Wee1 in *Trypanosoma cruzi*: evidence of conserved functions in cell cycle regulation and DNA damage response

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The classical model of eukaryotic cell cycle regulation relies on the sequential activation of cyclin–CDK complexes, coordinated by checkpoint mechanisms that ensure orderly phase progression. In trypanosomatids, cell cycle regulation involves cyclins and CRKs (Cdc2-related kinases); however, the underlying molecular interactions and regulatory mechanisms remain incompletely understood. Among negative regulators, the kinase Wee1 is, to date, the only kinase inhibitor identified by orthology in these organisms and is present in three trypanosomatids, including *Trypanosoma cruzi*. In model eukaryotes, Wee1 mediates inhibitory phosphorylation of CDKs, integrating intra-S, G2/M, and anaphase checkpoints, thereby coordinating cell cycle progression with DNA damage repair. Given that Chagas disease treatment relies on benznidazole, a DNA-damaging drug, and that Wee1 is present in *T. cruzi*, it is relevant to explore whether inhibition of this kinase could modulate parasite response to genotoxic stress, as observed in antitumor therapies where Wee1 inhibition sensitizes cells to DNA-damaging agents. A combinatorial approach targeting Wee1 and benznidazole constitutes a plausible strategy to enhance treatment efficacy, pending experimental validation. Despite its identification, the functional roles of Wee1 in *T. cruzi* remain largely unexplored. We aimed to characterize Wee1 expression and localization throughout the cell cycle and during metacyclogenesis, as well as to investigate the consequences of Wee1 loss on growth and genome stability. CRISPR/Cas9 was used to insert a c-Myc tag at the 5′ region of Wee1 and to generate hemi- and double knockout parasites. Edits were validated by PCR, Western blotting, and growth curves. Hydroxyurea synchronization revealed differential Wee1 expression, peaking in S phase. During metacyclogenesis, Wee1-Myc was undetectable by Western blot and confirmed by immunofluorescence. Throughout the cell cycle, Wee1 localized predominantly in the nucleus, except during mitosis. Knockout parasites exhibited increased growth, elevated DNA damage accumulation (49%), and a higher proportion of zoids (28%), reflecting conserved functions in DNA damage response and proliferation regulation. Treatment with the Wee1 inhibitor adavosertib (AZD1775) did not appear to inhibit *T. cruzi* Wee1, though further experiments are needed to confirm this. These findings reveal conserved patterns observed in model eukaryotes, including differential Wee1 expression during the cell cycle, nuclear exit during mitosis, and absence in non-proliferative cells. Loss of Wee1 impacts DNA damage accumulation, zoid formation, and growth, highlighting its role in conserved mechanisms of damage response and proliferation. Collectively, these observations indicate that Wee1 could play a role in controlling DNA integrity and proliferation in *T. cruzi*, highlighting a possible avenue for combinatorial therapeutic approaches with genotoxic agents.