

# Protein Interaction Between Basal Body and Kinetoplast DNA Complex Within *Trypanosoma brucei*

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## ABSTRACT

*Trypanosoma brucei* is responsible for the disease Human African Trypanosomiasis (HAT), also known as African Sleeping Sickness, in humans as well as N'gana in livestock. Our work was done on the sub-species *T. brucei brucei*, which is non-pathogenic in humans, and thus safe for undergraduate laboratory research. *Trypanosoma brucei* is part of a diverse group of flagellated protozoans known as the kinetoplastids. Kinetoplastids are named for the concentration of mitochondrial DNA located at the posterior end of the mitochondrion and adjacent to the flagellum, called the kinetoplast DNA (kDNA). The kinetoplast is connected to the basal body (motor) of the flagellum by proteins through both membranes of the mitochondrion. The flagella drive the process of binary fission (cell division), suggesting that the replicated kDNA is separated through these protein interactions. Specific proteins were tested for their participation in the connection between kDNA and basal body. Our genes of interest were inserted into plasmid vectors. The plasmids were then linearized, followed by transfection into trypanosomes. Immunofluorescence was used to identify where these proteins localized within the cell.

## INTRODUCTION

*Trypanosoma brucei* is responsible for the Human African Trypanosomiasis (HAT), also known as African Sleeping Sickness, in humans as well as N'gana in livestock. This parasite is present in 36 countries of Sub-Saharan Africa, putting approximately 60 million people at risk, and affecting an estimated 300,000 annually (World Health Organization, 2006). *T. brucei* also affects humans indirectly by decreasing the economic value of the livestock it infects. Infected cattle have decreased milk production and the milk that is produced is of lower quality. Strength and body weight is also decreased which provides less meat and affects their ability to plow fields. Because of the underproduction of cattle, an estimated \$4.5 billion is lost each year.

Trypanosome transmission to a human or animal host occurs mainly through the insect vector *Glossina morsitans*, the Tsetse fly. A form of *T. brucei* resides in the salivary gland of an insect vector. When an infected fly bites a mammalian host, the parasite is transmitted via the fly's saliva. Once in the host, the trypanosome goes through a series of transformations, evolving into the epidemiological *T. brucei* (Center for Disease Control, 2008). Initial symptoms include fever, severe headaches and extreme fatigue. The trypanosomes then attack the central nervous system, affecting mental functionality, leading to coma and eventually death.

*Trypanosoma brucei* is part of a diverse group of flagellated protozoans known as the kinetoplastids. Kinetoplastids are named for the concentration of mitochondrial DNA located at the posterior end of the mitochondrion and adjacent to the flagellum, called the kinetoplast DNA (kDNA). The kDNA consists of two types of circular DNA, one being "maxicircles" of about 25 kilobases and the other being "minicircles" of about 1 kilobase. Trypanosomes contain about 50 maxicircles and several thousand minicircles (Downey et al., 2005). Mini and maxi circles are intertwined in one another and must be separated for cell division. The kinetoplast is connected to the basal body (motor) of the flagellum by proteins through both membranes of the mitochondrion. The flagella drive the process of binary fission (cell division), suggesting that the replicated kDNA is separated through these protein interactions.

The objective of this study was to further our understanding of the kDNA and basal body complex. We tested specific proteins for their participation in the connection between these two structures. We did so by identifying where these proteins localized within the cell, and allowing for interpretation of protein function by blocking its production using RNA interference and studying the effects on the cell.

## METHODS

### *Protein Localization*

DNA was isolated from trypanosomes. Primers specific for our genes of interest were designed and used to amplify those specific genes via polymerase chain reaction (PCR). The amplified genes were then purified via PCR cleanup. Each gene was then inserted into the plasmid pLEW-82<sup>(1)</sup>. This plasmid was grown up in *E. coli*, purified and transfected into trypanosomes. The pLEW plasmid was engineered to have a hemagglutinin protein tag in frame with the gene of interest. The hemagglutinin tag was used as an antigenic target for immunofluorescence and thus localization of the protein within fixed trypanosomes.

