ICR The Institute of Cancer Research

UKRI Medical Research Council

PhD Project Proposal

Funder details

Studentship funded by: MRC DTP

Project details

Project title: Examining the mechanism of chromatin replication and assembly at telomeres

Supervisory team

Primary Supervisor: Max Douglas

Associate Supervisor(s):

Secondary Supervisor: Jessica Downs

Divisional affiliation

Primary Division: Cancer Biology

Primary Team: Telomere Biology

Site: Chelsea

Project background

Each human telomere is composed of 10-15 kb of repetitive DNA bound by a protein complex called shelterin, which forms a protective nucleoprotein cap at the chromosome end. In addition to shelterin, telomeric DNA is also wrapped around histone octamers in a closed, heterochromatic state that compacts telomeric DNA and represses transcription at the chromosome end (Tardat and Dejardin, 2018). Mutations that disrupt the assembly of chromatin at telomeres cause DNA damage and are found in essentially all ‘ALT’ type cancer cells (some 10-15 % of all tumour types), underlining the importance of chromatin in the function of telomeres.

At non-telomeric sites, chromatin is assembled during S-phase when chromatin remodelling factors and histone chaperones disassemble nucleosomes in front of the replication fork and reassemble them on newly synthesised DNA (Hoek and Stillman, 2003). Although telomeric chromatin is also assembled during S-phase, genetic studies show that a distinct set of chromatin remodelling factors are required for this process, suggesting the replication and reassembly of nucleosomes at telomeres occurs through a distinct mechanism.

The successful candidate will examine this mechanism using a combination of reconstitution biochemistry, biophysics and genetics. The starting point for the project is a reconstituted system for DNA replication that we have

recently developed in the Telomere Biology lab. Combining this system with chromatinised DNA templates and purified chromatin remodelling factors, we will examine i) how chromatin affects the human replication fork ii) how telomere-specific chromatin remodelling factors allow replication and reassembly of nucleosomes on telomeric DNA and iii) the consequences of disrupting these processes within cells. As opportunities arise, we will also collaborate with other groups to characterise replication intermediates using cutting edge biophysical and structural techniques.

Project aims

- Reconstitute chromatinised DNA templates containing telomeric and non-telomeric DNA
- Examine the impact of chromatin on the replication fork
- Examine the function of telomere-specific chromatin remodelling factors at the replication fork
- Characterise novel reaction intermediates using crosslinking mass spectrometry, electron microscopy and genetic approaches

Research proposal

Part I. Assembly and replication of chromatin in vitro.

We have recently developed a reconstituted system for human DNA replication, which has allowed us to use powerful in vitro techniques to study the replication and processing of telomeres in mechanistic detail for the first time. In the first part of this project, the successful candidate will incorporate chromatin into our replication system by using established protocols to reconstitute chromatinised telomeric or non-telomeric DNA templates which we will then replicate in vitro. The questions we will address will include:

• Is chromatin inhibitory for the human replisome? We will use nascent strand analysis to follow the progression of replication forks on telomeric and non-telomeric templates in the presence of absence of chromatin, examining whether replisome progression is affected by arrays of nucleosomes.

• Which chromatin remodelling factors promote chromatin replication in vitro? In vitro replication of chromatinised templates with a budding yeast replisome requires the FACT complex (Kurat et al., 2017). We will determine whether FACT is also sufficient for chromatin replication with a human replisome. If it is not, we will test the impact of other purified remodelling factors on the replication of chromatin in vitro.

• How is chromatin replication affected by histones variants? Telomeres are enriched for the histone variant H3.3, which can be assembled into chromatin in vitro. Using H3.3 nucleosomes and biochemical approaches we will examine whether DNA replication in the presence or absence of chromatin remodelling factors is affected by H3.3.

Part II. Characterisation of telomeric chromatin remodellers at the replication fork

Part I will develop a system for chromatin replication in vitro. In Part II, we will use this system to examine the role of telomere-specific chromatin remodelling factors during DNA replication. At non-telomeric sites, chromatin is disassembled and reassembled during DNA replication (Formosa, 2012, Hoek and Stillman, 2003); whether this is also the case at telomere is currently unclear. To examine these points, we will ask:

• Do chromatin remodelling factors affect DNA replication in vitro? We will introduce purified telomeric chromatin remodelling factors into replication reactions and examine their effect on the replication of telomeric chromatin.

• Do remodelling factors bind the replisome? We will use pulldown assays to examine whether telomeric chromatin remodelling factors directly bind the replisome. We will narrow down interaction sites and use cross-linking mass spectrometry (in collaboration with the ICR Functional Proteomics team) to interrogate interactions in high resolution. Making use of the outstanding structural biology facilities within the ICR, we will complement this work by examining stable complexes with electron microscopy.

- Do telomeric remodelling factors affect chromatin assembly during DNA replication? At other positions in the genome, chromatin remodelling proteins and histone chaperones assemble nucleosomes on newly replicated DNA during DNA replication. Telomeric remodelling factors also assemble chromatin during S-phase but whether this occurs during DNA replication is unknown. We will examine this question using our in vitro replication system.

Part III. Probing the role of telomeric chromatin remodelling factors in vivo.

Parts I and II will determine the role of telomeric chromatin remodelling proteins during DNA replication. In the last part of the project, we will use these insights to examine chromatin replication and assembly at telomeres within cells. The questions we will address will include:

- Do telomeric chromatin remodelling factors work at the replication fork in vivo? Using mutants defined in part II, we will determine whether chromatin remodelling factors that can't bind the replisome are defective in chromatin assembly at telomeres in vivo. Experimental approaches will include chromatin immunoprecipitation assays and immunofluorescence to examine the level of histones and the appearance of DNA damage markers at telomeres.

- Does binding of telomeric chromatin remodelling factors to the replisome play a role in the ALT phenotype? A common feature of 'ALT' type cancer cells is defects in telomeric chromatin. Using ALT-type cell lines and mutants defined in part II, we will examine whether the molecular processes examined above play a role in the survival of ALT cancer cells. This will involve using established assays to examine markers of the ALT phenotype when either wild-type or specific mutant versions of telomeric chromatin remodelling factors are expressed.

Literature references

FORMOSA, T. 2012. The role of FACT in making and breaking nucleosomes. Biochim Biophys Acta, 1819, 247-55.

HOEK, M. & STILLMAN, B. 2003. Chromatin assembly factor 1 is essential and couples chromatin assembly to DNA replication in vivo. Proc Natl Acad Sci U S A, 100, 12183-8.

KURAT, C. F., YEELES, J. T., PATEL, H., EARLY, A. & DIFFLEY, J. F. X. 2017. Chromatin Controls DNA Replication Origin Selection, Lagging-Strand Synthesis, and Replication Fork Rates. Mol Cell, 65, 117-130.

TARDAT, M. & DEJARDIN, J. 2018. Telomere chromatin establishment and its maintenance during mammalian development. Chromosoma, 127, 3-18.

Candidate profile

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1).

Pre-requisite qualifications of applicants: BSc or equivalent in biochemistry, cell biology or similar

Intended learning outcomes:

- Protein expression and purification
- Reconstitution biochemistry
- Protein biochemistry and analysis
- Nucleic acid biochemistry and analysis
- Mammalian cell culture techniques
- CryoEM analysis

Advertising details

Project suitable for a student with a background in:

- [x] Biological Sciences
- [ ] Physics or Engineering
- [ ] Chemistry
- [ ] Maths, Statistics or Epidemiology
- [ ] Computer Science