ICGEB International Centre for Genetic Engineering and Biotechnology | Developing Knowledge

ICGEB Research Grants awarded under the 2018 Call for applications

Title: Genomic reprogramming of bacterial growth
Principal Investigator: Dr. Alfonso Soler Bistue, Instituto de Investigaciones Biotecnologicas Dr. Rodolfo Ugalde, Universidad de San Martín, San Martín, Buenos Aires, Argentina
ICGEB Reference No. CRP/ARG18-06_EC
Abstract: Growth rate (GR) is a key measurement that reflects bacterial fitness and competitiveness, and the genetic factors that encode it are still unknown. Genome comparisons have shown that GR can be influenced by the number of rRNA operons (*rrn*) present and by the proximity of transcription and translation-related genes to the bacterial replication origin. This project will use genome-editing tools to alter both the genomic location of transcription and translation genes, and the number of rrns to attempt to reprogram bacterial GRs. *Vibrio cholerae*, the causative agent of cholera, normally divides every 17 minutes. We propose to slow this GR, while, in parallel, we accelerate the GR of the very slow-growing bacteria of the Bradyrhizobium genus. These are plant growth-promoting bacteria, which normally have a generation time of 20 hours. We propose to obtain fast-growing Bradyrhizobium derivatives that could have potential biotechnological applications. Our project will help to understand how genome organisation contributes to cell physiology, knowledge that will be essential in the context of developing artificial life forms.

Title: Understanding the incomplete extravillous trophoblast invasion in pregnancies complicated with severe and non-severe preeclampsia
Principal Investigator: Dr. Violeta Soljic, Department of Histology and Embriology, School of Medicine University of Mostar, Bosnia and Herzegovina
ICGEB Reference No. CRP/BIH18-03
Abstract: The fetal extravillous trophoblast (EVT), is a part of the placenta that invades maternal uterine tissues to allow transfer of oxygen and nutrients to the growing fetus. The capacity of the EVT to help balance the maternal immune response, permitting tolerance of the fetus while providing immune protection against infection, is important for adequate placentation. In preeclampsia (PE), both these mechanisms are deregulated. This research aims to test correlations between low invasive abilities and high apopototic potential of EVT cells, with their low expression of oncofetal RNA binding proteins IMP3 (Insulin-like growth factor 2 mRNA-binding protein 3, IGF2BP3) and Lin28 (lin-28 homolog A). We expect to see a decreased number of decidual CD8+ T cells that exhibit suppressor activity, which could be related to immunological disorders described in PE at the fetal-maternal interface.

Title: Investigation on the origin of the morphological pattern generated in Zika virus-derived brain malformations
Principal Investigator: Dr. Eduardo Sequerra, Brain Institute, Morro Branco, Natal, Brazil
ICGEB Reference No. CRP/BRA18-05_EC
Abstract: This project concentrates on testing hypotheses to explain the brain pattern seen in ZIKV-derived microcephalic children, where the anterior (frontal) brain is affected while caudal structures (the hind-brain-spinal cord) are preserved. In aim 1, we test the hypothesis that the virus uses the olfactory pathway to enter the brain. To test it, we are going to inject ZIKV into the amniotic fluid of developing mice while ablating the olfactory epithelium, to try to prevent brain infection. In aim 2, we will test if ZIKV activates the maternal immune reaction against the anterior fetal brain. To do this, we will react antibodies from the mothers of microcephalic patients against slices of the developing human brain, and also inject the antibodies into the brains of mouse embryos. For aim 3, we intend to characterise these experiments as animal models for ZIKV-derived epilepsies, using

electrophysiological techniques. With these strategies, we hope to contribute to the knowledge of how ZIKV-derived malformations and associated pathologies form.

Title: Transcriptional networks for mitochondrial dynamics related to human cardiomyocyte dysfunction
Principal Investigator: Dr. Valentina Parra, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Región Metropolitana, Chile
ICGEB Reference No. CRP/CHL18-04
Abstract: Heart failure (HF) occurs when the heart is unable to deliver sufficient blood to the body. It is a complex and expensive disease that progresses even when treated with the best currently available therapies. Although diverse in origin, a common feature of all HF is impaired mitochondrial function. Mitochondria, the organelles that generate ATP, are critical to maintain a normal heart function because of the heart's high demand for energy. The structure of mitochondria impacts their efficiency and can undergo continuous change through a series of processes often termed "mitochondrial dynamics". Our project's objective is to understand the impact of mitochondrial dynamics on nuclear gene expression, and vice versa, in human cardiac cells. Identifying transcriptional regulatory networks linked to cardiac mitochondrial dynamics will help us to better understand this fundamental aspect of heart disease and ultimately aid in the development of new pharmacological targets for the treatment of HF.