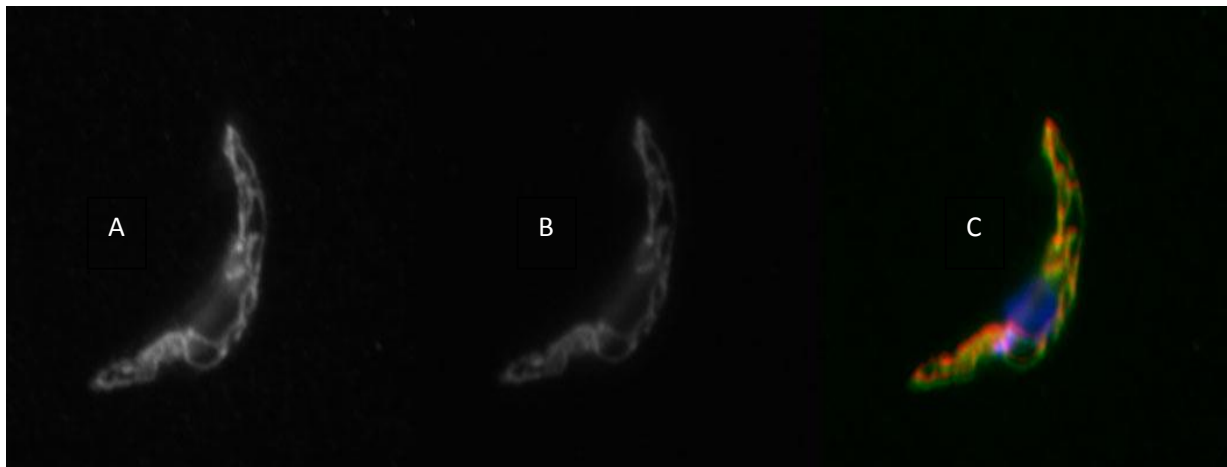
### *RNA Interference*

The purified PCR amplified genes were also inserted into the plasmid pZJM<sup>(2)</sup>. This plasmid is engineered to allow the production of double stranded RNA that leads to RNA mediated interference (RNAi) of protein production. RNAi was induced by the addition of tetracycline to cell cultures. The growth of these cultures was then monitored and compared to identical cultures that lacked the induction of RNAi. After a discrepancy was noticed in the growth rate between cultures possessing and lacking RNAi the cells were observed with confocal microscopy.

## RESULTS

### *Protein Localization*

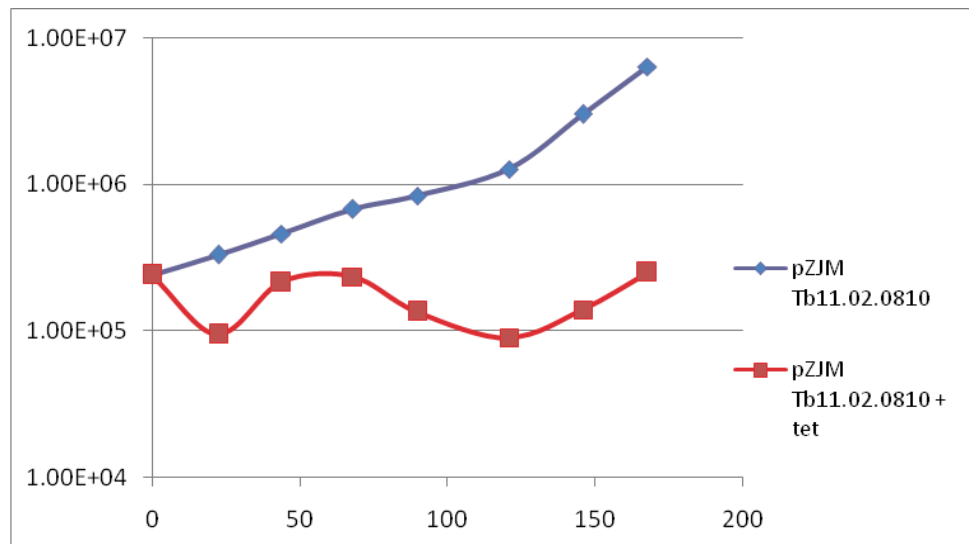
As shown in Figure 1, the protein of interest, Tb927.8.4400, was localized in accordance with the mitochondrial membrane. Our protein of interest was localized utilizing the conjugated hemagglutinin protein (Figure 1A). The mitochondrion was stained with mitotracker red (Figure 1B)



