CRUK Cambridge Centre MRes rotation project

Rotation Project Title	**Checkpoint control of DNA replication prevents mutagenesis in normal and cancer cells**
Head of Laboratory (PI) Name	Philip Zegerman
Second supervisor if applicable	
Programme	CMB
Supervisor's Email	Paz20@cam.ac.uk
Laboratory Location	Gurdon Institute

Project Outline	**Aims and objectives** Mutation is the root cause of cancer and is a consequence of many chemotherapies, acting as a driver of resistance. By understanding the root causes of mutagenesis we aim to identify pathways that are required to generate cancer mutations and therefore identify new ways to diagnose and treat patients. You will use our recently devised assay in budding yeast to rapidly screen for pathways required for an unexplained form of structural mutation caused by replication template switching. Preliminary data suggests that the checkpoint kinases are key regulators preventing this form of mutagenesis. This information will be used to test the onset of template switch-dependent mutations in both normal and cancerous human cells, before and after chemotherapy treatment. Together this project will provide new insights into the causes of cancer mutations and may provide ways to prevent such mutations during chemotherapy.
Experimental plan	Use established mutagenesis assays in yeast to rapidly screen for the role of multiple pathways in prevention of template switching. Use these strains to perform whole genome sequencing (WGS) to identify genetic loci that are prone to such mutations. With this information, inhibit these pathways in human cells and screen for structural mutations using WGS.
Main Techniques	• Mutagenesis and yeast genetics • Whole genome Illumina library prep and sequencing • Bioinformatic analysis of structural variants using multiple alignments • Human cancer cell culture
Key References	Helicase subunit Cdc45 targets the checkpoint kinase Rad53 to both replication initiation and elongation complexes after fork stalling. Can and Zegerman. Mol Cell. 2018. Under review Mechanisms underlying mutational signatures in human cancers. Helleday T[1] et al. Nat Rev Genet. 2014 Sep;15(9):585-98.