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ICGEB Research Grants awarded under the 2016 Call for applications

Title: Masters molecular regulators of polarized growth in plant cells
Principal Investigator: Dr. José M. Estevez, Fundación Instituto Leloir-FIL (IIBBA-CONICET), Buenos Aires, Argentina
ICGEB Reference No. CRP/ARG16-03
Abstract:
Both pollen tubes and root hairs are single plant cells that play crucial roles in the survival of plants either in fertilization and seed formation or the acquisition of nutrients and water. Their cell expansion is polarised and directional. This study project proposes to study the polarised cell expansion of both cell types in *Arabidopsis thaliana* as experimental model. Polarised cell expansion is managed and maintained by complex coordinated signalling master regulators that would control the flow of calcium ions (Ca^{2+}), producing reactive oxygen species (ROS) and pH oscillations in either cell apoplast of hair roots or in the cytoplasm of pollen tubes. Our main objective is to identify and characterise the nature of the Ca^{2+} transporters localised in plasma membrane and intracellular organelles involved in the growth of root hairs and pollen tubes. To this end, we will use pharmacological inhibitors, genetic tools and fluorescent biosensors. We propose to characterise the involvement of cytoplasmic Ca^{2+} (Ca^{+2}_{cyt}) and ROS production during polarised cell growth. Since root hairs are the cell entry gate of water and nutrient in the plant, the knowledge acquired in this project would have a great impact in plant growth and performance in general, and, specifically in the nutrient uptake in poor soils and in water stress conditions.

Title: The role of PRC2 in controlling T exhaustion during HIV infection
Principal Investigator: Dr. Renata de Meirelles Santos Pereira, Instituto de Microbiologia Professor Paulo de Góes, Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Rio de Janeiro, Brazil
ICGEB Reference No. CRP/BRA16-05_EC
Abstract:
Contrary to the tendency of decrease of new cases of the disease worldwide, the number of new cases of AIDS in Brazil increased of 11% between 2005 and 2013 (UNAIDS). A very serious problem that compromises the control of HIV is the fact the T cells are impacted by their overstimulation by antigens or inflammatory stimuli, which leads them to a state of cell exhaustion and unresponsiveness. PRC2 is a very important complex responsible for epigenetic modification in eukaryotes. Based on previous data, we hypothesise that PRC2 complex is important for CD8 T cells activation and function and counterbalances exhaustion during viral infection. Our aims are to investigate the function of PRC2 proteins in T CD8 cells function and to investigate if PRC2 member levels are reduced in HIV patients (as they are in mice during LCMV chronic infection), checking if the overexpression of these proteins can revert the T cell exhaustion phenotype.

Title: Dynamic changes in replication complex
Principal Investigator: Dr. Marina Nedelcheva-Veleva, "Acad. Roumen Tsanev" Institute of Molecular Biology of the Bulgarian Academy of Sciences, Sofia, Bulgaria
ICGEB Reference No. CRP/BGR16-03
Abstract:
One of the most important tasks of the living cell is to duplicate its genome and propagate. When there is an obstacle for correct replication, the genome stability is altered and genetic disorders or cancer can appear. That is why many proteins from the replication complex are under tight control in order to precisely execute the dynamic program of the replication machinery. This project proposal is focused on the study of the control over a key protein from the replication complex - Ctf4, in different organisms. It aims to find out whether Ctf4 has a constant presence at the replication complex, or is purposely destabilised or detached in order to perform a specific role related to the dynamics of the replication process. It questions whether the dynamics of Ctf4 follows a similar pattern as that of the replicative polymerases, helicase and other, harmonising their motion proteins in higher eukaryotes.