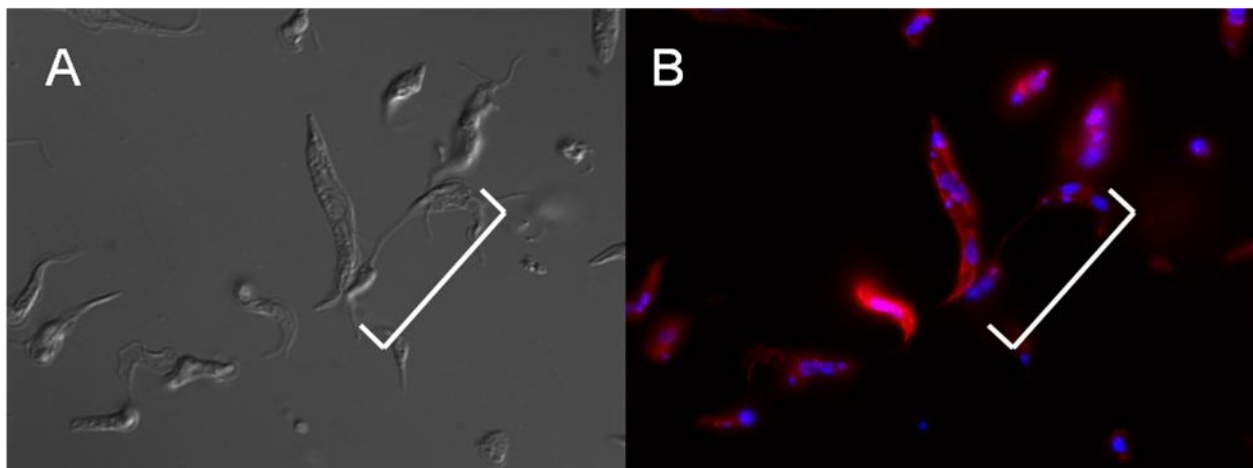
**Figure 1.** (A) Localization of gene Tb927.8.4400 using immunofluorescence via HA tag and GFP. (B) Localization of mitochondrion using Mitotracker Red. (C) Colorized merge of (A) and (B) with DAPI DNA staining.

### *RNA Interference*

After monitoring the growth rates of cells transfected with gene Tb11.02.0810 with and without tetracycline induction of RNAi, a growth curve was generated (Figure 2). Once a growth phenotype was observed, the cells were looked at under confocal microscopy to look for cell morphology abnormalities (Figure 3). These cells showed abnormal cellular projections, “giant” cells containing many nuclei (blue clumps) within one cell membrane, and cells trying but unable to divide.



**Figure 2.** Growth curves from cells transfected with gene Tb11.02.0810 grown with and without RNAi induction by tetracycline.



**Figure 3.** (A) Trypanosomes exhibiting RNAi phenotype of gene TB11.02.0810 viewed under direct interference contrast (DIC) microscopy. (B) Same cells viewed with colored merge of DAPI DNA stain and Mitotracker Red mitochondrial stain.

## CONCLUSION

Protein localization was observed in cells transfected with Tb927.8.4400. This protein appeared to localize to the mitochondrion as shown in Figure 1. Tetracycline induced RNAi showed retarded growth rates in the gene Tb11.02.0810. This data suggested that knockdown of our genes lead to the decreased growth rates. RNAi was shown to be effective in changing the growth phenotype of cells transfected with the pZJM plasmid containing Tb11.02.0810. Figure 3 (bracketed) shows the formation of giant trypanosomes containing multiple copies of genomic and kinetoplast DNA due to the inability to undergo complete cytokinesis. Limited results have been obtained with select genes, therefore further analysis needs to be done to determine specific gene function.

## ACKNOWLEDGEMENTS

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