2019) Effects of the Essential Oil from *Pistacia lentiscus* Var. *chia* on the Lateral Line System and the Gene Expression Profile of Zebrafish (*Danio rerio*). **Molecules** 24(21): 3919. doi: 10.3390/molecules

STRUCTURAL/COMPUTATIONAL EPIGENETICS: NUCLEAR ENVELOPE PROTEINS AND CHROMATIN



Anastasia Politou

GROUP MEMBERS

Postdoctoral Researcher

Katerina Soupsana

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Panagiotis Martzios

Eva Triantopoulou

Diploma thesis students:

Dimitra Chadou

Georgia

Georgiopoulou

Irini Meselidou

Vassiliki Tassou

Summary

Our main research interests lie in the area of Structural and Computational Epigenetics and Transcriptomics. In this context, we study the structure, dynamics and interactions of chromatin-associated and intrinsically disordered proteins in the cell nucleus, examining the gene expression profile of embryonic stem cells (ESCs) and differentiated cells lacking or overexpressing wild type or mutated forms of these proteins. Our approaches include:

I. Identification of structural determinants involved in epigenetic regulation.

It is believed that combinatorial patterns of post-translational modifications (PTMs) on histone tails constitute an epigenetic information code and can affect gene expression. We have been testing the structural basis of this hypothesis by analyzing modules of chromatin-associated proteins and their interactions with modified chromatin. In parallel, we investigate the conformational properties and association tendencies of specific histone PTM patterns by Molecular Dynamics simulations and biophysical assays.

II. Exploring and manipulating structure/function relationships in the intrinsically disordered, chromatin-associated nuclear envelope protein LBR

LBR, an integral membrane protein of the nuclear envelope (NE) tethers the inner nuclear membrane and the associated lamina structure to heterochromatin. We are investigating LBR association with specific genomic loci and its cooperation and complementarity with other nuclear structures in anchoring peripheral heterochromatin to NE. We are also studying the effect of site-specific PTMs on the conformation and the interactions of the protein and the organization of peripheral heterochromatin, using a series of mutants and knockouts and gene expression profiling.

Current Aims

Bivalent histone modifications

Our current work is focused on “bivalent” histone modifications, believed to cause silencing of developmental genes in ESCs, while keeping them poised for activation. To this end, we plan to:

- model by Molecular Mechanics and Dynamics the association between differentially modified histones carrying bivalent modifications;
- extend the calculations to modified histone tails attached to nucleosomes;
- experimentally test the theoretical predictions.

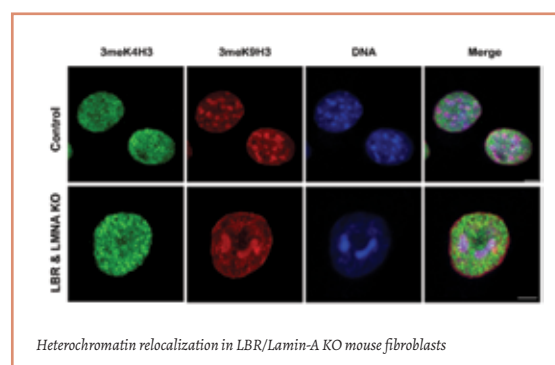
Nuclear envelope and chromatin

We seek to:

- explore the possibility that LBR cooperates with nuclear lamins and other components of NE in anchoring peripheral chromatin to the NE;
- investigate the cross-talk between LBR and partners with specific histone modifications required for correct spatial positioning of peripheral heterochromatin.

Progress in 2019-2020

- We have modeled the conformational properties and association tendencies of a novel histone modification pattern identified in mitotic somatic cells and haploid spermatids (PMM). PMM might act as a chromatin folding determinant, interlinking non-contiguous domains of chromatin. Consistent with the idea of a finely-tuned phosphorylation rheostat, our results indicate that PMM modifications allow tight “locking” of H3 tails that could potentially cross-link different chromatin domains.
- We have recently shown double knockout of LBR and Lamin-A causes a drastic rearrangement of heterochromatin in mouse fibroblasts (Figure).



