PhD Project Proposal

Funder details

Studentship funded by:	MRC DTP

Project details

Project title:	Cell cycle regulation of the WEE1 kinase

Supervisory team

Primary Supervisor:	Jonathon Pines
Associate Supervisor(s):	
Secondary Supervisor:	Jörg Mansfeld

Divisional affiliation

Primary Division:	Cell and Molecular Biology
Primary Team:	Cell Division
Site:	Chelsea

Project background

Entry to mitosis must be coordinated with pathways controlling DNA replication and DNA damage to ensure the generation of viable, undamaged daughter cells. Thus, the final trigger for mitosis activating Cyclin B-Cdk1, is tightly controlled by the antagonistic action of the CDC25 phosphatases and the WEE1 and PKMYT1 kinases[1,2]. WEE1 and PKMYT1 are of considerable interest for cancer therapy because many cancers, particularly those with replication stress, are vulnerable to their inhibition[3,4]. WEE1 inhibitors are in clinical trials, notably as agents to enhance radiotherapy, but have proven toxic to normal stem cells.

To analyse how WEE1 acts as part of the mitotic trigger mechanism we generated homozygous clones of epitope-tagged WEE1. We tagged WEE1 with a Flag tag for immunoprecipitation and mNeonGreen to assay protein levels and subcellular localisation. we analysed WEE1 phosphorylation through the cell cycle by mass spectrometry. We identified novel sites of phosphorylation that vary in a cell cycle-dependent manner and found that mutating these sites to alanines, or to acidic residues to mimic phosphorylation, either accelerated cells into mitosis, or delayed them in G2 phase. Further work revealed that phosphorylation increases WEE1 activity. This is a mechanism to regulate WEE1 that has not previously been described.

In collaboration with Norman Davey, we identified a novel motif in the N-terminus of WEE1 that binds to Cyclin A and Cyclin B, but whose affinity for Cyclin B markedly increases when an adjacent serine is phosphorylated[5]. This could be an important clue as to how WEE1 is converted from an inhibitor of Cyclin B1 to a substrate at the end of G2 phase.

We will pursue the mechanism and regulation of phosphorylation and of the Cyclin-binding motif in this project.

Project aims

• Determine the pathway regulating WEE1 activatory phosphorylation and how this controls S phase and mitosis.

• Determine how the switch from Cyclin A to Cyclin B1 binding is regulated and whether this determines the timing of the decision to enter mitosis.

• Determine the role of WEE1 after cells enter mitosis and in ensuring proper chromosome segregation.

Research proposal

1) Activatory phosphorylation:

We have raised phospho-specific antibodies to the novel phosphorylation site on WEE1 and will determine how and where WEE1 is phosphorylated on this site by immunoblotting and by immunofluorescence on cells synchronised by treatment and release from the Cyclin D-CDK inhibitor palbociclib. We will generate cell lines in which we replace endogenous WEE1 with alanine or aspartic acid mutants. To replace endogenous WEE1 we use a PROTAC that degrades endogenous WEE1[6] and have introduced a mutation in the exogenous WEE1 constructs that makes them resistant to the PROTAC. It takes about 1.5 hours to degrade WEE1 with the PROTAC, which will allow us to degrade it in G2 phase without interfering with the bulk of DNA replication. We will attempt to elucidate the pathway that regulates WEE1 phosphorylation. Our phospho-mass spectrometry results lead us to the hypothesis that WEE1 is phosphorylated in response to stress and may be responsible for the antephase checkpoint that delays mitosis[7]. This is relevant to cancer because the antephase checkpoint is absent in many cancer cells due to epigenetic silencing of its CHFR effector protein. If WEE1 phosphorylation is responsible for the mitotic delay induced by the antephase checkpoint, we will test whether it is directly phosphorylated by a p38 kinase family member, since p38 kinase activity is required for the antephase checkpoint[8]. The primary sequence of the WEE1 phosphorylation site indicates it is likely to be favoured by CK1 or, intriguingly, NIM1 kinase (Never in Mitosis kinase family).

