ICGEB Research Grants awarded under the 2021 Call for applications

Title: Long noncoding RNAs as non-transgenic biotechnological tools for acclimatization of vegetable crops
Principal Investigator: Dr. Federico Ariel, Instituto de Agrobiotecnología del Litoral (IAL), Santa Fe, Argentina
ICGEB Reference No. CRP/ARG21-01
Abstract: The advent of novel sequencing technologies has revealed that eukaryotic genomes are almost entirely transcribed into RNAs, although only a small fraction can be translated into proteins. The so-called long noncoding RNAs (lncRNAs) are critical integrators of external and developmental signals, influencing the expression of all genes within the plant genome (i.e., the 'transcriptome') in the dynamic response to the environment. The increasing frequency at which temperature extremes occur is a cause for concern worldwide and has a major effect on agriculture. Thus, understanding the role of functional lncRNAs for plant adaptation appears to be of paramount importance. Using the tomato as a vegetable crop model, this project aims to decipher how lncRNAs can shape the transcriptome in response to heat; and our ultimate aim is to develop non-transgenic approaches to enhance stress tolerance through the delivery of exogenous bioactive RNAs. The knowledge acquired should help smallhold farmers to safeguard the production of local landrace crops in a climate change context.

Title: Development of low erucic acid containing oilseed *Brassica* varieties in Bangladesh through genome editing
Principal Investigator: Dr. Tahmina Islam, Plant Breeding and Biotechnology Laboratory, Department of Botany, University of Dhaka, Dhaka, Bangladesh
ICGEB Reference No. CRP/BGD21-04_EC
Abstract: Erucic acid is a mono-unsaturated omega-9 fatty acid, present in the oil-rich seeds of Brassicaceae family, particularly rapeseed and mustard. Modern cultivated Bangladeshi *B. rapa* varieties contain high levels of erucic acid (40-52%), which is believed to be unsafe for human consumption and to be associated with major health problems. Owing to their anti-nutritive and detrimental nature, it is desirable to decrease the oil erucic acid content. Therefore, we strive to reduce erucic acid content (0-2%) in mustard (*Brassica rapa* L.) by knocking out the genes involved in erucic acid biosynthesis, using the contemporary CRISPR/Cas approach. Successfully edited mustard varieties are expected to have a low erucic acid content without any other metabolic imbalance. The genetic and metabolic changes in the edited plants will be monitored by sequencing and estimating erucic acid content, respectively. This project will develop the foundations of CRISPR/Cas technology and enhance the capacity for its use in future research and development in Bangladesh.

Title: ZNF429: A potential critical regulatory transcription factor for pathways in cancer
Principal Investigator: Dr. Mariana Boroni, Brazilian National Cancer Institute (INCA), Rio de Janeiro, Brazil
ICGEB Reference No. CRP/BRA21-01
Abstract: This project will investigate an integrant of the KRAB-ZNF (Krüppel box-associated zinc finger) protein family, which is believed to exert transcriptional repression activity by recruiting chromatin modifiers, playing essential roles in the evolution and biology of most organisms. Specifically, this will be the first study of the epigenetic mechanisms underlying the gene expression regulation mediated by ZNF429, and the functional consequences associated with its expression in tumour cells. Although this gene appears to have a critical impact on the progression of different tumour types, it has never been functionally characterized before. Using a combination of *in vitro* and high-throughput assays, we will characterize its specific targets, the pathways and phenotypes associated, and how it correlates with the prognosis of

tumors; with a particular emphasis on ovarian cancer, which is the most deadly gynecological malignancy, with increasing numbers of cases, not only in Brazil, but also globally.

