CRUK Cambridge Centre MRes rotation project

Rotation Project Title	**Regulation of human DNA replication**
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Programme	CMB
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Laboratory Location	Department of Zoology

Project Outline	**Aims and objectives** Chromosomal DNA replication is tightly regulated to allow successful cell proliferation and reduce the risk for cancer. In this project, we aim to investigate molecular mechanisms underlying the initiation of DNA replication in human cell nuclei. Previously, our laboratory has discovered that small non-coding Y RNAs are essential for the initiation of chromosomal DNA replication in vertebrate cells. Y RNAs have been implicated in cancer because they are overexpressed in human tumours, required for cell proliferation and down-regulated in quiescent cells. Y RNAs bind to cellular proteins to perform their function, but mechanistic details are still unclear. We have recently identified several Y RNA-binding proteins that are implicated in DNA replication, cell cycle regulation and cancer. Therefore, the objectives of this MRes rotation project are: 1) To identify direct molecular interactions *in vitro* between human Y RNAs and candidate Y RNA-binding proteins implicated in DNA replication, cell proliferation and cancer. 2) To characterise the functional relevance of these molecular RNA-protein interactions for chromosomal DNA replication in human cell nuclei. This MRes rotation project has the clear and exciting potential to be developed further into a full PhD project, aimed at characterising the molecular mechanisms by which Y RNAs regulate DNA replication and cell proliferation *in vitro* and *in vivo*.
Experimental plan	This project builds on a cell-free DNA replication initiation system from human cells, which we have established in our laboratory. In this system, template nuclei are isolated from synchronised late G1 phase human cells and incubated in a cytosolic extract prepared from proliferating human cells. Non-coding Y RNAs and additional replication proteins present in the cytosolic extract initiate DNA replication in the G1 phase template nuclei *in vitro*. Replicating nuclei are labelled by the incorporation of fluorescent nucleotides into chromosomal DNA and detected by confocal fluorescence microscopy. In this project, we will first employ immunodepletion to specifically remove candidate proteins from the cytosolic extract. We have already identified suitable candidate proteins implicated in DNA replication, cell proliferation and cancer. We will use these immunodepleted extracts in DNA replication initiation assays *in vitro* to determine which Y RNA-binding proteins are essential for DNA replication. Subsequently, we will map the interactions between Y RNAs and essential replication proteins by designing and synthesising wild-type and mutant Y RNAs *in vitro*.

	These synthetic RNAs will be coupled covalently to agarose beads and used to pull-down interacting replication protein(s) from the cytosolic extract. Western blotting will then be used to characterise the binding of RNA mutants to the candidate protein(s) and to map the Y RNA domain(s) required for binding to the candidate protein(s). These binding studies will be complemented by functional DNA replication experiments. We will examine if the *binding* of mutant Y RNAs to the candidate proteins is correlated to their *function* during DNA replication. To allow functional testing of mutant Y RNAs, endogenous Y RNAs will first be depleted from the cytosolic extract. This leads to an inhibition of DNA replication initiation in the cell-free system. We will then add *in vitro*-synthesised and purified mutant Y RNAs to the Y RNA-depleted extract and determine if they still initiate DNA replication *in vitro*. These experiments will allow us to determine experimentally whether interactions between Y RNAs and candidate replication protein(s) are essential for the initiation of human chromosomal DNA replication.
Main Techniques	• Protein techniques: cell culture; cell extract preparation and fractionation, chromatography and ultracentrifugation, immunoprecipitation, immunodepletion. • RNA techniques: *in vitro* synthesis of wild-type non-coding Y RNAs, *in silico* design of RNA mutants and *in vitro* synthesis for functional tests. • Reconstitution of DNA replication *in vitro* • Confocal fluorescence microscopy
Key References	**Review article:** Kowalski, M.P., and Krude, T. (2015). Functional roles of non-coding Y RNAs. Int J Biochem Cell Biol *66*, 20-29. **Original papers:** Christov, C.P., Dingwell, K.S., Skehel, M., Wilkes, H.S., Sale, J.E., Smith, J.C., and Krude, T. (2018). A NuRD complex from Xenopus laevis eggs Is essential for DNA replication during early embryogenesis. Cell Rep *22*, 2265-2278. Kheir, E., and Krude, T. (2017). Non-coding Y RNAs associate with early replicating euchromatin in concordance with the origin recognition complex. J Cell Sci *130*, 1239-1250. Christov, C.P., Trivier, E., and Krude, T. (2008). Noncoding human Y RNAs are overexpressed in tumours and required for cell proliferation. Br J Cancer *98*, 981-988. Christov, C.P., Gardiner, T.J., Szüts, D., and Krude, T. (2006). Functional requirement of noncoding Y RNAs for human chromosomal DNA replication. Mol Cell Biol *26*, 6993-7004.