Title: Autophagy, primary cilium and inflammation in neurons and astrocytes

Principal Investigator: Dr. Eugenia Morselli, Pontificia Universidad Católica de Chile, Departamento de Fisiologia, Santiago, Chile

ICGEB Reference No. CRP/CHL16-06

Abstract: Large portions of the population eat high fat diets (HFD) rich in unhealthy saturated fatty acids and poor in healthy polyunsaturated fatty acids. Consumption of HFD promotes obesity and associated diseases. The hypothalamus, the brain area that controls the amount of food we eat, is inflamed in obese people that consume HFD. When inflamed, the hypothalamus does not function properly promoting obesity. "Autophagy" is a process of recycle of intracellular material and it is modified by HFD. Consumption of HFD inhibits hypothalamic autophagy, driving inflammation and obesity. Autophagy regulates the activity of the primary cilium, a cell surface structure that controls different cellular functions. The objective of this project is to understand how eating HFD affects the activity of different hypothalamic cells, namely neurons and astrocytes. Using these cells and mouse models we will study how fatty acids modulate autophagy and regulate inflammation via the primary cilium driving metabolic dysfunctions.

Title: Identification of novel hypoxia induced radioresistance signalling for endemic nasopharyngeal carcinoma

Principal Investigator: Dr. XiangBo Wan, the Sixth Affiliated Hospital, Sun Yat-sen University, GuangZhou, China

ICGEB Reference No. CRP/CHN16-04_EC

Abstract: We had shown that hypoxia induced factor 1α (HIF-1α) induced nasopharyngeal carcinoma (NPC) radioresistance through Aurora-A--apoptosis or Beclin 1--autophagy pathways. Importantly, we also found that blocking these two pathways could partially sensitise the radioresistant NPC cell to irradiation, suggesting other novel signalling should be involved. To identify this signalling, we infected NPC cell with human genome CRISPR/Cas 9 Knockout (KO) Library Plasmid packaged lentivirus. In this way, only one gene was knocked out in single cell, however, the human genome 19276 genes were KO in the pooled NPC cells. Through screening whole genome KO pooled cell, we found that HIF-1α--nuclear EGFR--NONO--DNA repair signalling should be the key pathway to induce NPC radioresistance; targeting this signalling might be a promising way to sensitize NPC to radiotherapy. In this study we will elucidate the molecular mechanism between this novel pathway and radioresistence and address the potential to target this signalling to enhance NPC radiosensitivity.

Title: Regulation of adaptive immunity during a Brucellosis infection

Principal Investigator: Prof. Elías Barquero-Calvo, Research Center for Tropical Diseases (CIET), Faculty of Microbiology, University of Costa Rica, San José, Costa Rica

ICGEB Reference No. CRP/CRI16-02

Abstract: Cells, named neutrophils that ingest, and serum proteins that kill, are in the first line of defence against Brucellosis, a dangerous bacterial disease of domestic animals and humans caused by *Brucella* microbes. In spite of their function in the defence and in the immune response, very little is known of how neutrophils and serum proteins combat long lasting infections such as Brucellosis. To understand this, we propose to investigate both how *Brucella* resists the killing action of neutrophils and serum proteins and also the regulatory events that occur during the immune response. Our objectives are: (i) to determine the mechanisms by which *Brucella* interacts and resists the killing action of these elements; (ii) to determine the function of these elements in the modulation of host defences; (iii) to develop a model for exploring the role of infected neutrophils as vehicles for the dispersion of *Brucella* in the different organs.

Title: Biological factors determining Human Papillomavirus (HPV) driven carcinogenesis

Principal Investigator: Dr. Vjekoslav Tomaić, Ruder Bošković Institute, Zagreb, Croatia

ICGEB Reference No. CRP/HRV16-05_EC

Abstract: The first aim is to establish immortalised cell cultures of human keratinocytes from tonsils and from human foreskin keratinocytes (HFK) harbouring HPV-16 genomes. This will allow us to follow the progression of malignant potential over time in these two tissues and determine if there is a tendency for HPV-16 to initiate tumour progression more quickly in head-and-neck (HN) derived keratinocytes than in those derived from anogenital keratinocytes. This will also allow us to investigate potential novel prognostic markers associated with the development of HN and anogenital cancers. The second aim of our study is to perform proteomic analyses for E6 and E7 interacting partners in the tonsilar keratinocytes and to do comparative analyses with HFKs. This will allow us (i) to determine whether the target proteins of the virus oncoproteins are the same in cells from diverse tissue types and (ii) to identify potentially novel therapeutic targets of E6/E7 in HN and anogenital cancers.