Heterochromatin relocation in LBR/Lamin-A KO mouse fibroblasts

Other activities

- We have established and managed several tools and facilities that have been widely used by teams in Greece and abroad. These include the establishment of an integrated Biophysics Unit at the University of Ioannina and IMBB-BR. The Unit includes Circular Dichroism and Fluorescence Spectroscopy instrumentation, soon to be enhanced by the addition of Microscale Thermophoresis and Differential Scanning Fluorescence equipment. The facility is integrated in the National Research Infrastructure Program INSPIRED.
- We are coordinating the Ioannina team in the context of the National and the European ELIXIR consortium that supports biological data and management infrastructure.
- We have been evaluating novel synthetic compounds for efficient inhibition of Myc/Max interaction as part of a consortium coordinated by the Biomedical Research Foundation of the Academy of Athens

Web page

<http://www.imbb.forth.gr/politou>

Publications

Karanika E, et al. (2020) Haspin-dependent and independent effects of the kinase inhibitor 5-Iodotubercidin on self-renewal and differentiation. *Sci Rep.* 10(1):232



FACILITIES

IOANNINA

IMAGING FACILITY



Sofia Bellou
Senior Research Scientist

The IMAGING facility includes microscopes that provide researchers with the ability to visualize a variety of samples. In more detail, the facility is equipped with:

1. LeicaSP5/STED

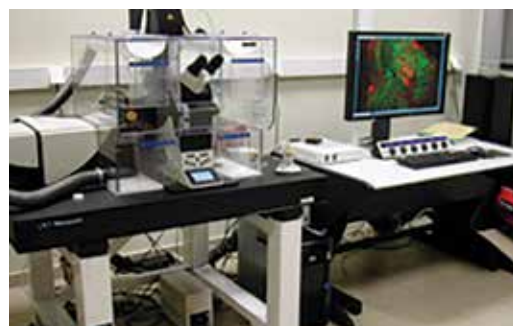
This microscope is an inverted fully motorized confocal microscope from Leica (SP5) combined with a super resolution STED module (Stimulated Emission Depletion) (Vrettos, Karampelas et al. 2021). In addition to the basic applications, with the SP5 we can perform additional microscopy applications. Software wizards for FRAP (Fluorescence Recovery After Photobleaching) and FRET (Fluorescence Resonance Energy Transfer) are available (Karanika, Soupsana et al. 2020).

2. TIRF Microscope

Total internal reflection fluorescence microscopy permits high signal-to-noise imaging of fluorescently-labeled molecules at surfaces and interfaces. The facility houses Leica AF 7000 TIRF system equipped with a 100x/1.45 NA Plan Apochromat TIRF objective lens (in addition to 10x, 20x, 40x, and 60x lenses), DIC & phase optics, UV, FITC, TRITC, a low-profile stage incubator (to facilitate live cell imaging) and a CCD camera. The system integrates four laser lines; 405nm, 488nm, 561nm, and 635nm with fast AOTF control.

3. IncuCyte ZOOM System

The IncuCyte ZOOM® system is a live-cell imaging and analysis platform that enables automated quantification of cell behaviour over time (from hours to weeks) by automatically gathering and analyzing images (phase contrast, bright-field and/or fluorescence) around the clock. The system provides insight into active biological processes in real-time which is not possible using single-point and end-point measurements. The system resides within the controlled environment of a standard cell incubator. All imaging is completely non-invasive and non-perturbing to cell health (Verigos, Karakaidos et al. 2019).



Publications

Karanika E, et al. (2020) Haspin-dependent and independent effects of the kinase inhibitor 5-Iodotubercidin on self-renewal and differentiation. **Sci Rep.** 10(1):232

Verigos J, et al. (2019) The Histone Demethylase LSD1/KappaDM1A Mediates Chemoresistance in Breast Cancer via Regulation of a Stem Cell Program. **Cancers** (Basel) 11(10)

Markou M, et al. (2020) Tissue engineering using vascular organoids from human pluripotent stem cell derived mural cell phenotypes. **Front Bioeng Biotechnol, section Tissue Engineering and Regenerative Medicine** 8:278 doi: 10.3389/fbioe.2020.00278 eCollection

HUMAN EMBRYONIC (HESCS) AND INDUCED PLURIPOTENT STEM CELL (HIPSCS) FACILITY



Carol Murphy

We have established a facility which is dedicated to hESCs and hiPSCs. We study the biology of these pluripotent cells as well as their differentiation into various cell types, including endothelial cells, mural cells, and neurons. This facility harbours 4 incubators dedicated for work with these cell types, a dissection hood and 2 biosafety flow hoods. A stereo microscope for isolation of clones, a Zeiss microscope for visualization and imaging are also available and an automated cell counter. Stem cells are handled separately from other cell types and liquid nitrogen storage containers harbouring cell stocks are also dedicated to stem cells to avoid potential contamination.

