

Identification of gene expression profiles and co regulation events in the cell cycle of epimastigotes *Trypanosoma cruzi* using Microarray technology

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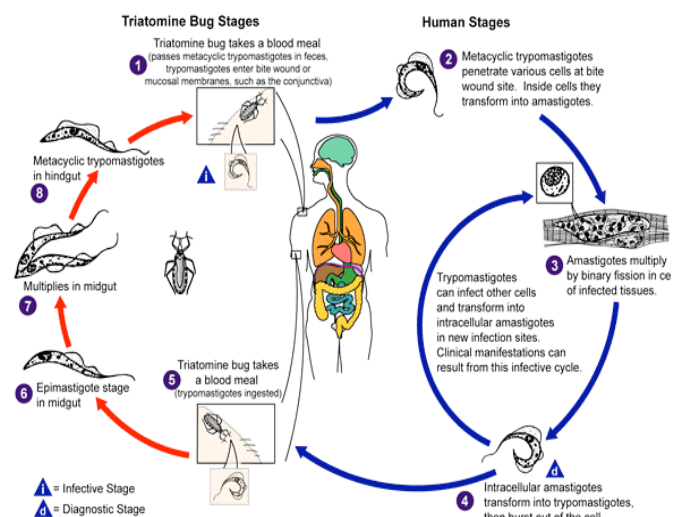
Abstract

Trypanosoma cruzi, a parasitic protozoa is the leading cause of early death due to heart disease throughout Central and South America (Perez 2005). Approximately 18 to 20 million people are currently infected with *T. cruzi*. Of those chronically infected, half of these infections go on to develop the severe gastrointestinal, and cardiomyopathy diseases characteristic of Chagas' disease. There is no vaccine available and chemotherapeutic treatments are toxic and ineffective at clearing host tissue of parasites. Although *Trypanosoma cruzi* has been studied since 1909, not much is known concerning the details of its complex life cycle. Fortunately the recent sequencing of the *T. cruzi* genome has afforded a new range of genetic approaches. Through use of techniques such as Hydroxyurea-induced synchrony, and DNA microarray I hope to identify gene expression patterns and co-regulation events in the different stages of *T. cruzi* cell cycle.

Introduction

Trypanosoma cruzi, the parasite protozoan responsible for Chagas disease exhibits a very complex life cycle. In the mammalian host, *T. cruzi* replicates intracellularly as a nearly spherical amastigote in many cell types. When the host cell ruptures, the parasites develop into non-dividing trypomastigotes.

Trypomastigotes circulate and infect other cells in remote organs and tissues. When ingested by insect vectors, trypomastigotes transform and replicate as epimastigotes in the digestive tract (Tyler, and Engman 2004). This stage is not



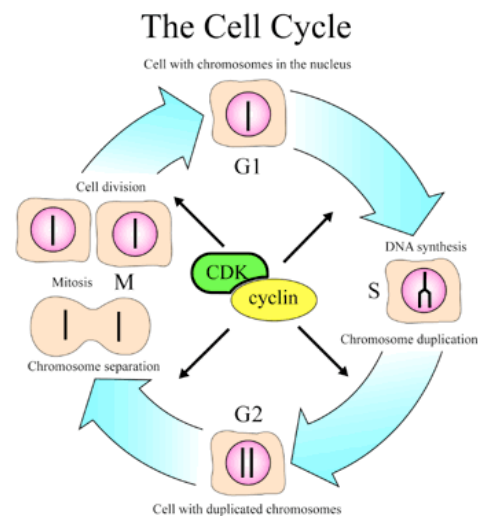
infective for mammalian hosts and is rapidly lysed by complement (Nogueira et al., 1976). Conditions in the colon and rectum of the reduviid bug vector induce metacyclogenesis, or differentiation of non-infectious epimastigotes into infectious metacyclic trypomastigotes. The cycle is completed when the bug deposits contaminated excreta near the bite wound and the metacyclic trypomastigotes are mechanically introduced into the host. The metacyclic trypomastigotes are complement resistant (Nogueira et al., 1976) and circulate in the host briefly prior to infecting a host cell. While many events of the life cycle of the parasites

have been characterized, the cell cycle remains poorly understood. In mammalian cells, the cell cycle represents the series of events between one cell division and the next. It consists of four phases: G1, S, G2 (collectively known as interphase) and M. M phase is composed of mitosis and cytokinesis. Cells that have stopped dividing are said to have entered a state of quiescence called G0. The molecular events that control the cell cycle are ordered and directional and cyclins and cyclin-dependent-kinases are believed to be the key elements on the cell's progress through the cell cycle.

In this proposal I intend to gain understanding in the genetic events associated to the cell cycle of *T. cruzi* by combining several approaches including microarray technology and bioinformatics tools. Ultimately I expect to provide scientific knowledge to better identify and exploit genetic properties of the parasite, perhaps to the extent of developing an effective therapy.

Methods

T. cruzi epimastigotes forms at different stages of its growth (log phase and late log phase) will be arrested in its cell cycle using Hydroxyurea-induced synchrony. This method involves the application of Hydroxyurea (HU), which targets the enzyme



ribonucleic reductase inhibiting DNA replication. (Calanti et.al, 1994) Following the arrest for 24 hrs, we will incubate the parasites in LIT medium supplemented with 10% fetal calf serum triggering the cell cycle. RNA collected immediately after (0hr), 6, 14, and 24 hours after release of the cell cycle (estimated length 18-24 hrs) will be extracted, labeled and hybridized to a 10,000 oligonucleotide microarray representing nearly all of the non-redundant *T. cruzi* genes. The resulting images will be collected and analyzed using a combination of several bioinformatical tools, including RMA (robust multi-array analysis) and SAM (significance analysis of microarrays), to determine which genes are differentially regulated during these experiments.

Potential results

By revealing co-regulatory events as well as expression patterns, the analysis of gene expression during various cell cycle stages will afford better understanding to the complex *T. cruzi* life cycle providing must need insight into the cell cycle of the parasite and perhaps answering questions such as: What genetic differences distinguish one cell cycle stage from the next? Are there one or more crucial genes that directly regulate the transformation from the replicative exponential phase epimastigote into the infectious non-replicative Trypomastigote?

Future applications include identifying genetic patterns that correlate with molecular biological data may provide the framework for the makings of a treatment by which differentiation of the parasite to its infective stage is inhibited.

Summer 2006 results and future direction

Experiment attempts yielded successful hybridizations for only the 0hr, and 14 hr RNA extractions. While an incomplete set of data prevents holistic characterization of the epimastigote cell cycle, certain differentially expressed genes noted from my preliminary analysis support biological assumptions of parasite replication. For instance, an upregulation of DNA polymerase, cytoskeleton components, and cAMP specific phosphodiesterase at the time of release from Hydroxyurea (0 hr) is indicative of a cell

preparing for synthesis of DNA (S phase). Results at 14 hours after release from Hydroxyurea also produced results that verified biological assumption; particularly noteworthy is the upregulation of Mitotic centromere-associated kinesin, a currently putative protein in the *cruzi* genome sequence. The upregulation of this gene may not only provide a marker for identifying epimastigotes in the late M phase, but provides an affirming biological context for the identity of this particular gene.

Duplicate experiments of RNA extraction and hybridization from various time points will be performed and to attain a complete data set, and provide statistical backing. Upon securing complete data set and replicates, analysis will be run using programs such as TMEV and TIGR to characterize different points of the cycle based on differentially expressed genes and co-regulation events. I will then confirm the observations made from analysis using RT PCR as well as visualize parasite replicative states using immunofluorescence microscopy.