Title: Development of microbial-mediated RNA interference as a potential insect control method of the coffee berry borer, *Hypothenemus hampei* (Coleoptera)
Principal Investigator: Dr. Lucio Navarro Escalante, National Coffee Research Center (CENICAFE), Manizales, Caldas, Colombia
ICGEB Reference No. CRP/COL18-04_EC
Abstract: Among several biological factors limiting coffee production worldwide, the coffee berry borer (CBB) is the most relevant pest insect, especially for Colombia. Despite the use of current insect control methods, there is a growing need for development of novel strategies to control the CBB. This research proposal aims to develop a new potential method for this purpose. We propose to use bacteria present in the gut of the CBB as a mechanism to deliver toxic molecules that interfere with vital physiological processes of the insect. As a proof-of-concept, we will first develop the methodological conditions to use a common experimental bacteria to deliver these molecules in the CBB gut, and evaluate their capacity to negatively affect or kill the insect. In parallel, we will also identify bacteria species present in the CBB gut that would be suitable for the genetic manipulations necessary to deliver toxic molecules and cause insect mortality as a potential insect control strategy.

Title: Development and validation of a genetically encoded fluorescent reporter for Dengue and Zika virus NS2B-NS3 protease activity and its application to explore the phenotypic diversity of viral infection in terms of replication, cell death induction and antiviral drug response
Principal Investigator: Prof. Rodrigo Mora Rodríguez, Centro de Investigación en Enfermedades Tropical (CIET), Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica
ICGEB Reference No. CRP/CRI18-02
Abstract: Dengue and Zika virus are responsible for important human diseases including severe haemorrhagic cases and congenital defects. Currently, there is no drug available to treat these infections and the discovery of new drugs is limited by the lack of sensors able to report how these viruses replicate and kill the host cells. A sensor is a system that turns the cells bright once the virus infects them. This sensor is placed in the cells, turning them into great tools to detect the infection of these viruses in the lab, and it could also be placed in animals to study how the virus affects the entire organism. We propose to develop this sensor system and test it in cells infected with different types of Dengue and Zika virus, to study the variety of cellular effects and assess the potential of this system for antiviral drug development.

Title: Genetic screening approaches to investigate cellular mechanisms that protect against DNA damage and associated diseases
Principal Investigator: Dr. Menattallah Elserafy, Center for Genomics, Zewail City of Science and Technology, 6th of October City, Giza, Egypt
ICGEB Reference No. CRP/EGY18-05_EC
Abstract: When cells of living organisms suffer DNA damage, it must be rapidly repaired otherwise the organism has to face a lot of negative consequences. The most common type of DNA damage is misincorporation of ribonucleotides (rNTPs) into the DNA, which should consist only of deoxyribonucleotides (dNTPs). We are particularly interested in the mechanisms that prevent and repair ribonucleotide incorporation in the genome to avoid the development of associated diseases.
One of the autoimmune diseases associated with defects in rNTP misincorporation repair is systemic lupus erythematosus: an incurable disease affecting children and adults in the world and in Egypt. We aim to identify new players/genes in rNTP misincorporation repair, using screening methods and a variety of molecular biology techniques. Finally, we will analyse systemic lupus erythematosus samples to test whether the identified genes are mutated in the patients. Our findings will open doors for improving diagnostics and developing personalised therapies for this incurable disease.

Title: Generating blast resistant Finger Millet plants through gene-specific genome editing
Principal Investigator: Dr. Wilton Mwema Mbinda, Department of Biochemistry and Biotechnology, Pwani University, Kilifi, Kenya
ICGEB Reference No. CRP/KEN18-01
Abstract: Finger millet is an important stable cereal crop for millions of people in the semi-arid tropics of East Africa, playing an important role in the economy of subsistence farmers in the region. Blast disease caused by *Magnaporthe oryzae* is one of the most destructive diseases, causing serious damage to global finger millet production. The adoption of host resistance has proven to be the most economical and effective approach to control finger millet blast. In recent years, sequence-specific nuclease technologies have been demonstrated to be powerful tools for the improvement of crops via gene-specific genome editing, and the clustered regularly interspersed short palindromic repeats (CRISPR)/Cas system has emerged as the most effective sequence-specific nuclease tool. This project is designed to improve finger millet blast resistance by engineering a CRISPR/Cas9 targeting the ethylene response factor transcription factor gene in finger millet. The developed system should be adaptable to other grass crop improvements.

