

Epigenomics in *Trypanosoma cruzi*: Role of Methylation in Gene Expression

Introduction

The Chagas disease is still one of the most deadly diseases in South America today. The parasite responsible for this disease is the *Trypanosoma Cruzi* which is carried by the triatomine bug. The disease affects millions of people in central and South America [1]. One intriguing characteristic of the parasite is its ability to thrive and replicate within muscle cells for 30 years before killing its host. In 30% of the cases, individuals develop severe damage to the digestive tract, cardiac and smooth muscles. The 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 epimastigotes which are extra cellular and also replicative and then the infective form of the protozoan, the trypomastigote [2]. A lot of research efforts are being made to understand the complex biological functions of this parasite. However, information on the epigenomic events like DNA methylation is limited.

While some data show 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 up regulated or down regulated in the presence of a demethylation agent like (5azaC) and the extent to which it affect the parasitic ability of the protozoan remains unknown.

It has been found that there are methylated bases in the *T. Cruzi* genome specifically, 5-methylcytosine ($m^5\text{Cyt}$) and possibly N6- methyladenine ($m^6\text{Ade}$) [2]. The occurrence of methylated bases are in sites that are different from CpG sites as they are characterized in other organisms. In *T. Cruzi*, methylated bases are found in regions possibly XCGX. [2]. Building on some of this evidence, our quest this past summer was to identify genes that are under the control of a Methylation. In this task, we sought to block methylation with the use of a potent demethylation agent, namely 5-azaCytidine (5-azaC). The drug works by blocking the action of an enzyme that transfers a methyl group.

Progress Report.

In our experiment, we cultured epimastigotes in the demethylation drug (5-azaC) for 3-6 days. One set was treated with the drug and the second set was not treated, however all other conditions were kept constant. The first set and the second set were cultured for three to six days respectively. The reason for treating the epimastigotes at a three day interval was to capture the exponential and stationary stage of the cell cycle. Predictably, these stages would have different gene expression profiles. In growing the parasites, we noticed a proliferation of the parasites. This has been noticed in other research involving 5-azaz [3]. In order to examine the genome wide effects of DNA methylation on gene expression in the parasite, we run a transcriptional profiling of parasites treated with and without 5-azaC as mentioned above. The parasites were treated with 25uM of the drug. The extracted RNA was hybridized to 12000 short oligonucleotide microarrays. We identified 1035 genes (10%) that were affected by the treatment of 5-azaC, suggesting that these genes were potentially under the control of methylation.

In addition, we noticed the presence of cyclin and cyclin associated genes. Since cyclin genes are linked to the cell cycle, this could explain the increased number of cells when the drug is present. In the process of our experimentation, one of the microarray slides had a very high background; as a result we were not able to use it in our statistical analysis.

Future Directions.

For the upcoming academic year, the focus of this project would be to repeat this experiment three more times to increase the statistical significance of our results. With this in mind, an appropriate data analysis program would be sought out to further analyze the results we obtain. The repeated experiment would be followed up with the selection of genes of interest. A methylated specific PCR (MSP) would be performed to confirm the methylated status of the selected genes. In the above mention process, DNA is modified by sodium bisulfite treatment [3] converting unmethylated cytosines to uracil.

References:

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