Title: Subtype-specific differences in HIV-1 Gag-protease and Nef Function of virus lineages circulating in Cameroon: the origin of the HIV-1 group M epidemic

Principal Investigator: Dr. Marcel Tongo Passo, Center for Research on Emerging and Re-Emerging Diseases (CREMER), Yaounde, Cameroon

ICGEB Reference No. CRP/CMR21-02

Abstract: Global HIV-1 group M (HIV-1M), genetic diversity and evolution form a major challenge to treatment and prevention efforts, which are essential for reaching the UNAIDS 90:90:90 targets. Cameroon is one of the countries in the world where this diversity is accumulating. It has an extraordinary and unevenly distributed amount of viral diversity, including some unusual and rare strains. Despite this broad spectrum of HIV-1 diversity, very little is known about the biological properties of the virus strains circulating in the country. In this project, we propose to perform comparative studies of HIV-1M biological functions, based on the variants circulating in Cameroon. Specifically, we will test the hypothesis that rare and unusual viruses may have decreased transmissibility compared with dominant lineages. Data from this study may help to explain differences in epidemic spread of HIV-1M subtypes and may have important implications for biomedical prevention and treatment strategies against HIV, both for the country and the world.

Title: The development of ultrasound based micro-array technology for high throughput screening

Principal Investigator: Dr. Liangfei Tian, Zhejiang University, Hangzhou, China

ICGEB Reference No. CRP/CHN21-04_EC

Abstract: Indexing is critical in experiments that require the identification and monitoring of hundreds to millions of samples, over a range of timescales and chemical/physical environments. Effective indexing allows the high-throughput screening of chemical/biochemical and cellular processes, and the digital measurement of rare events in a large sample. A straightforward approach to labelling such samples is to systematically arrange them into 2D micro-arrays, in which the addressability of each object can be indexed on the basis of one or more spatial variables. Based our previous investigations, the proposed research project aims to develop a new type of ultrasound-based 2D micro-array platform, capable of generating different micro-arrays and sustaining one or more constant concentration gradients of diffusible substances within the droplet array. The potential applications of this platform in the fields of chemical/biochemical analysis, cell screening and drug discoveries, as well as in bottom-up synthetic biology, will also be accordingly explored.

Title: Elucidating structural components of extracellular vesicles associated with inflammatory responses in patients with seropositive rheumatoid arthritis as potential therapeutic targets and biomarkers

Principal Investigator: Dr. Diana Castaño Monsalve, Universidad de Antioquia, Medellin, Antioquia, Colombia

ICGEB Reference No. CRP/COL21-01

Abstract: Rheumatoid arthritis (RA) is a common autoimmune disease in Colombia, with catastrophic implications for quality of life, and high costs for the health system. Patients with RA may be classified as seropositive or seronegative. Seropositive patients have more severe disease and higher levels of the cytokine TNF-α than seronegative patients. Blocking antibodies against TNF-α is a highly effective RA treatment; however, this therapy has been associated with several adverse effects. We propose that specific blood components that stimulate the production of TNF-α could be better candidates for therapeutic intervention than blocking the cytokine. Extracellular vesicles (EVs) from seropositive patients are potent inducers of TNF-α production by phagocytes. Characterizing the protein and lipid components of the EVs responsible for this response, and also determining the signaling pathways activated by EVs, could lead to the identification of more specific therapeutic targets with fewer side effects, and could also identify potential biomarkers for patients with RA.

Title: Anaerobic Digestate of Wastewater Sludge (ADWS) and microalgae-bacteria coculture as a zero-waste model for biofuel production

Principal Investigator: Dr. Eladl Eltanahy Eltanahy, Mansoura University, Mansoura, Egypt

ICGEB Reference No. CRP/EGY21-03

Abstract: This industrial biotechnology research project aims to optimize an zero-waste model for biofuel production, based on waste recycling. The anaerobic digestate of wastewater sludge contains high levels of ammonia and phosphate, and thus has potential as a nutrient medium for co-culturing microalgae and bacteria to enhance their lipid accumulation. The biomass produced could be used as a sustainable source of biodiesel, while the remaining defatted biomass can be used as a feedstock to induce the anaerobic digestion of wastewater sludge, using its carbohydrate content to enhance the production of biomethane. In this project we will bioprospect relevant bacterial and microalgal strains to identify the most effective combinations to use in this process. Also, transcriptomic analysis will be used to confirm the co-culture effect on genes

responsible for biodiesel induction, while microbiome analysis will reveal the changes in the microbial community during these processes. This model will be used commercially as an eco-friendly, cheap source of sustainable biofuels from wastes.

