Cell cycle synchronization in African trypanosome

Max Pendlebury, Chris de Graffenreid, Mick Urbaniak

Abstract

Trypanosoma congolense and Trypanosoma brucei are vector-transmitted parasites that cause Animal African Trypanosomiasis (AAT) – a livestock wasting disease that results in significant economic losses in sub-Saharan Africa. The regulation of the cell cycle is not fully understood in T. brucei, and much less understood in T. congolense. Greater understanding of the regulatory mechanisms underpinning the cell cycle could provide novel targets for treatment of African Trypanosomiasis. Study of the trypanosome cell cycle could be improved by utilizing reliable cell synchronization techniques.

The main drawback with synchronization protocols relying on chemical inhibition, such as hydroxyurea synchronization, is the potential for chemically induced artifacts. Centrifugal counter-flow elutriation (CCE) avoids this by separating cells based on sedimentation velocity and has been used to monitor the changes in phosphorylation status of proteins in T. brucei. CCE also avoids the drawbacks of flow cytometry cell-sorting methods which are more time consuming and result in a majority non-proliferative population. No synchronization methods have been described for T. congolense, and much less is known about the cell cycle, including the timing of nuclear and kinetoplast genome division and the changes in cell morphology.

We show data demonstrating the synchronization of both bloodstream form and procyclic form T. brucei by CCE and compare it to our current progress in synchronizing T. congolense. We also provide data comparing the synchronization of T. brucei and T. congolense by both CCE and hydroxyurea mediated arrest. Success of synchronization is judged by the uniformity of cell size and DNA content within synchronized populations, which is assessed by flow cytometry. Cell lines have also been generated in T. brucei with YFP tagged PUF9 and PLK – which have been shown to change in either abundance or localization throughout the cell cycle. Synchronization can then be assessed by western blots to show changes in abundance, and immunofluorescence microscopy to show changes in localisation. In the future, these will be compared to tagged T. congolense cell lines to assess the differences in PLK and PUF9 localisation between the species.