Dual-PhD program between Sussex and HKUST

Functional and quantitative post-translational modification (PTM) proteomic and interactomic study of the post-DNA replication damage recovery in *Schizosaccharomyces pombe*

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Summary

We are looking for student interested participating in a joint PhD project between Hong Kong University of Science and Technology (HKUST; Li laboratory) and the University of Sussex (UoS; Carr laboratory) to explore the role of post-translational modification of proteins in the response to, and during recovery from, replication stress. The project will involve exploiting mass spectrometry to identify protein post translational modification in fission yeast cells recovering from replication stress (expertise of the Li laboratory) and subsequently analysing the function of these modifications by exploiting molecular and classical genetics (expertise of the Carr laboratory).

Scientific background

DNA replication stress and how cells recover from such stress is an important subject in basic cancer research. Most cancer cells are subjected to DNA replication stress as a consequence of the activation of oncogenes, a phenomenon known as oncogene-induced stress (OIS). In the aetiology of cancer it is proposed that an early event is a stochastic mutation that activates an oncogene. This "unbalances" the growth signalling pathways and results in cells replicating their DNA under suboptimal conditions. The resulting OIS causes a high level of replication errors and thus a significant increase in mutations such as base changes, insertions, deletions and translocations. This increased mutagenic load promotes the multiple changes that need to occur in order for cells to become fully cancerous.

In recent years, there has been much interest in exploiting the phenomenon of OIS to specifically target cancer cells: the fact that cancers cells are undergoing OIS

distinguishes them from non-cancerous cells and may provide opportunities to enhance the "therapeutic window" such that cancer can be targeted effectively with minimal disruption to normal proliferating tissue. Many strategies are being explored in the scientific community, including synthetic lethality approaches and better targeted chemotherapy based on the precise nature of the individual tumour. All these strategies demand that we have a clear understanding of how cells tolerate replication stress and the biochemical mechanisms that are at play.

Project

We propose a collaborative approach to explore the time course of post-translational modifications (PTMs) that occur during recovery from replication stress. This will complement other studies that have looked at PTMs in cells exposed to agents that induce replication stress and help to elucidate what happens in the period of time during which cells are recovering from replication stress. We will explore this in a model organism, the fission yeast *Schizosaccharomyces pombe*.

We will identify changes to PTMs as cells are recovering from replication stress caused by exposure to either hydroxyurea or methyl methanesulfonate. We will examine Sumoylation and phosphorylation changes during the recovery period. We will also study which pathways are promoting these modifications by the use of specific mutant cells that are defective in specific genes. For example, in the case of *S. pombe*, Sumoylation is promoted by a small number of "E3" targeting enzymes, such as Siz1, Siz2 and Nse2. By studying changes in these mutants, we will be able to identify key pathways and link cause and effect to provide both a map of changes and a mechanistic framework that will inform future research.

The methods that will be used include stable isotope labelling-based quantitative proteomic workflows *and MudPIT* (Multiple Dimensional Protein Identification Technology). These will be applied to samples enriched either for sumoylated peptides or phosphopeptides from nuclei isolated from yeast cells. The objective is to obtain approximately 5,000 PTM peptides. The genes encoding the post-translationally modified proteins will be subjected to molecular biology and genetic studies in the fission yeast to elucidate the roles of PTM proteins in the post-DNA replication damage recovery.

Proposed time-line for the students:

Summer of 2018-Fall of 2020: the PG student in the joint program will take courses at HKUST and conduct quantitative PTM proteomic study on yeast in Ning Li's lab.

Spring of 2020 – Summer of 2022: the student will conduct molecular biology studies on PTM proteins in the DNA damage recovery. Preparation for thesis and publication papers.

Summer of 2022: Defence of PHD thesis either at University of Sussex or HKUST.