Title: Unlocking the secretes of low virulence of SARS-CoV-2 in Ethiopia by sequencing and Spike binding efficiency test

Principal Investigator: Dr. Molalegne Bitew, Ethiopian Biotechnology Institute, Addis Ababa, Ethiopia

ICGEB Reference No. CRP/ETH21-01*

Abstract: Since the beginning of 2020, COVID-19 has become a pandemic affecting more than 200 countries and territories in the world, infecting more than 130 million of people. One of the measurements to control the disease is early detection using RT-qPCR in tracing, testing, and treating in the community. Ethiopia confirmed its first case of COVID-19 on 13 March 2020 and two days later the WHO declared a pandemic of the disease. On 8 April 2020 the Ethiopian government declared a five-month state of emergency, but allowed economic activities to continue during the public health crisis. Currently, on 18 April 2021, the country tested 2,494,258 suspects, of whom 240,236 (9.6%) cases had been confirmed positive and of these, 3,328 (CFR = 1.38%) died and 178,705(74.38.15%) recovered. However, we don't know exactly which kind of strain is circulating in our country. It has been observed that the case fatality rate is still very low as compared with other parts of the world. This research project is formulated with the objectives of (i) sequencing and molecular characterization of Covid-19 causing SARS-CoV-2 virus circulating in Ethiopia; studying the SARS-CoV-2 genome variability occurred in Ethiopia using bioinformatics tool, (iii) evaluating the local SARS-CoV-2 virus Spike protein binding efficiency with the receptor and (iv) establishing the virus genomics and bioinformatics training in collaboration with scientists at ICGEB Trieste and EBTI.

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Title: From young to old and vice versa - Neuronal autophagy in human ageing

Principal Investigator: Dr. Karolina Milena Pircs, Semmelweis University, Budapest, Hungary

ICGEB Reference No. CRP/HUN21-05_EC

Abstract: With increasing life expectancy, incurable age-related diseases, like Alzheimer's disease, are a growing burden in our society. A better understanding of ageing is thus an utmost necessity. In this project, I will focus on autophagy and its relevance to ageing. Autophagy (meaning "eating itself") normally breaks down old, non-functioning, or harmful proteins inside the cells. However, in ageing and in age-related diseases autophagy does not function normally. The goal of this project is to understand what aspects of autophagy are changed during ageing, and how this damages the cell, thus paving the way for new effective treatments for cell recovery in the brain. I will use a new model system to study ageing: the method produces brain cells from skin cells using advanced but well-proven reprogramming methods. This project will lead to an increased understanding of ageing and to the development of much-needed new treatment methods for age-related neurodegenerative diseases.

Title: Screening lead compounds for activity against the multiplicative and dormant stages of Apicomplexan parasites*: Plasmodium falciparum* and *Toxoplasma gondii*

Principal Investigator: Dr. Swati Patankar, Indian Institute of Technology Bombay, Mumbai, India

ICGEB Reference No. CRP/IND21-02

Abstract: Malaria and toxoplasmosis are diseases that affect millions of people globally. These diseases can be treated with anti-parasitic drugs, however, resistance to these drugs is an ever-looming problem. Furthermore, there are few/no drugs to treat some of the latent stages of these parasites. These two problems thwart the ongoing attempts to eradicate malaria and control toxoplasmosis. Hence, the drug discovery pipeline needs constant replenishment. We propose to test a panel of small molecules that could potentially add to the drug discovery pipeline. These small molecules inhibit a pathway required for transporting proteins into the nucleus of the parasites, and this pathway has never been targeted before. The ICGEB grant aims to develop sensitive assays for testing small molecules on the two parasites that cause these devastating diseases.