Title: Phytochemical and anti-tumour activity of two endemic species of Lecythidaceae in Ecuador: *Grias neuberthii* and *G. peruviana*

Principal Investigator: Dr. Natalia Catalina Bailon-Moscoso, Department of Health Sciences, Universidad Técnica Particular de Loja, Loja, Ecuador

ICGEB Reference No. CRP/ECU16-01_EC

Abstract: The general objective of our study is to determine anti-tumour activity of extracts and secondary metabolites of *Grias neuberthii* and *Grias peruviana*, both endemic species of Ecuador. The specific objectives of the research are as follows: (i) to isolate and provide chemical characterisation of the secondary metabolites isolate of *G.*

neuberthi and *G. peruviana* with cytotoxicity activity in cancer cell lines; (ii) to understand the molecular mechanisms underlying the apoptotic and autophagic effect of extract and secondary metabolites isolate of *G. neuberthi* and *G. peruviana* and the modulation of p53 family members (p53, p63 and p73), which are well-known regulators of cell proliferation, cell cycle arrest, cell survival, cell death, cell metabolism, and autophagy.

Title: Exosomes in the inflammatory intestinal stem cell niche
Principal Investigator: Dr. Zoltán Wiener, Semmelweis University, Department of Genetics, Cell and Immunobiology, Budapest, Hungary
ICGEB Reference No. CRP/HUN16-04_EC
Abstract: Inflammatory bowel disease (IBD) has an increasing incidence in Hungary. The chronic inflammation associated with IBD critically affects the regenerating capacity of the intestinal epithelium. This is fuelled by stem cells, residing in a specific microenvironment that is established by the surrounding other cells, such as fibroblasts. The recently discovered extracellular vesicles (EVs) are small vesicles enclosed by biological membranes, representing a novel cell-cell communication mechanism. In this project we will test the hypothesis that fibroblast-derived exosomes, representing a special EV subpopulation, have a central role in (i) the formation of stem cell niche and (ii) in modifying the effects of inflammation on the intestinal epithelium, focusing on stem cells. Thus, the project aims at understanding how the specific microenvironment for intestinal stem cells is formed, which has an outstanding importance in the identification of novel intervention possibilities to regulate stem cell functions in processes like epithelial regeneration under inflammation.

Title: Bioprospecting Mauritian phytomedicines for anticancer leads
Principal Investigator: Dr. Joyce Govinden Soulange, University of Mauritius, Reduit, Mauritius
ICGEB Reference No. CRP/MUS16-01
Abstract: Mauritius Island is considered to be one of the famous biodiversity hotspots with an indigenous flora of 60-65%. The people of Mauritius use ethno-medicine for treating diseases, minor ailments and other diseases including diabetes, kidney stones, pain and gastric disorders. In Mauritius cancer has become the third major health threat after diabetes and cardiovascular diseases. The search for new compounds with mode of actions better than the existing ones in the treatment of cancer is essential. Drugs currently used to treat cancer are derived from natural products such as terpenes, which have been detected in some endemic plants of Mauritius. In this study, we propose to screen two plant families (*Asteraceae* and *Sapotaceae*) for their pharmacological potential with the aims of detecting promising plants for the identification of anticancer leads. Moreover the phylogenetic relationships among members of these plant families will be reviewed and linked with their respective bioactivity.

