Protein Kinase Involvement in *Leishmania* Cell Cycle Regulation Revealed Using Chemical Genetics

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Cell division is a core biological process for both multicellular and unicellular organisms. It is a conserved process in eukaryotes, but trypanosomatids are evolutionary diverse, consequently they have some unique aspects to their cell division that is reflected in the repertoire of proteins involved in the process. Here, we used genetic and chemical approaches to explore the role of some essential protein kinases in cell cycle progression. We used CRISPR-Cas9 to perform precision editing of the *L. mexicana* genome to generate analogue sensitive mutants suitable for chemical genetic inhibition. For the kinetochore protein kinase KKT2, the cyclin-dependent kinase CRK9 and a CMGC family proteins kinase CMGCa, a replacement of the bulky gatekeeper methionine residue with a glycine in the ATP-binding site makes the enzymes sensitive to the bulky inhibitor 1NM-PP1. For the kinetochore protein kinases CLK1 and CLK2 (also known as KKT10 and KKT19, respectively) replacement of a cysteine near to the ATP-binding domain prevents binding of the covalent Michael-acceptor in the inhibitor AB1, validating the specificity of this compound against CLK1/CLK2. The chemical validation demonstrated that these protein kinases are essential for the promastigote and intracellular amastigote stages of the parasite. The specific inhibition of CLK1/CLK2, KKT2 and CMGCa caused a cell cycle arrest in G2/M stage of the promastigote. A further investigation, by fluorescence microscopy labelling the mitotic spindle, revealed that KKT2 inhibition is followed by a significant accumulation of cells in early mitosis, where mitotic spindle coordination in the nucleus failed. Furthermore, it was observed that CMGCa inhibition also impaired chromosome segregation, but mitosis reaches a more advanced stage, suggesting CMGCa activity is required later in mitosis than KKT2. In addition, CLK1/CLK2 inhibition doesn't affect the coordination of the mitotic spindle, but it blocks cell cycle progression in cytokinesis. These studies bring new insights into the essential biological process of cell division in *Leishmania* and provide a source of new potential therapeutic targets.