Title: Aptamer-guided plant polyphenol nanoassemblies for effective cancer targeting

Principal Investigator: Dr. Suhair Sunoqrot, Al-Zaytoonah University of Jordan, Amman, Jordan

ICGEB Reference No. CRP/JOR21-03

Abstract: Cancer is one of the leading causes of death worldwide. By the end of 2020, female breast cancer became the most diagnosed cancer type. Here we aim to design nanoscale particles from plant polyphenols, such as those found in teas and spices, which have shown promising activity against a variety of human diseases, including breast cancer. The efficacy of these particles will be enhanced by decorating them with a homing moiety: an aptamer that can specifically recognize breast cancer cells while sparing healthy ones. Overall, the results from this study are expected to lay the groundwork for the design of a smart nanomedicine derived from ubiquitous natural compounds for more efficient delivery to, and treatment of, breast tumours.

The project will also provide training opportunities to junior researchers and contribute to capacity building in the field of nanomedicine and translational research in Jordan, through national and international collaborations.

Title: To elucidate the effect of RhoH Y38X mutation in regulating Rac-induced BAFF-R signalling in PID malignancyp

Principal Investigator: Dr. Ana Masara Binti Ahmad Mokhtar, Research Creativity and Management Office (RCMO), Penang, Malaysia

ICGEB Reference No. CRP/MYS21-04_EC

Abstract: 3.6% PID (Primary Immune Deficiency) patients have been diagnosed with cancer and the most prevalent (66.7%) of these are haematological malignancies. Current approaches in therapy for PID malignancies relies on the use of small-molecule inhibitors or immuno-modulatory agents that focus more on the relief of the symptoms without properly addressing the underlying causes. Thus, to ensure that future therapeutics are efficacious, a complete picture of the pathogenesis of PID malignancies is urgently needed; especially important is information on the effect of mutations that may promote cancer progression. Amongst these is the RhoH Y38X mutation, wherein the patient was shown to develop Burkitt lymphoma together with the immune-defect observed. This arose potentially through deregulation of Rac activities in regulating BAFF-R signalling, a common defect observed in haematological malignancies. It is expected that elucidating the mechanistic understanding of PID malignancies, would help to identify a new therapeutic target that could lead to the development of new therapies in the future.

Title: Unveiling the miRNAs of the medicinal *Mascarene* aloes and *in vivo* testing of their neuroprotective attributes in *Drosophila* models

Principal Investigator: Dr. Vijayanti Mala Ranghoo-Sanmukhiya, The University of Mauritius, Reduit, Mauritius

ICGEB Reference No. CRP/MUS21-01

Abstract: Research on the medicinal profiles of endemic Mascarene Aloes at the University of Mauritius has unveiled their rich phytochemical content, explaining their antimicrobial, antioxidant, neuroprotective and cytotoxic properties. So far, research on these medicinal plants has mainly focused on the pharmacological functions of their natural chemical ingredients. The therapeutic potential of plant miRNA is well established and in the quest to unveil this possible molecular pathway, we initiated a collaboration with the miRNA research team at ICGEB New Delhi. This research collaborative agreement culminated in the training and co-supervision of a PhD student who has now been trained in RNA extraction. The present project proposes to sequence the extracted miRNA and set up an in-house facility for fostering miRNA research for national and regional development. In addition, it will start the in-vivo *Drosophila* Parkinson Disease model assay to confirm the reported neuroprotective properties and free radical scavenging abilities of the Mascarene Aloes.