Title: Metabolic regulation of chromatin dynamics in obesity
Principal Investigator: Dr. Lorena Aguilar Arnal, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Ciudad de México, Mexico
ICGEB Reference No. CRP/MEX16-05_EC
Abstract: Metabolic diseases and obesity are emerging as a XXI century epidemic, promoted by modern society lifestyles, including unhealthy intake of high calorie content meals and dysregulation of biological rhythms. The molecular mechanisms underlying these processes are the subject of study in this proposal. Precisely, we want to investigate how chromatin is influenced by cellular metabolism to determine expression of circadian genes, which are crucial for the maintenance of metabolic homeostasis. Spatial positioning of genes inside of the nucleus is determinant of its transcriptional state. Here we will assess a potential role for metabolic cues in relocating circadian genes in the nuclear space, thereby influencing its regulation and transcriptional levels. For these studies, we will use a diet induced obesity (DIO) mouse model combined with cell culture, microscopy and molecular biology techniques.

Title: Establishment of a laboratory facility for the diagnosis and surveillance of Arbovirus infections in Nigeria
Principal Investigator: Prof. Marycelin Baba, University of Maiduguri Teaching Hospital, Maiduguri, Borno State, Nigeria
ICGEB Reference No. CRP/NGA16-03
Abstract: Arboviruses are pathogens delivered by insect vectors such as mosquitoes, ticks and flies. Some of these pathogens are most widespread world-wide with serious socio-economic impact in both humans and animals. Examples of such pathogens include Dengue viruses, Yellow fever virus and Zika virus, which is currently causing outbreak in Latin America. Early symptoms of arbovirus infections are often confused with other common diseases in Africa such as malaria or typhoid fever, leading to inappropriate use of anti-malarial drugs and antibiotics. However complications, which depend on the pathogen, could lead to haemorrhagic bleeding, encephalitis and microcephaly in newborns. Differential diagnosis of febrile illnesses for arboviruses in Nigeria has remained a mirage over several decades, despite evidence of the circulation of these pathogens in the country. Therefore, the need to enhance systematic surveillance for arboviruses in Nigeria as an early warning mechanism against any pending outbreaks marked the decision for this project.

Title: SYK combinatory treatments against brain cancer
Principal Investigator: Dr. Gerald Moncayo, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología, Clayton, Ciudad del Saber, Panama

ICGEB Reference No. CRP/PAN16-01_EC

Abstract: The immune system and cancer are highly interrelated. Initially the immune system has anti-tumour function, eliminating most malignant cells before tumour formation; however, when the tumour is established the immune system may promote tumour growth. It is well known that cancers are highly infiltrated with immune cells. Nevertheless, we have shown that some of the immune response signatures found in tumours are derived from the tumour cells themselves. In addition, inhibiting a signalling molecule called SYK found downstream of many immune receptors, had a strong anti-migratory effect on tumours. However, while this treatment did have an effect on tumour progression it did not cure the mice. We will, therefore, test if combining other treatments such as phagocyte depletion, vimentin inhibition and immune checkpoint blockade with SYK inhibition will enhance the response and positively affect brain tumour progression.

Title: Molecular mechanism of *T. cruzi* virulence factors

Principal Investigator: Dr. Juan Manuel Lopez Smith, Pontificia Universidad Católica del Perú, San Miguel, Lima, Peru

ICGEB Reference No. CRP/PER16-05_EC

Abstract: Chagas is a trypanosomiasis disease inflicted by *T. cruzi* parasite. In Latin America, at least 10 million people are infected and 10,000 die annually. In order to infect its host, the parasite secretes several virulence factors. We propose to study the molecular mechanism of *T. cruzi* virulence factors, such as TcMIP (Macrophage Infectivity Potentiator) and the prolyl oligo-peptidase Tc80, involved in the transmigration of the parasite through the extracellular matrix. A better understanding of the molecular mechanism, by which these two macromolecules facilitate the penetration and dissemination process, could contribute to the exploration of new chemotherapeutic approaches to combat the ailment.

