PhD Project Proposal

Funder details

Studentship funded by:	MRC DTP

Project details

Project title:	Redox control of DNA replication: how mitochondrial ROS tune replisome function to preserve genome stability

Supervisory team

Primary Supervisor:	Jörg Mansfeld
Associate Supervisor(s):	Karla Vuina
Secondary Supervisor:	Wojciech Niedzwiedz

Divisional affiliation

Primary Division:	Cell and Molecular Biology
Primary Team:	Post-translational Modifcactions and Proliferation
Site:	Chelsea/ CBL

Project background

Reactive oxygen species (ROS) were long regarded as harmful by-products of mitochondrial metabolism, but growing evidence—including our own—shows that ROS also signal to core processes such as cell-cycle control and DNA replication. Mitochondrial production of aspartate, a nucleotide precursor whose synthesis generates ROS, has been proposed as the essential mitochondrial function even when ATP is not limiting (Birsoy et al., 2015) . Consistent with this metabolic–replication coupling, we demonstrated that mitochondrial ROS activate the key S-phase kinase CDK2 and prevent its inhibition by oxidising p21 (Kirova et al., 2022; Vorhauser et al., 2025) These findings suggest that physiological ROS help match nucleotide supply with replication demand, thereby limiting replication stress and preserving genome stability.

Using cell-cycle–resolved quantitative redox proteomics, we found that multiple replication-fork proteins—including PCNA, Bloom helicase (BLM) and MCM6—undergo S-phase-specific oxidation in non-transformed cells (Vorhauser et al., 2025). Replication is exquisitely sensitive to redox imbalance: insufficient ROS slows fork progression, whereas excess ROS promotes DNA damage. However, the mechanisms by which regulated cysteine oxidation tunes replication remain unclear nor is it known how mitochondrial ROS can reach the nuclear localised replication machinery. A plausible ROS-sensing mechanisms at the replication fork could involve the antioxidant peroxiredoxin antioxidant proteins (PRDX). PRDX2 localises to replisome and oligomerises in response to ROS to promote Timeless recruitment to increase replication speed (Somyajit et al., 2017) . However, beyond ROS scavenging, peroxiredoxins can catalyse disulphide transfer to client proteins (Sobotta et al., 2015) , raising the possibility that fork-resident PRDXs direct selective oxidation of replication factors to modulate fork dynamics and

maintain genome integrity. This PhD research proposal will investigate how replication proteins can be specifically oxidised during S-phase, and elucidate the functional consequences of oxidations for DNA replication and genome stability by focusing on two main objectives:

1) **Determine if and how PRDX proteins act as highly sensitive H_2O_2 sensors at the replication fork and mediate replication protein oxidation**
2) **Investigate how oxidation of replication proteins regulates their localisation and activity and the resulting consequences for replication fidelity and genome stability under physiological versus cancer-like ROS levels**

Project aims

- **Define PRDX dependence of replication-protein oxidation**.
- **Map PRDX-mediated oxidation targets at the replisome.**
- **Determine redox sensitivity of DNA replication by local ROS control.**
- **Link redox state to localisation and activity of ROS-targeted replication factors.**
- **Compare physiological vs cancer-like ROS/O_2 regimes.**

Research proposal

Objective 1: Determine whether PRDX proteins act as highly sensitive H_2O_2 sensors at replication forks and mediate replication-protein oxidation.

Hypothesis and rationale:
Peroxiredoxins (PRDXs) react with H_2O_2 ~10^6–10^8× faster than most cysteines and can relay oxidative equivalents to client proteins via disulphide exchange. Cytoplasmic PRDXs are readily oxidised by mitochondrial H_2O_2 and can transit to the nucleus. We propose that fork-proximal PRDXs transmit mitochondrial redox signals to key replication factors (e.g. PCNA, BLM, MCM6) to tune fork dynamics and preserve genome stability.

Experimental strategy:
(1) Confirm PRDX localisation and interaction with the replisome under defined redox states.
We will validate reported interactions of PRDX1/2/6 with replication proteins (Dam et al., 2021; Somyajit et al., 2017) by immunoprecipitation and streptavidin pull-downs in non-transformed epithelial cells (RPE-1, MCF10A) and in breast cancer cells with lower or higher steady-state ROS (MCF-7, CAL-51). Validated antibodies and epitope-tagged replication proteins (e.g. PCNA) are available. Assays will be performed under reducing and non-reducing SDS-PAGE to detect disulphide-linked complexes. In parallel, we will monitor PRDX1/2/6 localisation to replication foci (PCNA co-staining, EdU) by live-cell imaging and immunofluorescence.

Outcome: Identification of replisome-associated PRDXs and how their fork interactions respond to cellular redox state.
(2) Test PRDX sensor/relay necessity.
We will knock out PRDX1/2 by CRISPR/Cas9 and rescue with catalytically inactive, sensor-dead (peroxidatic Cys→Ser) and relay-dead (resolving Cys→Ser) mutants, followed by quantitative redox proteomics on S-phase-enriched cells to verify known sites, determine oxidation stoichiometry, and define PRDX-dependent events. As a complementary approach, oxidised recombinant WT and mutant PRDXs will be added to fully reconstituted human replication reactions (in collaboration with Max Douglas) prior to redox-proteomic site mapping. A postdoc in my lab is currently developing proximity-ligation–based detection of specific oxidation sites. If feasible, we will apply this technique to PRDX knockout/mutant rescue approaches to determine in situ localisation of replication proteins that we already found are oxidised in S phase (PCNA, BLM, MCM6) and the top candidates identified by redox proteomics.