Title: Study of the mechanisms that coordinate tracheal and epidermal development in the embryogenesis of *Drosophila melanogaster*

Principal Investigator: Dr. Luis Daniel Rios Barrera, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Coyoacán, Mexico

ICGEB Reference No. CRP/MEX21-04_EC

Abstract: For organs to be functional, the cells and tissues that constitute them must effectively communicate with each other and coordinate their behaviours. Here, we focus on the development of the tracheal system of the fruit fly *Drosophila melanogaster* to study these interactions. The tracheal system consists of a network of tubes that allows gas exchange by forming hollow branches throughout the body of the animal. We aim to understand how tracheal branches interact with other tissues, specifically, the epidermis; and to determine how these interactions influence each other's behaviours and development. Besides their structural similarities, tracheal branches resemble blood vessels in that they use similar genes to build tubes. Understanding how tracheal tubes interact with other organs will allow us to better understand the mechanisms that tissues like blood vessels use to interact with their own targets.

Title: The molecular role of Human papilloma virus association with head and neck squamous cell carcinoma (HNSCC) in Namibia

Principal Investigator: Dr. Lamech Mwapagha, Namibia University of Science and Technology, Windhoek, Namibia

ICGEB Reference No. CRP/NAM21-02

Abstract: Head and neck squamous cell carcinoma (HNSCC) is one of the most common cancers in developing countries, with the incidence globally projected to increase by >1 million new cases annually by 2030. The Human papillomavirus (HPV) has been established as one of the main causative agent for HNSCC. Knowledge regarding the mechanistic aspects of HPV strains and their association with cancer is important for setting the baseline data to identify the need for appropriate strategies in combatting the high burden

of HNSCC in Sub-Saharan Africa. This study aims to investigate the molecular mechanisms of HPV-associated HNSCC in Namibia. RNAseq and proteomics analysis will be performed to identify gene variants, signalling pathways and protein interaction profiles, while biochemical assays, RT-PCR, western blotting, flow cytometry and microscopy experiments will also be utilised to gain further insights into the mechanisms involved in the development of HNSCC where HPV is involved.

Title: Characterization of metabolic markers of prostate cancer as determinants of tumour pathobiology in an African population

Principal Investigator: Dr. Oseremen Aisuodionoe-Shadrach, University of Abuja, Abuja, Nigeria

ICGEB Reference No. CRP/NGA21-01

Abstract: Prostate cancer (CaP) is the leading cancer in both incidence and mortality among men of African ancestry (MAA), and evidence shows that MAA suffer disproportionately from CaP compared with men of other races. In Nigeria, CaP is both the most common and most deadly cancer with 32.8 cases and 16.3 deaths per 100 000 men annually. The mutational landscape of CaP varies substantially by race, supporting the observation that biological factors may uniquely influence incidence and tumour aggressiveness in Africans. This project will identify the metabolic signatures associated with CaP in Nigerian patients of different ethnic groups, it will characterize the metabolic signature of CaP aggressiveness and investigate the consequent metabolic signature changes that occur over time during disease progression. Understanding the metabolic perturbations in the African population may guide treatment decisions and lead to the development of metabolic markers for CaP in African patients.

Title: Exploring novel genes and mechanism behind genetic epilepsies for implementation in the Pakistani health care system

Principal Investigator: Dr. Ambrin Fatima, Aga Khan University, Karachi, Pakistan

ICGEB Reference No. CRP/PAK21-05_EC

Abstract: Genetic epilepsies (GP) are heterogenous disorders of the developing brain that typically result in cognitive and motor delay regression, and sometimes death. The incidence of GP is far higher in Pakistan than in the rest of the world, as a result of frequent, culturally-sanctioned, consanguineous marriages. This high incidence generates enormous stresses, both for the healthcare system and for the families concerned and imposes a serious socio-economic burden on the developing economy. However, due to the lack of genomic diagnostic facilities locally, the relevant genetics remain unknown, thus impeding such preventative options as career screening, prenatal diagnosis and genetic counselling. Here, we take a holistic approach; from identifying the causative genes/gene variants, to unravelling the pathophysiological mechanisms involved, with the prospective of developing therapeutic interventions using next-generation sequencing and human induced pluripotent stem cells (iPSCs), CRISPR/Cas9 and multi-omics techniques. We anticipate that this project will unravel a number of disease-associated biomarkers, indicating pathways and factors that may serve as candidates for the development of personalized medicine and novel treatment options for epilepsy.

