Quantitative High-Resolution Analysis of Cell Cycle Regulated Protein and Phosphorylation Abundance in Non-Transformed Cells

By

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A Master’s Thesis

Barts and The London School of Medicine and Dentistry

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August 2025

Word count:

# Acknowledgements

I would like to express my sincere thanks to Doctor Tanya Soliman of the Barts Cancer Institute for providing the needed data for the project and for her helpful advice and supervision throughout.

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# Abstract

The cell cycle consists of a highly regulated sequence of events that result in the cell splitting into two daughter cells. It consists of several stages, each of which is covered by changes in protein and phosphorylation abundance.

Various papers have addressed changes in these abundance levels in cancerous cells. Here we analyse non-transformed cells to offer a baseline reference for these papers. We achieve this by analysing changes in abundance in proteomic and phosphoproteomic in TODO – copy from Tanya. with a higher greater temporal resolution than the bulk of other investigations. This has revealed various significant findings regarding proteins and phosphorylation events involved in the cell cycle, including oscillations

# Introduction

The reproduction of cells is achieved through a highly regulated sequence events known as the cell cycle [1]. In eukaryotic cells this process is highly complex and an area of ongoing study, having various aspects that still poorly understood. It consists of a sequence of discrete stages. The first and longest is G1, consisting of duplication of the majority of various organelles (ribosomes, mitochondria) and an increase in cell size [2]. It may then proceed to the S phase and duplication of the chromosomes into two sister chromatids. The cycle then progresses to the G2 phase, a period of rapid growth, before proceeding to the highly complex M phase. The M (for mitosis) phase itself consists of five phases (prophase, prometaphase, metaphase, anaphase and telophose) culminating in splitting of the nucleus into two identical nuclei. This is then rapidly followed by fission of the cell during cytokinesis.

Progression through the cell cycle is controlled by interactions between a complex network of proteins and phosphorylation events. Protein abundances are tightly controlled by transcription, translation and degradation, with phosphorylation levels being regulated by kinases and phosphatases [3]. Amongst the most significant of these are cyclin dependent kinases (CDKs) [4] which are themselves subject to phosphorylation.

# Aims and Objectives

The aim of the study was to identify proteins and phosphorylation events relevant to the cell cycle process.

# Materials and Methods

The data consists of two spreadsheets of abundances, one for proteins and one for phosphopeptides. Each entry has readings for ten timepoints within the cell cycle. These data are analysed by software written in Python, pytest, Django, Postgres, Docker and D3.js. The steps in the analysis consist of:

Clean and import protein abundances by timepoint from proteomics spreadsheet.

Clean and import phosphopeptide abundances by timepoint from phosphopeptides spreadsheet.

Calculate medians for raw protein and phosphopeptide data.

Calculate log2 means and various metrics (ANOVA and Fischer p value and q values TODO - confirm) for proteins and phosphopeptides.

Calculate protein and phosphopeptide oscillations.

Identify relevant proteins and phosphopeptides – those with low q values (TODO – confirm ideal q values) and high curve fold changes (> 1.2)

Classify by group, complex and subcellular localisation (TODO page 6 ‘Biological Role of cell-cycle dependent proteins – function written for CORUM).

Identify those of above that are high/low variability and oscillation.

Generated various charts from the data (Fig TODO)

SEE ALSO page 14, Defining cell cycle-dependent (CCD) proteins and phosphorylation sites

Outline how the curve fold threshold of 1.2 was determined, if done (Page 15, Protein curve fold change cut-offs were defined by quantifying theenrichment of the‘cell cycle’ term).

# Results

Where the phosphorylation sites are and whether they’re disordered or accessible (end of page 8).

# Discussion

Page 12

# Conclusion

# Additional Information

# References