Outcome: A set of PRDX-dependent oxidation sites at the replisome and prioritised candidates for functional analysis.

Objective 2: Investigate how oxidation of replication proteins regulates their localisation and activity and the resulting consequences for replication fidelity and genome stability under physiological versus cancer-like ROS levels.

Hypothesis and rationale:
Site-specific cysteine oxidation functions as a reversible regulatory switch that controls recruitment, residence time and enzymatic activity of key replication factors. We predict that spatially confined H_2O_2 fine-tunes fork dynamics, whereas elevated or poorly buffered ROS flux—typical of many cancers—disrupts replication fidelity and genome stability.

Experimental strategy:
(*1) Determine redox sensitivity of DNA replication by local ROS control.*
We will generate tetracycline-inducible cell lines expressing a PCNA-nanobody fused to the ROS-producing enzyme DAAO and to the antioxidant enzyme TRX to precisely control the local redox state at the replisome. We have previously applied the same strategy to fine-tune the redox state of p21 (Vorhauser et al., 2025) . In parallel, we will fuse the most sensitive H_2O_2 sensor HyPer7 to the PCNA nanobody to read out oxidation locally at the replication fork in non-transformed and cancer cells. Subsequent read-outs include replication fidelity and DNA fibre assays (fork speed, symmetry, origin firing/restart; in collaboration with the lab of Wojciech Niedzwiedz), PCNA and EdU replication-foci dynamics by live-cell imaging and IF, detection of replication stress and DNA damage by IF (RPA, pChk1, γH2AX, 53BP1), and genome stability (e.g. comet assays, micronuclei, DNA content). In this set of experiments, we will also monitor the localisation of oxidised replication proteins from Objective 1 by live-cell imaging of fluorescently tagged proteins and by IF. Notably, ROS production by DAAO can be standardised with known amounts of H_2O_2; therefore, these experiments will provide the local ROS concentrations required to regulate and perturb DNA replication, allowing us to relate this information to the distinct redox states of ROS-high and ROS-low cancer cells.

Outcome: effect of local redox changes at the replication fork on replication fidelity and DNA damage.

(*2) Link redox state to localisation and activity of ROS-targeted replication factors*.
Using the mapped oxidation sites from Objective 1, we will engineer Cys→Ser mutants in candidate replication proteins by CRISPR/Cas9-based genome engineering using efficient negative-selection approaches established in the lab (Vorhauser et al., 2025). Live-cell imaging and FRAP will quantify recruitment and residency at replication foci. This will be complemented by determining the association of WT and Cys→Ser mutant candidates with newly synthesised DNA at replication forks using an EdU variant that can be clicked to biotin for subsequent purification and proteomic and/or Western blot analysis. Replication fidelity and DNA fibre assays (in collaboration with the lab of Wojciech Niedzwiedz) will be used to determine the functional consequences of a lack of oxidation. Further, proximity-ligation assays in *Objective 1* will show whether non-oxidisable mutants localise differently from WT.

(3) Compare physiological vs cancer-like ROS/O₂ regimes.
We will perform the cell-biological experiments from Objectives 1 and 2 in both non-transformed (RPE-1, MCF10A) and cancer lines (e.g. MCF-7, CAL-51) under varying oxygen concentrations using a controllable oxygen workstation available in the lab: (i) 0.5–2% (hypoxia; typical for solid breast tumours); (ii) 6.8% (physioxia of the mammary epithelium); and (iii) 21% (hyperoxia), to contrast results with previous studies. We will investigate in particular how cell lines with non-oxidisable replication proteins maintain genome stability (γH2AX/53BP1 foci, comet assays, micronuclei).

Outcomes (2 and 3): Linking the redox state of individual cysteines and replication factors to replication-fork fidelity and genome stability, and determining how oxygenation and the individual redox state of cancer cells impact DNA replication.

Risk mitigation:

There is already robust published evidence that PRDX1, PRDX2, and PRDX6 interact with replisome-resident proteins, although these experiments have not yet been performed in our cell models (MCF10A and MCF-7, CAL-51), which will allow us to directly compare high- and low-ROS cell lines. Further, if the PRDX disulphide-transfer hypothesis does not hold, the work in Objective 2 will not be affected because (i) we already know the S-phase-specific oxidation sites on several replication proteins from our recent cell cycle-dependent redox proteomics (e.g. PCNA, BLM, MCM6), and (ii) by using replication-localised DAAO and TRX we can fine-tune the redox state at the replisome. We recently published such approaches for the CDK inhibitor p21 (Vorhauser et al.). In that case, the mechanism by which replication proteins are oxidised would remain unclear, but we would nevertheless be able to study the functional consequences. Notably, all techniques required for this proposal are already established in our lab or in the labs of our collaborators Wojciech Niedzwiedz and Max Douglas.

Literature references

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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: Candidates must have a first class or upper second class honours BA or BSc Honours/MSc or equivalent in chemistry or biological sciences

Intended learning outcomes:	• Profound expertise in cell cycle and redox fields
	• State of the art molecular and cell biology techniques
	• CRISPR/Cas9-mediated genome engineering
	• Advanced human cell culture techniques
	• Biochemical skills
	• Single cell and high-resolution microscopy Automated image analyses

Advertising details

Project suitable for a student with a background in:

- ☒ Biological Sciences
- ☐ Physics or Engineering
- ☒ Chemistry
- ☐ Maths, Statistics or Epidemiology
- ☐ Computer Science