Introduction:

Trypanosoma Cruzi is the protozoan agent responsible in the deadly chagas disease which is mostly found in South American countries. The triatomine vector puts 90 million people at risk of the disease; from the southern tip of the United States of America to the province of Chubut in Argentina. However incidence of the disease by the vector is rare in the United States because of better living conditions [1]. This disease agent exhibits three phenotypic characteristics during its life cycle. These include the amastigote form, which is found inside of the host cell and is replicative, the epimatigotes which are extracellular and also replicative and then the infective stage of the protozoan which is the trypomatigote [2]. Over the decades, research has been done to understand the unique characteristics of this protozoan. One biological process that has not been widely studied in trypanosomes is DNA methylation. Methylation is an enzyme induced modification of DNA that occurs after replication and epigenetically affects transcriptional modification [3]. While some data shows that methylation in trypanosomes have some kind of effect on DNA protein interaction and DNA conformation and subsequent effect of gene expression, the extent of the effect is still not known; specific genes that are turned on or off in the presence of demethylation agents like (5Azac) and the extent to which it affect the parasitic ability of the protozoan remains unknown. Genes can be silenced by the activity of DNA methylation, this is done by the direct obstruction of the interaction between transcription factors and their regulatory sequences. DNA methyltranferases are enzymes that catalyze DNA methylation in humans and higher eukaryotes, this usually occurs in C5- cytocine (m5C) in CpG dinucleotides. Recently, a paper was published that demonstated that the

protozoan parasite *Entermeoba histolytica* had an attenuated virulence signal when treated with 5-azacytidine, however, protozoan growth was not significantly affected [3]. The incidence of attenuated virulence has not yet been seen *trypanosoma cruzi,* on the other hand, some research show that *T. Cruzi* epimatigotes multiply when treated with (5Azac) as it is seen in the increased number of cells and the inclusion of [3H-methyl] thymidime in DNA [. Our question in this investigation is to identify which genes are regulated by methylation and its role in the life cycle of the parasite.

Methods:

The experimental method that would be used in this investigation would be similar to the one done M. V. Rojos and Norbel (DNA methylation in *T. Cruzi*). Trypanosomes in the epimastigote stage of growth will be cultured for 5-7 days and treated with 5-azacytidine (5AzaC). The candidate genes would be isolated and examined using microarray analysis. Two cultures of epimatigotes would be grown the presence of (5AzaC) and the RNA would be extracted. Oligonucleotide array would be used a probe to find RNA compliments. The data that is received from the from the oligonucleotide array would be analyzed for differentially expressed genes (DEG). TMEV (Tigr multi-experiment viewer) would used to study the levels of gene expression.

Possible results and Implications

In this experiment, it is expected that we would find specific genes that are critical in methylation activities, evidence of genes that are up regulated and down regulated during methylation would also be expected. This result would elucidate our understanding of the effects of methylation and how it affects gene expression and parasitic activity.

References:

1)João Carlos Pinto Dias (in: Chagas Disease - American Trypanosomiasis: its impact on transfusion and clinical medicine. S. Wendel, Z. Brener, M.E. Camargo, A. Rassi (Edt.). ISBT BRAZIL'92, SAO PAULO, BRAZIL).

2)Rojos M, Galatini N,(1990) DNA methylation in *Trypanosoma Cruzi* (1-7), (12-15) [1]

3)Ibne Karim M Ali1, Gretchen M Ehrenkaufer1, Jason A Hackney1 and Upinder Singh*(2007), Growth of the protozoan parasite Entamoeba histolytica in 5-azacytidine has limited effects on parasite gene expression.Zigman Brener, S.