Title: Vagus nerve stimulation: Investigation on cholinergic anti-inflammatory activity and macrophage polarisation in rats with induced myocardial infarction
Principal Investigator: Dr. Mohd Kaisan Bin Mahadi, Faculty of Pharmacy, Universiti Kebangsaan Maalaysia, Kuala Lumpur, Malaysia
ICGEB Reference No. CRP/MYS18-04_EC
Abstract: One of the contributing factors for heart failure is cardiac tissue injury resulting from restricted blood supply, which invokes activation of local immune responses. Active inflammation in the injured cardiovascular region may spread locally and progress to adverse cardiac remodelling, leading to inefficient cardiac pumping. We propose a study to examine the novel cardio-protective mechanism of non-invasive nerve stimulation at the ear through inhibition of active inflammatory responses in ischemic-induced rats. The activated anti-inflammatory pathway will be identified using pharmacological ablation of the nicotinic receptor. This receptor, primarily located in the central nervous system, may also be present in the macrophage, an activated immune cell. The genetic subtype of macrophages in the injured cardiovascular region will be quantified, with and without ear stimulation. The findings of our study are expected to elucidate the mechanism of cardio protection by auricular neurostimulation through anti-inflammatory action, which could potentially be used as an inexpensive management procedure for heart failure patients.

Title: Unraveling the molecular therapeutic mechanisms of resveratrol rescue-driven in *Drosophila melanogaster* models of Parkinson's disease
Principal Investigator: Dr. Amos Abolaji, Drosophila Laboratory, Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Nigeria
ICGEB Reference No. CRP/NGA18-02
Abstract: Parkinson's disease (PD) affects the nerves in the brain that produce dopamine, and can lead to death. Search is currently ongoing for cheap and effective compounds from plants to treat PD. This is because conventional drugs employed to treat PD patients do not completely cure the disease. Resveratrol is a compound found in grapes and several other plants. Its beneficial role has been reported in alleviating PD symptoms in animals, including *Drosophila melanogaster* (fruit flies). A preliminary study in our laboratory showed that resveratrol restored different antioxidant enzymes in chemical-induced PD in the flies. In this study, four different types of fruit flies that have been genetically modified to exhibit PD symptoms will be exposed to diets supplemented with resveratrol. Thereafter, several markers of PD such as the antioxidants will be evaluated. We will also carry out studies on the neurons of the flies using standard protocols to identify the genes affected by resveratrol supplementation in the flies. This information will help to unravel the molecular therapeutic mechanisms of resveratrol, which will aid in the discovery of a treatment regimen for PD.

Title: QM/MM Approach for remodeling of biocatalytic specifity
Principal Investigator: Dr. Alexander Gabibov, Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia
ICGEB Reference No. CRP/RUS18-01
Abstract: The design of novel biocatalysts has become an important fundamental and biotech goal in the Life Sciences. Previously, there was only a limited number of approaches for improving catalytic efficiency and re-programming catalytic specificity. Most of these were realised by the rational design of mutants, based on refined 3D structures. Screening representative libraries of enzyme active sites and immunoglobulin combining sites may give an extraordinary opportunity to generate improved biocatalysts. In order to enhance this approach, we developed the screening of virtual libraries, using a supercomputer powered by QM/MM simulation of catalytic chemical transformation. We propose to generate *de novo* catalytic bioscavengers directed toward organophosphorus compounds, with modified specificity based on the butyrylcholinesterase and Ig active sites. We will estimate optimal variants of these templates using QM/MM predictions, and will change the stereospecificity of the catalyst and improve its catalytic properties. We will attempt to arrange the catalytic re-programming of selected reaction.

