The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	What does it take to trigger mitosis?
SUPERVISORY TEAM	
Primary Supervisor:	Professor Jonathon Pines
Other supervisory team members:	Dr Jyoti Choudhary and Professor Jessica Downs
DIVISIONAL AFFILIATION	
Primary Division:	CANCER BIOLOGY
Primary Team:	Cell Division

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

The decision to begin cell division (mitosis) is highly conserved through evolution. The core principal is that a mitotic Cyclin-dependent kinase (Cdk) is activated and its antagonistic phosphatases are inhibited: this acts as a bi-stable switch that triggers the fundamental changes in the architecture of the dividing cell. Although this core principle has been defined through experiments in many different model systems, how the trigger is pulled in mammalian cells is made complicated by the presence of multiple isoforms of the components, many of which are overexpressed in cancer cells. With the advent of CRISPR/Cas9 gene editing it is now feasible to take a leaf out of Sir Paul Nurse's fission yeast book to knockout all but one isoform of each mitotic regulator (Cyclin A, Cyclin B, Cdc25, Wee1) to generate the 'minimal' mammalian cell. We will combine this with an analogue sensitive version of Cdk1 to obtain a cell in which we can exactly control of the activity of Cdk1.

We have already begun to generate the minimal cell: we have removed two isoforms of Cdc25 (B & C) and found that cells are viable just with Cdc25A - they are slightly slower through G2 phase but otherwise normal – and we have fluorescently tagged Cdc25A. We have clones of cells where we have removed the Myt1 kinase, which means that cells are viable with just Wee1. We are currently testing which of the mitotic cyclins is essential; already we are confident that we can remove Cyclin B2. We have also tested the mutations required to make Cdk1 analogue-sensitive but preserve kinase activity. Thus, we are confident that our strategy Is viable.

Our first aim will be to define the minimal machinery required for proper mitosis; subsequently we will determine how altering component levels, or introducing other components, affects the control of cell division. We will achieve our aims by combining CRISPR/Cas9 gene-targeting with time-lapse fluorescence imaging and proteomic analyses to define exactly what it takes for the cell to trigger mitosis.

PROJECT AIMS

• Generate the minimal mammalian cell cycle machinery with only one isoform of Cdc25 (Cdc25A), Wee1, Cyclin A

and Cyclin B

• Add a fluorescent protein or Halo tag to each component of the mitotic trigger to analyse their localisation and their proteolysis during the cell cycle; mutate Cdk1 to generate an analogue-sensitive kinase

• Determine the phosphorylation state and interacting partners for each component through the cell cycle by mass spectrometry

• Determine the activity of the mitotic trigger using biosensors to generate a kinetic model of the decision to enter mitosis

• We will subsequently work towards determining how altering the level of individual components, or introducing other isoforms, alters entry to mitosis and the fidelity of chromosome segregation

RESEARCH PROPOSAL

We will continue our strategy of using CRISPR/Cas9 to introduce frame shifts close to the start codon of mitotic regulators to generate null mutants. We select these CRISPR/Cas9 targeted clones by FACS and validate them by genomic PCR followed by immunoblotting and/or mass spectrometry analysis. We characterise the cell cycle in the clones using time-lapse fluorescence microscopy: we measure the length of G1, S and G2 phases using a fluorescent PCNA marker, and the length and fidelity of mitosis using DIC and a Sir-DNA marker. In particular, we analyse clones to determine whether the length of G2 phase is changed, which would indicate whether mitosis is triggered earlier or - more likely - later than normal. Subsequently, we analyse whether clones are more or less sensitive to insults such as DNA replication poisons or DNA damage, and how rapidly the clones can recover from the damage to begin mitosis. With respect to the fidelity of mitosis, we analyse whether chromosomes are captured efficiently or whether there is a delay; if there is a delay we analyse whether this is dependent on the spindle assembly checkpoint. Once cells have captured all their chromosomes we analyse the speed with which they exit from mitosis, the ability of cells to perform proper cytokinesis, and finally whether daughter cells remain separate and correctly reform their nuclei.

