DESCRIPTION DU PROJET DE RECHERCHE :

Dissecting the roles of histone variants in the closest relatives of animals, the choanoflagellates

DNA in all eukaryotic cells is package into nucleosomes composed of the 4 core histones: H3, H4, H2A and H2B. This packaging not only allows for organisation of DNA into chromatin in the nucleus, but histones are also the substrate for the regulation of processes including transcription, DNA replication and DNA repair. One important aspect of this regulation is the presence of non-canonical or variant histones which can replace the canonical core histones in order to exert specific functions. One notable example is CENPA which is a H3 variant found exclusively at centromeres where it functions to epigenetically specify the identity of these important genomic domains. Although histone variants have been extensively studied, their evolutionary history is often less well understood.

In this project I aim to analyse the variant-histone compliment of the choanoflagellate *Salpingoeca rosetta*. Choanoflagellates are the sister group to animals and therefore have a pivotal position for understanding early animal evolution. *S. rosetta* has become an attractive model due to the recent development of advanced functional tools. The Gahan lab studies the evolution of diverse chromatin-regulated processes in choanoflagellates in order to trace the evolutionary origins of animal gene regulation. Previous work has shown that *S. rosetta* possess a small compliment of variant histones including a replication-independent H3 variant, a H2A variant known as H2Az and two CENPA paralogs. The genomic localization and function of these variants are, however, totally unknown.

The project will have several discreet aims:

1. Using newly developed methodologies, generate *S. rosetta* lines in which the histone variants are endogenously tagged with GFP or small epitope tags.
2. Using these newly developed lines:
    a. Determine the localization of variant histones in the nucleus by microscopy.
    b. Interrogate their genome-wide distributions using ChIPseq.
3. Generate mutant lines that can be used in the future to study the function of variant histones in *S. rosetta*.

The outcomes of the proposed research project will provide novel insights into the function of variant histones in choanoflagellates and, via comparison with animals, reveal new or ancestral roles for those histones. In addition, the tools developed will enable future research in this area. Finally, the proposed research will provide me with skills including working with choanoflagellates, CRISPR-Cas9 genome editing, advanced microscopy and epigenomic analyses.

Description of the project – Lou Ruffino in Pr Stephen Simpson Laboratory (Sydney, Australia)

Dietary Macronutrients and the Male Reproductive Proteome

Background: Dietary macronutrients affect all aspects of metabolic and reproductive physiology. In particular, diets that perturb metabolic function, such as high-fat obesogenic diets, are likely to be detrimental to reproductive health (1). Epidemiological studies in humans, for instance, have shown that obesity is strongly linked with decreased male fertility (2). Strikingly, it has been recently shown that obesogenic diets can also act inter-generationally, with both the maternal and paternal diet before conception inducing metabolic effects in the offspring (3). Understanding how, and which aspects of the diet, are causal determinants of these effects has emerged as a critical question in nutritional biology and reproductive health.

Aims: The aim of this project is to gain insight into the molecular mechanisms by which dietary macronutrients alter male reproductive function using an innovative proteomics work-flow, coupled with a the unique experimental model of nutritional geometry in the mouse.

Approach:

The Nutritional Geometric Framework: To date, most experimental models for nutrition research have focussed on simplistic two-, or three-way tests of diets. Such approaches are highly reductive and can conflate the experimental effects of diet composition and amounts. This project will use samples from a cutting-edge experimental design, termed the Nutritional Geometric Framework (NGF) (4). Specifically, it will use an isocaloric 10-diet NFG design, which holds dietary energy density constant, and covaries the energy from dietary protein, carbohydrates and fats. This design allows the user to separate the effects of total amount of energy within the diet from the energy source.

Model Organism and Experimental Tissues: This study will utilise reproductive tissues from male C57Bl6J mice. Six male mice were held on each of the 10-isocaloric diets from 5 weeks of age until 20 weeks of age. Previous work has shown that over this period the animals show a metabolic and reproductive response to the diet (5; 6). At 20 weeks of age the animals were culled and testicular and seminal -vesicle tissue was sampled and frozen. For future experiments, other tissues, such as brain and adipose tissue, were also collected and frozen.

Proteomic Quantitation: Tissues will be lysed, and total protein content assessed by BCA. Based on the BCA samples will be added, with buffer, to fresh well plates and frozen for storage. Once all samples have been prepared, protein quantitation will be undertaken using Sciex 7600 instrument coupled to an EvoSep liquid chromatography system.

Bioinformatic Pipeline: Bioinformatics and biostatistical analysis will be undertaken in R. Proteins will be filtered for significance in response to dietary macronutrient content using the R package 'limma'. For those proteins that are statistically significant we cluster the model coefficients using a k-means, or c-means, clustering algorithm. Finally, cluster-average responses will be plotted as GFN surfaces for qualitative interpretation.

Proteomic data will be analysed to identify gene pathways that are altered by certain macronutrient compositions. Function will be imputed from the identified pathways to get insight into the effect of macronutrient on the specific functions in the reproductive organs.

References:

1. Crean AJ, Senior AM. 2019. High-fat diets reduce male reproductive success in animal models: A systematic review and meta-analysis. Obesity Reviews 20:921-33
2. Du Plessis SS, Cabler S, McAlister DA, Sabanegh E, Agarwal A. 2010. The effect of obesity on sperm disorders and male infertility. Nature Reviews Urology 7:153-61
3. Drake AJ, Liu L. 2010. Intergenerational transmission of programmed effects: public health consequences. Trends in Endocrinology & Metabolism 21:206-13
4. Simpson SJ, Raubenheimer D. 2012. The Nature of Nutrition: A Unifying Framework from Animal Adaptations to Human Obesity. Oxford, UK: Princeton University Press
5. Crean AJ, Senior AM, Freire T, Clark TD, Mackay F, et al. 2024. Paternal dietary macronutrient balance and energy intake drive metabolic and behavioral differences among offspring. Nature communications 15:2982
6. Crean AJ, Afrin S, Niranjan H, Pulpitel TJ, Ahmad G, et al. 2023. Male reproductive traits are differentially affected by dietary macronutrient balance but unrelated to adiposity. Nature Communications 14:2566

Descriptif du projet de stage :

Large scale immunogenomics analysis for the characterization of immune landscape and determinant of tumor immune infiltrate in pediatric tumors

Immune checkpoint blockades (ICBs), molecules that restore the immune system's ability to recognize and eliminate tumor cells, have revolutionized the treatment for some adult cancers. To be efficient, ICBs need the tumor to be primarily infiltrate with immune cells, especially activated «inflamed» T-cells. Only a small percentage of pediatric tumors are sensitive to ICBs. We believe that ICBs sensitivity is highly dependent on tumor immune composition and can be anticipated to select the children that are more likely to benefit from ICBs and to promote personalized immunotherapy in pediatric oncology.

Dr. Santiago's lab has developed strategies to classify tumor immune phenotypes across pediatric solid tumors. His works has identified subgroups of pediatric tumors that could be candidate for immunotherapy, either in monotherapy or in combination.

My role in this project would be to identify molecular signature associated to each phenotype to better characterize them and understand the drivers of immune recognition or evasion specific to pediatric tumors. For this, I will use multi-omic and machine learning approaches to aggregate gene expression, DNA methylation, and genomics datasets from hundreds of childhood solid tumors.

This internship will be a part of a collaborative project involving clinicians and researchers from several centers across Canada and is intended to have clinical and therapeutic impacts.