Title: Mesenchymal stem cells and myxoma virus in oncolytic melanoma therapy

Principal Investigator: Dr. Joanna Jazowiecka-Rakus, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland

ICGEB Reference No. CRP/POL16-02_EC

Abstract: Our project verifies a novel therapy responding to the need for an efficient and less expensive treatment of metastatic melanoma. It is based on oncolytic action of a virus encapsulated in bone marrow-derived mesenchymal stem cells (MSC). Oncolysis (tumour-destruction) will be achieved by myxoma virus (MYXV), genetically engineered to produce a fusion protein (interfeukin 15 and part of its receptor) and triggering strong immune response, which enhances tumour cells killing. MYXV is not pathogenic to humans and it destroys human cancer cells leaving normal tissues intact. "Naked" MYXV construct administered intravenously might, however, undergo elimination by the host immune defences. Therefore, the therapeutic MYXV will be encapsulated ("hidden") in MSC cells. They show tropism towards tumour and shield the therapeutic in transit thus improving, upon release, its oncolytic action. This "Trojan horse" therapy would consist of administering MSC cells loaded with MYXV therapeutic construct to mice bearing experimental metastatic melanoma tumours.

Title: Iba-1 (+) macrophages contribution to peripheral neuropathic pain development

Principal Investigator: Dr. Violeta Ristoiu, University of Bucharest, Faculty of Biology, Department of Anatomy, Physiology and Biophysics, Bucharest, Romania

ICGEB Reference No. CRP/ROU16-04

Abstract: Lesion of the peripheral nervous system triggers activation of Iba-1 (+) endogenous macrophages at the dorsal root ganglia (DRG) level, which presumably sensitise neurons and subsequently contributes to neuropathic pain. Iba-1, a cytoskeleton protein involved in cell mobility and phagocytosis, is a specific marker for macrophages and microglia, which seems to be crucial for their survival and pro-inflammatory activity. In preliminary studies we have shown that Iba-1 mRNA can be silenced using siRNA delivered intrathecally, and that its silencing has analgesic effects by interfering with Iba (+) macrophages in the DRG. In this project we aim to investigate if the spinal nerve ligation-induced neuropathic pain can be reduced by intra-ganglionic delivery of Iba-1 siRNA possibly by altering the activation profile of the macrophages, and to explore if communication between Iba-1 (+) macrophages and the surrounding cells (neurons or satellite cells) is mediated by tight contacts or ATP-activated P2 receptors.

Title: New synergistic strategy to treat chronic wound infections

Principal Investigator: Dr. Lidija Senerovic, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

ICGEB Reference No. CRP/SRB16-02

Abstract: Chronic wounds (e.g., diabetic foot ulcers, decubiti, etc.) are one of the major health care problems nowadays. There are no effective therapies to prevent chronic wound formation and only partially effective treatments are available to help their healing. Delay in wound healing is usually caused by biofilm-forming antibiotic-resistant bacterial infections. Our project aims to discover anti-infective drug candidates targeting bacterial virulence instead of viability. The new drugs should exhibit anti-biofilm activity with low potential to develop resistance. Therefore, we will explore soil bacteria diversity for structurally new molecules and, based on structure-activity studies, derivatise selected anti-virulence drugs to improve their performance. The best candidates will be conjugated with novel two-component multifunctional nano-carriers to improve their therapeutic index and enable simultaneous bacterial biofilm disruption and clearance of deep tissue infection. This synergistic strategy is expected to provide effective therapy of difficult-to-treat chronic wound infections, thus helping their timely healing.

