PhD Project Proposal

Funder details

Studentship funded by: MRC DTP

Project details

Project title: Determining the role of Cyclin B2 in Genomic Stability

Supervisory team

Primary Supervisor: Jon Pines

Associate Supervisor(s):

Secondary Supervisor: Jörg Mansfeld

Divisional affiliation

Primary Division: Cancer Biology

Primary Team: Cell Division

Site: Chelsea

Project background

Mitosis is controlled by the major mitotic kinase CDK1 and its regulatory partner, Cyclin B. There are two B-type cyclins in vertebrate cells: Cyclins B1 and B2. Cyclin B1 is essential but the role of Cyclin B2 is controversial. Using CRISPR/Cas9 gene editing, this project will investigate the role of Cyclin B2 in cell division and genome stability.

In my research group, we originally showed that Cyclin B2 localises to the Golgi apparatus and promotes its disassembly in mitosis, whereas Cyclin B1 was able to reorganise the whole intracellular architecture. This more circumscribed role of Cyclin B2 agreed with the results of genetic knockouts in the mouse, where Cyclin B1 is essential for cell division but Cyclin B2 loss only results in a reduced litter size. More recent studies, however, have reported more important roles for Cyclin B2 in genomic stability, including regulating centrosome separation; MAD2-dependent recruitment to the kinetochore to regulate chromosome segregation; inhibiting separase; and functionally replacing Cyclin B1 in somatic cells. Increased levels of Cyclin B2 are also associated with poor prognosis in a variety of cancers.

This multi-disciplinary project will combine cell biology, biochemistry and proteomics, with a strong emphasis on quantification for the fluorescence time-lapse microscopy and proteomics data.

Project aims

To determine the role of Cyclin B2 in somatic cell division by:

- Tagging endogenous Cyclin B2 and determining its localisation and stability through the cell cycle
- Identifying the interactome of Cyclin B2 in the presence and absence of Cyclin B1
- Determining the extent to which Cyclin B1 and Cyclin B2 can substitute for each other
- Determining the effect of increased levels of Cyclin B2 on genomic stability

Research proposal

Experimental approaches:

We will use CRISPR/Cas9 gene editing to introduce a fluorescent protein, an epitope tag, or a mini-auxin-inducible degron (AID) into the endogenous Cyclin B2 protein. The fluorescent protein tag will be inserted between the unstructured N-terminus and the first cyclin fold, which has been shown to preserve all the properties of B-type cyclins in fission yeast. We will characterise the behaviour of the tagged Cyclin B2 through the cell cycle and determine its localisation and the timing of its degradation. This will require quantitative fluorescence microscopy.

With the AID-tagged Cyclin B2 we will analyse the behaviour of cells in which Cyclin B2 is rapidly depleted at specific points in the cell cycle. We will visualise different cytoskeletal elements in the cell using Golgi, ER and microtubule-targeted fluorescent probes. We will also AID-tag Cyclin B1 in the genetic background of fluorescently-tagged Cyclin B2 to determine whether Cyclin B2 can compensate for Cyclin B1 and whether it changes its subcellular localisation to do so.

In parallel with these cell biological studies, we will use CRISPR/Cas9 gene editing to introduce a biotin ligase tag into the Cyclin B2 locus in the genetic background of wild type and AID-Cyclin B1 cells. We will use these cell lines to perform BioID labelling to determine the interactome of Cyclin B2 in the presence and absence of Cyclin B1 through the cell cycle. This will give further insight into the degree of specialisation and redundancy between the two B-type cyclins. Proteins interacting specifically with Cyclin B2, and proteins that only interact in the absence of Cyclin B1, will be prioritised for further characterisation.

Depending on the results of these analyses, we may find that Cyclin B2 is indeed primarily associated with the membrane compartment. If so, we will identify the region of Cyclin B2 required to bind to this compartment and determine how preventing membrane binding alters Cyclin B2's properties. Membrane association would also raise the intriguing proposition that Cyclin B2-CDK1 complexes would be mostly under the control of the MYT1 kinase that is ER-associated, rather than WEE1 that is mostly nuclear. Cells have been shown to be particularly reliant on MYT1 to prevent premature mitosis when Cyclin E is overexpressed, and we will characterise the behaviour of Cyclin B2 in cells over-expressing Cyclin E. We will compare this to the effect on cell division of over-expressing Cyclin B2 itself and whether this can contribute to tumourigenesis.

Literature references

[1] Jackman M, Firth M, Pines J . (1995). Human cyclins B1 and B2 are localized to strikingly different structures: B1 to microtubules, B2 primarily to the Golgi apparatus. EMBO J 14: 1646–1654.

[2] Draviam VM, Orrechia S, Lowe M, Pardi R, Pines J . (2001). The localization of human cyclins B1 and B2 determines CDK1 substrate specificity and neither enzyme requires MEK to disassemble the Golgi apparatus. J Cell Biol 152: 945–958.

[3] Brandeis, M. et al. Cyclin B2-null mice develop normally and are fertile whereas cyclin B1-null mice die in utero. Proc. Natl Acad. Sci. USA 95, 4344–4349 (1998).

[4] Bellanger, S., de Gramont, A. & Sobczak-Thépot, J. Cyclin B2 suppresses mitotic failure and DNA re-replication in human somatic cells knocked down for both cyclins B1 and B2. Oncogene 26, 7175–7184 (2007). https://doi.org/10.1038/sj.onc.1210539

[5] Nam, HJ., van Deursen, J. Cyclin B2 and p53 control proper timing of centrosome separation. Nat Cell Biol 16, 535–546 (2014). https://doi.org/10.1038/ncb2952

[6] Liu, S., et al. Mad2 promotes Cyclin B2 recruitment to the kinetochore for guiding accurate mitotic checkpoint. EMBO Reports (2022) 23:e54171 https://doi.org/10.15252/embr.202154171
[7] Li J, Ouyang YC, Zhang CH, Qian WP, Sun QY (2019) The cyclin B2/CDK1 complex inhibits separase activity in mouse oocyte meiosis I. Development 146: dev182519
[8] Hégarat et al., Cyclin A triggers Mitosis either via the Greatwall kinase pathway or Cyclin B. The EMBO Journal (2020) 39:e104419 https://doi.org/10.15252/embj.2020104419
[9] Gallo, D., et al., CCNE1 amplification is synthetic lethal with PKMYT1 kinase inhibition. Nature 2022 604 (7907):749-756. doi: 10.1038/s41586-022-04638-9

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: Bachelor's degree in biological science

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:

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