Chemical genetic approaches to study the role of protein kinases in *Leishmania* cell cycle regulation.

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Cell division is a core biological process during the development of both multicellular and unicellular organisms. It is a conserved process throughout eukaryotes, which has diverse evolutionary roots, resulting in a unique repertoire of protein component in Trypanosomatids, including *Leishmania*. Some of these components has been identified in *Leishmania*, but the extension of its repertoire and their role in the coordination of the cell cycle remains unclear. 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, CRK9 and CMGCa 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 specific inhibition of CLK1, CLK2, KKT2 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 the cell body development 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.