Title: Cell-specific naked gene delivery mediated by DNA/RNA aptamers

Principal Investigator: Dr. Martin Panigaj, Institute of Biology and Ecology, P.J. Safarik University in Kosice, Faculty of Science, Kosice, Slovakia

ICGEB Reference No. CRP/SVK16-01_EC

Abstract: Delivery of a correct gene to sick cells has a great potential to treat diseases caused by missing or improperly working genes. However, selective gene delivery to such cells is challenging. The classic approach using engineered viruses is demanding on the safety and production. Recently, nucleic acid-based aptamers selected to bind relevant cell-surface proteins were shown to be effective cell-type-specific drug carriers. This inspired us to propose that aptamers can also be a novel and simple tool to bring beneficial genes to appropriate cells. In this project we want to find an optimal way to link aptamers with a nucleic acid strand encoding a gene and to test the ability of our system to provide effective but safe gene delivery. The advantage of these suggested aptamer-gene particles is that their nucleic acid-based composition significantly reduces production costs and allows easy assembly of aptamers with genes adapted towards user need.

Title: Lysosomal proteases in semaphorin signalling and cell polarity

Principal Investigator: Prof. Boris Turk, Department of Biochemistry and Molecular and Structural Biology, Institute Jozef Stefan, Ljubljana, Slovenia

ICGEB Reference No. CRP/SVN16-01

Abstract: Cancer is one of the major health problems worldwide. Among factors, which have major roles in cancer progression, are proteases. It has been demonstrated that gene ablation and pharmacological inhibition of cysteine proteases, in particular lysosomal cysteine cathepsins, have dramatically reduced tumour growth and metastasis spread, validating the cathepsins as promising anticancer targets. However, the molecular signalling pathways they utilise to promote cancer progression are still unclear. The major aim of the project is, therefore, to unravel the role of endolysosomal cysteine proteases, secreted from the immune or cancer cells, in semaphorin signalling and cell polarity, two processes known to largely contribute to cancer progression. More specifically, we will focus on cathepsin K and legumain, and partially to cathepsins S and L, and their transmembrane substrates affecting semaphorin signalling and cell polarity, thereby identifying molecular mechanisms underlying cancer cell migration and invasion mediated by the extracellular cysteine proteases.

Title: Genetic contributors to Diabetes Mellitus in Indo-Trinidadians

Principal Investigator: Dr. Rajini Haraksingh, The University of the West Indies, St. Augustine, Trinidad and Tobago

ICGEB Reference No. CRP/TTO16-04_EC

Abstract: Trinidad and Tobago has a high prevalence of Diabetes Mellitus (DM) and the Indo-Trinidadian subpopulation, comprising ~35% of the population, is particularly prone to this disease. However, the underlying genetic causes of DM in this population have never been investigated. This study aims to understand the genetic causes of the forms of diabetes that are attributed to mutations in a single gene in the Indo-Trinidadian population. Two thousand DM patients would be screened to identify ~20 families where diabetes is inherited in a straightforward fashion. All ~35,000 genes of 2-3 members of each family would be searched to identify the genetic cause of the disease and this will be confirmed in the remaining family members. The resulting causative genetic mutations, whether known or novel, could be used for genetic screening for early detection of DM in this population. This will be the first investigation into the genetic architecture of this unique population.

Title: Wheat eTMs and roles on miRNA activity under drought stress

Principal Investigator: Dr. Turgay Unver, Izmir International Biomedicine and Genome Institute (iBG-izmir), Dokuz Eylül University, Izmir, Turkey

ICGEB Reference No. CRP/TUR16-03

Abstract: MicroRNAs (miRNA) are small, non-coding RNAs that regulate expression of specific target genes at post-transcriptional level. Plant miRNAs are known to function in a diverse set of biological processes including response to abiotic stresses such as drought. As of a defence system, plants may reprogram expression of certain genes by miRNAs upon drought stress. A recently discovered mechanism revealed that miRNA activity is controlled by another class of RNAs known as eTMs (endogenous target mimics). The aim of this project is the identification of eTMs and experimental validation of miRNA:eTM network in different tissues of a drought-resistant bread wheat cultivar after drought application. For this purpose, small RNA sequencing will be performed and the data will be analysed together with transcriptome data of the same tissues/conditions obtained in a TUBITAK Project (113O016) to explain the role of eTMs in regulation of miRNA activity and these eTMs will be correlated to drought stress.