***Organising the cell cycle in the absence of transcriptional control: Dynamic phosphorylation co-ordinates the Trypanosoma brucei cell cycle post-transcriptionally***

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*PLoS Pathogens* 15(12): e1008129. <https://doi.org/10.1371/journal.ppat.1008129>

**Abstract**

 The cell division cycle of the unicellular eukaryote *Trypanosome brucei* is tightly regulated despite the paucity of transcriptional control that results from the arrangement of genes in polycistronic units and lack of dynamically regulated transcription factors. To identify the contribution of dynamic phosphorylation to *T. brucei* cell cycle control we have combined cell cycle synchronisation by centrifugal elutriation with quantitative phosphoproteomic analysis. Cell cycle regulated changes in phosphorylation site abundance (917 sites, average 5-fold change) were more widespread and of a larger magnitude than changes in protein abundance (443 proteins, average 2-fold change) and were mostly independent of each other. Hierarchical clustering of co-regulated phosphorylation sites according to their cell cycle profile revealed that a bulk increase in phosphorylation occurs across the cell cycle, with a significant enrichment of known cell cycle regulators and RNA binding proteins (RBPs) within the largest clusters. Cell cycle regulated changes in essential cell cycle kinases are temporally co-ordinated with differential phosphorylation of components of the kinetochore and eukaryotic initiation factors, along with many RBPs not previously linked to the cell cycle such as eight PSP1-C terminal domain containing proteins. The temporal profiles demonstrate the importance of dynamic phosphorylation in co-ordinating progression through the cell cycle, and provide evidence that RBPs play a central role in post-transcriptional regulation of the *T. brucei* cell cycle.

Data are available via ProteomeXchange with identifier PXD013488.

**Proteomic data availability:**

 To make our data accessible to the scientific community, we uploaded our study to TriTrypDB (<http://www.tritrypdb.org>) and deposited the Thermo RAW files and search engine output into ProteomeXchange (<http://www.proteomexchange.org>) consortium via the Pride partner repository with the dataset identifier PXD013488, enabling researchers to access the data. More detailed metadata of the proteomic files are given below

**Metadata and associated files**

 The following Excel files describe the metadata for each RAW file for the proteomic and phosphoproteomic analysis.

PURE-metadata.xls

 The following Excel files are the processed and analysed data presented in the supplementary material given in the associated publication

S2 Table. 5,949 Phosphorylation sites quantified at all six time points.

S3 Table. 3,619 Proteins quantified at all six time points.

S4 Table. 917 cell cycle regulated phosphorylation sites.

S5 Table. 443 Cell cycle regulated proteins.

S6 Table. Immunoprecipitation of *T. brucei* CSBPII