Title: The brain-gut axis linking inflammatory bowel disease with anxiety and depression: the inflammation-microbiome network

Principal Investigator: Dr. Elena Milanesi, Victor Babes National Institute of Pathology, Bucharest, Romania

ICGEB Reference No. CRP/ROU21-01

Abstract: Inflammatory Bowel Diseases (IBD), including ulcerative colitis and Crohn's disease, are conditions in which the intestines become chronically inflamed, leading to diarrhoea and abdominal pain. IBD are associated with high rates of anxiety and depression, with a decreased quality of life for these patients. In this project we aim to clarify the mechanisms underpinning the crosstalk between IBD and mood disorders; investigating markers related to inflammation, the circulating miRNA profile, and the gut microbiome that consists of the collective genome of microbes inhabiting the gut. To this end, we will study a cohort of IBD patients with and without anxiety or/and depression symptoms and a cohort of non-IBD patients with depressive and anxiety symptoms, as well as a control group without any symptoms. The expected results will allow us to identify molecular patterns that may either be unique to IBD or associated with the complex phenotypes of IBD with anxiety and depression.

Title: The role of distinct isoforms of PHF10 subunit of PBAF chromatin-remodeling complex in MYC dependent transcription activation.

Principal Investigator: Dr. Sofia Georgieva, Engelhardt Institute of Molecular biology, Russian Academy of Sciences, Moscow, Russia

ICGEB Reference No. CRP/RUS21-01

Abstract: MYC is a proto-oncogene encoding a transcription factor that contributes to the progression of many different types of cancer. We have found previously that the PHF10 protein, which was described in our

previous studies, interacts with MYC and maintains MYC-dependent proliferation of melanoma tumour cells. The goal of this project is to reveal the mechanism of the MYC - PHF10 collaboration in melanoma progression.

Title: Dissecting the molecular underpinnings of centriole amplification in multiciliated epithelial cells
Principal Investigator: Dr. Elif Nur First Karalar, Koc University, Istanbul, Turkey
ICGEB Reference No. CRP/TUR21-01
Abstract:
In mammals, multiciliated epithelium is found in the airway, the oviduct and the ventricular system of the brain. Multiciliated epithelial cells have tens to hundreds of centrosomes, which are essential for assembling motile cilia. Deregulation of centrosome and cilium biogenesis and functions in these cells causes multisystemic genetic diseases, known as "motile ciliopathies", such as Primary Ciliary Dyskinesia (PCD). These diseases have diverse clinical manifestations, including respiratory problems caused by impaired mucociliary clearance, left-right laterality defects, infertility and hydrocephaly. Importantly, the majority of genes mutated in PCD encode components of the centrosome and cilia. Although genetic studies have described many proteins mutated in motile ciliopathies, relatively poor understanding of their functions and mechanisms impedes the development of therapeutic approaches. The major goals of the proposed project are to dissect the molecular underpinnings of centriole duplication and to provide insight into mechanisms associated with its deregulation, such as developmental disorders and cancer.

Title: Tweaking proline metabolism in plants to increase their tolerance to stress
Principal Investigator: Dr. Santiago Signorelli-Poppolo, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay
ICGEB Reference No. CRP/URY21-04_EC
Abstract:
Alfalfa is the main forage legume used worldwide to feed cattle and is hence of major economic importance. Proline accumulation is known to be a conserved stress-response in plants, having multiple beneficial roles, but this response has been poorly exploited for the development of more resilient crop varieties. Therefore, in this project we propose to develop a stress-resistant genotype of alfalfa by adjusting its proline metabolism through CRISPR/Cas9 gene editing. The edited plants will be transgenesis free, making them more acceptable to the society. Future applications of these results include the use of this legume in the livestock industry. As a sub-product we will also obtain knock-out plants for the gene P5CS2 (i.e., with reduced proline accumulation), which will be a valuable resource to support different ongoing basic science projects in our group and the group of our collaborators.