The facility also specialises in the three-dimensional (3D) culture of cells and more specifically the generation of vascular (Markou et al., 2020) and forebrain organoids (Gkogkas lab, unpublished). These organoids can recapitulate development and disease, and in the case of vascular organoids, can be used in the field of tissue engineering. For the analysis of organoids, among other techniques, we use cryotome sectioning, confocal and light sheet fluorescence microscopy.

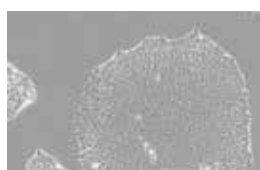


Fig. 1: Human embryonic stem cell colonies.



Fig. 2: Dissection hood containing a stereo microscope, Zeiss imaging system and the Countess III automated cell counter.



Fig. 3: A Biosafety hood dedicated to human stem cell work.



Fig. 4: CO₂ incubator for culturing of forebrain organoids, including shaking platforms.

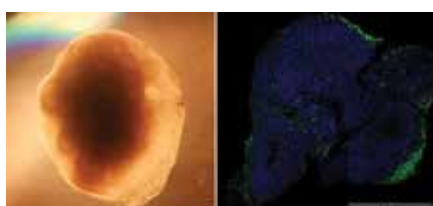


Fig. 5: A forebrain organoid in culture (left). Confocal image of a forebrain organoid slice immuno-stained with fluorescent markers (right), Gkogkas lab. Size bar 1mm.

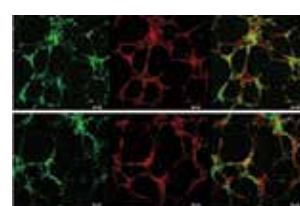


Fig. 6: Endothelial (red) and Mural cells (green) both differentiated from hiPSCs form a vascular network in vitro. Murphy & Fotsis lab. Size bar 100 microns.

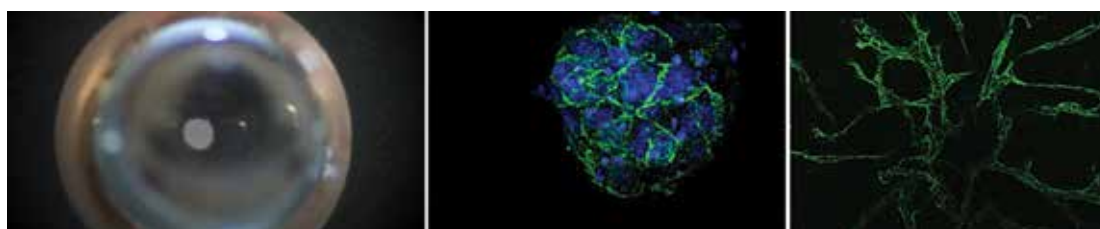


Fig. 7: A vascular organoid in culture as hanging drop (left). Confocal image of a whole vascular organoid immuno-stained with fluorescent markers (middle panel). Immunofluorescence analysis of sections from vascular organoid implants in vivo. Murphy & Fotsis lab. Size bars from left to right panels 100, 20, 20 microns.

Publications

Markou M, et al. (2020) Tissue engineering using vascular organoids from human pluripotent stem cell derived mural cell phenotypes. **Front Bioeng Biotechnol, section Tissue Engineering and Regenerative Medicine** 8:278 doi: 10.3389/fbioe.2020.00278 eCollection

Papadopoulos A, et al. (2020) Combined transcriptomic and phosphoproteomic analysis of BMP4 signaling in human embryonic stem cells. **Stem Cell Res.** 50:102133 doi.org/10.1016/j.scr.2020.102133

Many thanks to our Administration and Support Personnel



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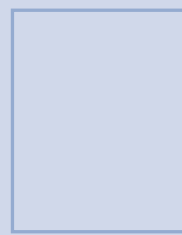
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