2) Cyclin-binding motif

We will test our hypothesis that phosphorylation of the novel Cyclin-binding motif, potentially working in combination with the first RxL motif (at residues 180-182) is responsible for converting WEE1 from an inhibitor to a substrate of Cyclin B-CDK1. We have raised antibodies to the serine adjacent to the Cyclin-binding motif and will express motif and alanine or aspartic acid mutants in cells as above to determine their effect on changing interaction partners and the decision to enter mitosis. Our hypothesis is that phosphorylation allows Cyclin B1 to bind to the N-terminus in a conformation that makes WEE1 a substrate of Cyclin B1-CDK1 instead of an inhibitor. Should our data support this hypothesis we will consult with our Structural Biology colleagues about using single particle EM to solve the relevant structures. In the same way as for the activatory phosphorylation, we will attempt to elucidate the pathway that controls Cyclin-binding motif phosphorylation. Our initial hypothesis is that Cyclin A is the kinase responsible because the site conforms to a CDK consensus site.

3) WEE1 activity in mitosis

Our phospho-mass spectrometry analyses showed that some WEE1 activity remained in mitotic cells, and we found that adding a WEE1 inhibitor perturbed mitotic exit. Thus, it appears that WEE1 may also have a role in inactivating Cyclin B-CDK1 to promote exit from mitosis. We will analyse this in relation to the activatory mutation in particular, since our pilot experiments show that mutations at this site perturb chromosome segregation. This aspect of the project will integrate with other work in the lab on the control of Cyclin B1 destruction by the Anaphase Promoting Complex/Cyclosome.

Overall, this project will explore novel and potentially important pathways and mechanisms that control WEE1. This has obvious relevance to genome stability, since the proper control of WEE1 is crucial to prevent premature mitosis and ensure equal segregation of fully replicated and undamaged chromosomes.

Literature references

1) Crncec, A. & Hochegger, H. Triggering mitosis. *FEBS Lett.* **593,** 2868–2888 (2019

2) Wieser, S. & Pines, J. The Biochemistry of Mitosis. Csh Perspect Biol 7, a015776 (2015).

3) Matheson, C. J., Backos, D. S. & Reigan, P. Targeting WEE1 Kinase in Cancer. Trends Pharmacol Sci 37, 872–881 (2016)

4) Gallo, D. et al. CCNE1 amplification is synthetic lethal with PKMYT1 kinase inhibition. Nature 604, 749–756 (2022).

5) Örd, M., Winters, M. J., Subbanna, M. S., Garrido, N. de M., Cushing, V. I., Kliche, J., Benz, C., Ivarsson, Y., Greber, B. J., Pryciak, P. M. & Davey, N. E. High-throughput investigation of cyclin docking interactions reveals the complexity of motif binding determinants. *Nat. Commun.* **16,** 7622 (2025).

6) Li, Z., Pinch, B. J., Olson, C. M., Donovan, K. A., Nowak, R. P., Mills, C. E., Scott, D. A., Doctor, Z. M., Eleuteri, N. A., Chung, M., Sorger, P. K., Fischer, E. S. & Gray, N. S. Development and Characterization of a Wee1 Kinase Degrader. *Cell Chem. Biol.* **27,** 57-65.e9 (2020)

7) Mikhailov, A., Shinohara, M. & Rieder, C. L. Topoisomerase II and histone deacetylase inhibitors delay the G2/M transition by triggering the p38 MAPK checkpoint pathway. J Cell Biol 166, 517–526 (2004).

8) Matsusaka, T. & Pines, J. Chfr acts with the p38 stress kinases to block entry to mitosis in mammalian cells. J Cell Biology 166, 507–16 (2004)

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 BA in Biochemistry or Molecular Cell Biology

Intended learning outcomes:

- Proper experimental design: concept of the assay and the control
- Quantitative data analysis and interpretation
- CRISPR/Cas9 gene editing
- Time-lapse fluorescence microscopy

	Protein biochemistry (affinity purification, protein kinase assays, interaction mapping by mass spectroscopy)

Advertising details

Project suitable for a student with a background in:	☒ Biological Sciences ☐ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science