We will introduce fluorescent and affinity (Flag) tags into each of the key mitotic regulators and use affinity chromatography and cross-linking to isolate the proteins that interact with each regulator at different stages in the cell cycle. Using cross-linkers will allow us to identify more transient interactions. An alternative to cross-linking is to use Turbo Bio-ID to tag key regulators with a biotin ligase that will enable us to identify those proteins that are in close proximity. These mass spectrometry studies will be carried out in collaboration with Dr Jyoti Choudhary. The fluorescent tags will allow us to measure the levels of the proteins through the cell cycle, and whether they change in response to cell cycle perturbations, as well as to determine their subcellular localisation. Thus we will build up a map of the dynamic interactions of the minimal network of proteins that trigger mitosis. To measure the kinetics with which the trigger is pulled we will use our specific Förster Resonance Energy Transfer (FRET) biosensor to assay the increase in Cyclin B-Cdk1 protein kinase activity.

We will use the data on protein accumulation and degradation, kinetics of enzyme activation and inactivation, and changes in subcellular localisation, to generate a model of how entry to mitosis is triggered. Based on our current knowledge, this is likely to involve a series of positive and double negative feedback loops that depend on Cyclin-Cdk activity. We will be able to test this using the 1NM-PP1 inhibitor to adjust the activity of our analogue-sensitive version of Cdk1. Using this inhibitor we will be able to test models of ultra-sensitivity and bi-stability for entry to mitosis, i.e. that more Cdk1 kinase activity will be required for cells to enter mitosis than for cells to remain in mitosis. The Cdk1as cells will also enable us to test the hypothesis that different thresholds of Cdk1 activity activate different macro-molecular machines to reorganise the cell as it enters mitosis in a coordinated fashion.

Once we have built up an understanding of the minimal machinery required to coordinate entry to mitosis we will begin to perturb the system to identify what happens in cancer cells when specific components are overexpressed. We will determine the effect on chromosome stability and on the ability of cells to respond correctly to replication stress or DNA damage. We are particularly interested in expressing some of the meiotic components, such as Cyclin A1, since meiotic genes are often inappropriately activated in cancer cells.

By the end of this study we should have an understanding of exactly what it takes to trigger mitosis, to coordinate the changes in the mitotic cell, and to ensure equal chromosome segregation to two daughter cells. We should have built a model for how mitosis is triggered in mammalian cells, and we should have begun to understand how this is deregulated in cancer cells.

LITERATURE REFERENCES

Strauss, B., Harrison, A., Coelho, A.A., Yata, K., Zernicka-Goetz, M., and Pines, J. (2018)
Cyclin B1 is essential for mitosis in mouse embryos, and its nuclear export sets the time for mitosis
J. Cell Biol., **217**, 179-193
Pagliuca, F., Collins, M.O., Lichawska, A., Zegerman, P., Choudhary, J.S. and Pines, J. (2011)
'Quantitative Proteomics Reveals the Basis for the Biochemical Specificity of the Cell Cycle Machinery'
Mol Cell, **43**, 406-417.
Gavet, O. and Pines, J. (2010)
'Progressive activation of Cyclin B1-Cdk1 coordinates entry to mitosis'
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'Activation of Cyclin B1-Cdk1 synchronizes events in the nucleus and the cytoplasm at mitosis'
J. Cell Biol. **189**, 247-259.
Reviews:
Wieser, S. and Pines, J. (2015) 'The biochemistry of mitosis'
Cold Spring Harbor Perspectives in Biology a015776
Pines, J. and Hagan, I. (2011) 'The Renaissance or the Cukoo clock'.
Philosophical transactions of the Royal Society of London. Series B, Biological sciences (Special issue in honour of Paul Nurse) **366**, 3625-3634**.**

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)	Higher second or first class degree in biological science or biophysics
Intended learning outcomes:	Experimental design Critical data analysis Literature review and critique Live cell microscopy Image processing and analysis Proteomic analysis