Title: The tryptophan kynurenine pathway-theraputic strategy for neuroprotection in tauopathies
Principal Investigator: Dr. Andrej Kovac, Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia
ICGEB Reference No. CRP/SVK18-01

Abstract: Tauopathies are a group of progressive neurodegenerative disorders. Current studies indicate the importance of the tryptophan/kynurenine pathway in the pathology of neurodegenerative disorders, including tauopathies. Under physiological conditions, the amino acid tryptophan is metabolised in the brain to form neuroprotective kynurenic acid. In the process of neurodegeneration or neuroinflammation, this pathway is disturbed and tryptophan is metabolised to form neurotoxic quinolinic acid. The main aim of our project is to analyse how administration of a synthetic analog of kynurenic acid will affect the metabolism of tryptophan and thus modify the process of neurodegeneration in an animal model for tauopathies. These findings will help us to better understand the role of tryptophan metabolism in neurodegeneration, especially its role in tauopathy progression. Our results from tau transgenic models could contribute to the development of new therapeutic approaches in neurodegenerative diseases that are so desperately needed in clinical practice now.

Title: Sonogenetics: ultrasound-inducible transcriptional regulation
Principal Investigator: Dr. Iva Hafner Bratkovic, National Institute of Chemistry, Ljubljana, Slovenia
ICGEB Reference No. CRP/SVN18-01
Abstract: We will develop tools for empowering cells to specifically respond to ultrasound stimulation. Such tools will replace optogenetics for stimulation in deep tissue, since ultrasound, unlike light, can penetrate tissue. Ultrasound has been used for decades in diagnostics without adverse side effects and can be focused in the deep tissue. We will first identify the mechano-transduction channels, which increase the sensitivity of mammalian cells to ultrasound. Next, ultrasound stimulation will be coupled to a designed signal transduction pathway, and finally tested *in vivo*. The main aim of the project is to develop a synthetic biology toolkit, which will allow gene transcription to be turned on or inhibited in ultrasound-stimulated cells. The project will provide an important advance in the construction of ultrasound-responsive cellular devices that could have an impact on medicine and life sciences and open many avenues for heterogeneous research and therapy.

Title: The role of insect gut microbial communities in overcoming the chemical defences of host plants by the pine emperor moth *Nudaurelia cytherea*
Principal Investigator: Dr. Almuth Hammerbacher, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa
ICGEB Reference No. CRP/ZAF18-06
Abstract: Plants produce an impressive variety of chemical compounds to deter insects and mammals from eating them. Despite the presence of these harmful chemicals, insects occur on almost all plants and successfully complete their life cycle by feeding on them. In order to survive and thrive on plants, we hypothesise that insects form stable, as well as temporary, associations with yeasts and bacteria in their intestinal tract. The aim of this research is to study the diversity, population dynamics and metabolic activities of microorganisms in the intestinal tract of the South African pine emperor moth *Nudaurelia cytherea*, a large and colourful caterpillar with a voracious appetite for the foliage of pine, guava, apple, eucalyptus and many other tree species. Results from this study will reveal how insects adapt to different food sources by forming incidental associations with microbes from their environment, and how the pine emperor moth can be controlled without the use of pesticides.

Title: Genetic dissection of polyethylene degradation ability of *Perenniporia* sp. isolated from decaying hard woods in Sri Lanka
Principal Investigator: Dr. K.P. Renuka Nilmini Attanayake, Department of Botany, University of Kelaniya, Dalugama, Sri Lanka
ICGEB Reference No. CRP/LKA18-03
Abstract: Each year trillions of plastics bags are consumed and dumped in landfills, creating severe social and environmental problems worldwide. Traditional 3R concepts have failed in finding sustainable solutions, but microbial-assisted polyethylene biodegradation has gained recent attention among scientists. From our preliminary studies, it was found that *Perenniporia* spp. and another, as yet, unidentified genus isolated from decaying hard woods of Sri Lankan dry zone forests are consistently growing and creating microscopic holes in polyethylene sheets (20-microns) *in vitro*. However, the exact mechanism of degradation is not clear. This project aims to uncover the molecular basis of the polyethylene biodegradation process. The objectives of this project are to search for more fungal species that can grow on polyethylene and identify those to the species level, to identify which genes are responsible for the degradation, and to clone an efficient gene from *Perenniporia* to a bacterium. Our ultimate goal is to provide a sustainable solution for polyethylene accumulation worldwide.

