

Title of Project:	Towards a molecular understanding of piRNA-instructed transposon methylation	
Cell Mechanism Supervisor Name	Dónal O'Carroll	
Quantitative Supervisor Name	Atlanta Cook	

Summary of project
<p>In mammals, transposons are silenced throughout most of life by DNA methylation. The mammalian germline is derived from somatic cells early during development. To reset genomic DNA methylation patterns, genome demethylation and <i>de novo</i> methylation is required in germline cells as the embryo develops. In the male germline, the PIWI protein MIWI2, along with its associated small non-coding piRNAs, direct DNA methylation to silence young, active transposons. We have defined a high confidence MIWI2 interactome from gonocytes that are undergoing <i>de novo</i> DNA methylation. Through the analysis of this dataset we have identified the first essential nuclear executors of MIWI2:piRNA-directed transposon <i>de novo</i> DNA methylation. This discovery now permits the exploration of the molecular mechanisms and design principles underpinning piRNA-directed transposon methylation. In this project, we propose to define, through biochemical methods, what proteins directly interact with MIWI2 and a novel factor. We also propose to explore molecular and structural basis of key interactions. Finally, we aim to understand how these constituents of the complex contribute to function by employing mouse genetics to generate alleles that encode point mutations that uncouple the respective factors from their key interacting proteins. In summary, the goal of the proposed project is to define, mechanistically and at high resolution, this process that is essential for the immortality of the germline and the continuity of mammalian life.</p>

What quantitative skills will the student acquire or develop during their PhD project?
<p>Cell biology skills</p> <ul style="list-style-type: none"> • Gene editing to generate novel mouse alleles • Histological analysis of spermatogenesis • Confocal immunofluorescence microscopy on tissue sections • Southern and northern blotting <p>Quantitative skills</p> <ul style="list-style-type: none"> • Generation and analysis of RNA-seq and small RNA-seq libraries • FACS analysis and sorting of germ cell populations • Biochemical and biophysical characterisation of intermolecular interactions • Structural studies using X-ray crystallography and/or cryo-EM