The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	Defining replication mechanisms at the telomere end
Short Project Title:	**Defining replication mechanisms at the telomere end**

SUPERVISORY TEAM	
Primary Supervisor(s):	Max Douglas
Backup Supervisor:	Wojciech Niedzwiedz
Lead contact person for the project:	Max Douglas

DIVISIONAL AFFILIATION	
Primary Division:	Cancer Biology
Primary Team:	Telomere Biology

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Eukaryotic genomes are divided into individual linear chromosomes that terminate in two DNA ends. As free DNA ends potently activate checkpoint responses and DNA repair mechanisms, specialised structures called telomeres have evolved to shelter chromosomal termini from recognition as a form of DNA damage in eukaryotic cells. The importance of these structures is highlighted by the range of human diseases associated with telomeric defects, including dykeratosis congenita, pulmonary fibrosis and essentially all types of cancer[1].

Whilst telomeres have been extensively studied genetically, our molecular understanding of how these dynamic complexes are organised and processed each cell cycle is currently limited. To address this question, the Telomere Biology group in the Division of Cancer Biology is using a multidisciplinary approach that employs reconstitution biochemistry, structural biology and genetics to study telomeres in molecular detail. The successful candidate will address a fundamental question in telomere biology: what happens when a replication fork reaches the end of the chromosome? This process of telomeric 'replication termination' plays an important role in human health and disease, because the unreplicated gap left behind at the chromosome end after replication is complete (the 'end replication problem'[2]) leads to telomere shortening in somatic human cells, which limits proliferative capacity and is a major barrier to cancer[1].

To gain mechanistic insight into this process, the successful candidate will build on current work within the group and use a recently developed in vitro system for eukaryotic DNA replication[3] to i) define the series of events that take place when a replication fork reaches a DNA end and ii) examine how these events are affected by telomere-associated factors. Complementing this biochemical approach, you will use budding yeast genetics to test mechanistic findings in vivo, and collaborate with other groups to characterise reaction intermediates using cutting edge biophysical and structural techniques.

PROJECT AIMS

- Use an in vitro DNA replication system to examine how the eukaryotic replisome terminates at a DNA end
- Use purified telomere binding proteins to examine how this termination process is affected by specific telomere-associated factors.
- Employ budding yeast genetics to test the mechanistic insights we develop in a cellular context
- Collaborate with other research groups to characterise novel reaction intermediates using crosslinking mass spectrometry and electron microscopy

RESEARCH PROPOSAL

Part I. Defining the events that occur when the eukaryotic replisome meets a DNA end

A major research focus for the Telomere Biology group is understanding how telomeres are replicated semiconservatively, and how the canonical replisome is altered by telomere-associated factors. To do this, we are studying the mechanism of semiconservative telomere replication using an in vitro replication assay in which a budding yeast replisome is reconstituted using more than 20 purified proteins and protein complexes[3]. Building on our expertise with this system[4,5], the successful candidate will develop DNA templates ending in telomeric DNA, and use biochemical approaches to examine what happens when a replication fork reaches a DNA end. The major experimental approaches you will use, and the questions you will address are as follows:

- Where does the replisome terminate leading and lagging strand synthesis at a DNA end? We will pull down radiolabelled replication products from in vitro replication reactions and use polyacrylamide gels to examine replication products with single nucleotide resolution, determining where the leading strand terminates and the final primer on the lagging strand is placed.
- Are these processes sensitive to the type of template being copied? We will modify the template end and examine whether the termination of leading or lagging strand synthesis is affected by template sequence or structure, using the experimental approach above.
- Can the replisome freely translocate off a DNA end? Using pulldown assays with replicated DNA, we will use Western blotting to determine whether replisome components remain on a linear template after DNA replication has taken place. If they do, we will use DNase I footprinting assays to probe the position of stalled replication components, and budding yeast extracts to characterise a replisome disassembly activity at telomeres.

This work will describe how replication terminates at a DNA end and allow us to build a first mechanistic picture of the end replication problem.

Part II. How do telomere-associated factors influence the termination process at a DNA end?

In vivo, telomeric DNA is bound by specific protein factors[6], which are likely to influence how replication terminates at a DNA end. The successful candidate will examine this process by:

- Purifying specific telomere proteins from budding yeast using classical fractionation approaches and affinity- and size exclusion chromatography.
- Developing reconstituted telomeric substrates by combining purified telomere proteins with bead-immobilised budding yeast telomeric DNA.
- Combining these templates with the in vitro replication system, and using experimental approaches described in part I to examine whether specific steps in replication termination (e.g. leading/lagging strand

termination sites and translocation of the replisome off a DNA end) are affected by specific telomere-associated components.
- When an effect is found, using deletion mapping and mutagenesis to identify point mutations that disrupt this effect.

A major focus of this analysis will be the Cdc13:Stn1:Ten1 (CST) complex. CST is a conserved trimeric complex that is known to bind the very end of the chromosome and promote semiconservative replication at telomeres, but the interplay between CST and the replication fork is not well understood[7].

Part III. Testing mechanistic insights in vivo

Parts I and II will define the mechanistic basis of the end replication problem at telomeres. To ensure that our findings accurately reflect how replication terminates at telomeres in vivo, the successful candidate will compliment this work with genetic experiments, making use of the wide range of well-developed genetic assays available with budding yeast. Key assays will include:

- The TPX method[8] to measure single stranded telomeric overhangs, the length of which is affected by where the final lagging strand primer is placed.
- Southern blotting to measure the length of telomeric repeats and follow telomere shortening over time, which is affected by where the final lagging strand primer is placed.
- MNase footprinting assays to determine the position of replisome components on telomeres in vivo and examine replisome disassembly in a cellular context.

Part IV. Collaborative projects with other groups

The ICR has outstanding facilities for mass spectrometry and electron microscopy. As opportunities arise, you will be able to work collaboratively with other groups within the institute, including Ed Morris in the Structural Biology Division and Jyoti Choudhary in the Cancer Biology division, to characterise the architecture of novel protein complexes and reaction intermediates using electron microscopy and cross-linking mass spectrometry respectively. These complementary approaches have the potential to provide atomic level structural insight that will in turn inform our biochemical experiments.

LITERATURE REFERENCES

1. Shay, J. W. & Wright, W. E. Semin Cancer Biol 21, 349-353, (2011).
2. Watson, J. D. Nat New Biol 239, 197-201, (1972).
3. Yeeles, J. T., Janska, A. *et al.* Mol Cell 65, 105-116, (2017).
4. Douglas, M. E., Ali, F. A. *et al.* Nature 555, 265-268, (2018).
5. Douglas, M. E. & Diffley, J. F. X. J Biol Chem 291, 5879-5888, (2016).
6. Wellinger, R. J. & Zakian, V. A. Genetics 191, 1073-1105, (2012).
7. Chen, L. Y. & Lingner, J. Nucleus 4, 277-282, (2013).
8. Soudet, J., Jolivet, P. *et al.* Mol Cell 53, 954-964, (2014).

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: e.g. BSc or equivalent in specific subject area(s)	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 - Budding yeast genetics and cell cycle analysis - Use of electron microscopy and mass spectrometry in collaboration
ADVERTISING DETAILS	
Project suitable for a student with a background in:	☒ Biological Sciences ☐ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science ☐ Other (provide details)
Keywords:	**1. Telomere PhD**
	2. DNA replication PhD
	3. Biochemistry PhD
	4. Telomere Replication
	5. In vitro DNA replication
	6. Reconstitution biochemistry