Title: Inter-epidemic Transmission Dynamics of Rift Valley, Dengue and Chikungunya viruses in Tanzania
Principal Investigator: Dr. Jaffu Othniel Chilongola, Kilimanjaro Clinical Research Institute, Moshi, Tanzania
ICGEB Reference No. CRP/TZA18-04
Abstract: Arboviruses are a growing health threat globally and have been responsible for large epidemics in many sub-Saharan countries. The viruses cause focal epidemics with high case fatalities that are difficult to predict and control. The epidemics are usually associated with the changes in the distribution of the vector, the availability of a source of infection, and the presence of a susceptible vertebrate/human host.
In addition, for some arboviruses, outbreaks have been associated with particular lineages. The virus vectors are prevalent in many regions with no known history of previous transmission, creating the potential for future epidemics, since it is not known where the viruses hide during inter-epidemic periods. We aim to understand the Transmission Dynamics of Dengue (DENV), Chikungunya (CHIKV) and Rift Valley Fever (RVFV) viruses across their hosts in different seasons of the year. Such information will form the basis for targeted control of transmission between vertebrate and invertebrate hosts.

Title: Cetuximab-mediated molecular targeted cancer therapy through nanoceria

Principal Investigator: Dr. Hilal Yazici, TUBITAK-Marmara Research Center, Genetic Engineering and Biotechnology Institute, Gebze, Kocaeli, Turkey

ICGEB Reference No. CRP/TUR18-03

Abstract: Nanoparticle-based therapies and drug-delivery systems for anti-cancer agents, especially those functionalised with monoclonal antibodies (mAbs), have become an emerging field in cancer therapy. Nanoceria has enormous potential as an antioxidant and radioprotective agent for cancer applications. It has remarkable selectivity properties between cancerous and healthy cells that distinguish nanoceria from other chemotherapeutic agents. Due to the limitations of currently available nanoparticle delivery systems, there is a route shift not only applying new nanocarriers but also utilising mAbs as molecular targeted immunotherapy. Cetuximab, as a therapeutic monoclonal antibody, has been used for colorectal, lung, and head-and-neck cancer, with combination of other chemotherapeutic agents and radiotherapy respectively. The objective of this proposed research is to develop nanoparticle-based therapies and drug-delivery systems through functionalisation of dextran-coated nanoceria with Cetuximab. This system allows the enhancement of Cetuximab efficiency without any other chemotherapeutic agent, utilising only the antioxidant, radioprotective and selective properties of nanoceria for colorectal and lung cancer treatment.

Title: Novel mechanisms of inflammatory macrophage proliferation

Principal Investigator: Dr. Álvaro Díaz, Área Inmunología, Departamento de Biociencias, Facultad de Química, Universidad de la República, Montevideo, Uruguay

ICGEB Reference No. CRP/URY18-02

Abstract: Inflammation is necessary for defence against infections, but also a major factor in currently important diseases. An important cell type that usually accumulates in inflammation is the macrophage. Inflammatory macrophages usually arise from monocytes that arrive in tissues from the blood. However, it was recently discovered that macrophages can also accumulate because they proliferate (divide) locally. Macrophage proliferation is known to take place in important medical conditions with inflammatory backgrounds, namely atherosclerosis and obesity. Certain molecular signals that drive inflammatory macrophage proliferation are known, but these are ineffective alone *in vitro*, and the additional signal required is unknown. We have a hypothesis for the identity of this additional signal, backed by clear initial results. We propose to study this mechanism in-depth, to satisfactorily connect certain established elements of immunological knowledge that have not hitherto been connected. We also propose to validate this mechanism in models of atherosclerosis and obesity.

Title: Identification of target autoantigens for narcolepsy type I

Principal Investigator: Dr. Xuan-Hung Nguyen, Vinmec Research Institute of Stem Cell and Gene Technology (VRISG), Hanoi, Viet Nam

ICGEB Reference No. CRP/VNM18-05_EC

Abstract: Narcolepsy type I (NT1) is a chronic sleep disorder characterised by a selective loss of orexinergic neurons from the hypothalamus. Although the genetic and epidemiological evidence strongly supports an autoimmune basis for NT1, the exact autoimmune process underlying the selective neuronal destruction is largely unknown. Our long-term goal is to elucidate the immune mechanisms that trigger NT1, potentially leading to the development of new diagnostics and therapies for the disease. Our specific aims are: (i) to identify the potential autoantigens in NT1, using integrated approaches that couple phage-displayed random peptide library and human induced pluripotent stem cell (hiPSC) technology; and (ii) to identify the autoantigen-specific pathogenic T cells in NT1. Identification of the target autoantigen(s) will be a major breakthrough in the understanding of NT1 pathogenesis. The hiPSC technology developed from this study will set up a basis for promoting the hiPSC research and application in Vietnam.