

Introduction

T. brucei is a eukaryotic pathogenic that infects mammals and livestock, causing sleeping sickness in human and nagana in cattle. *Trypanosoma* is carried between hosts by the tsetse vector which initiates in the midgut after feeding on blood (mammalian host biting) and then migrates to the salivary glands (Ooi et al., 2015). This is the place where the parasite is prepared for transmission to another mammalian host following the stages (Fig 1).

The gene *C21ORF2* is crucial for the development of flagellum (Davidge et al., 2006). The gene controls the shape structure of the cells. The aim of the project is to explore a phenotype analysis where the gene for *C21ORF2* will be overexpressed.

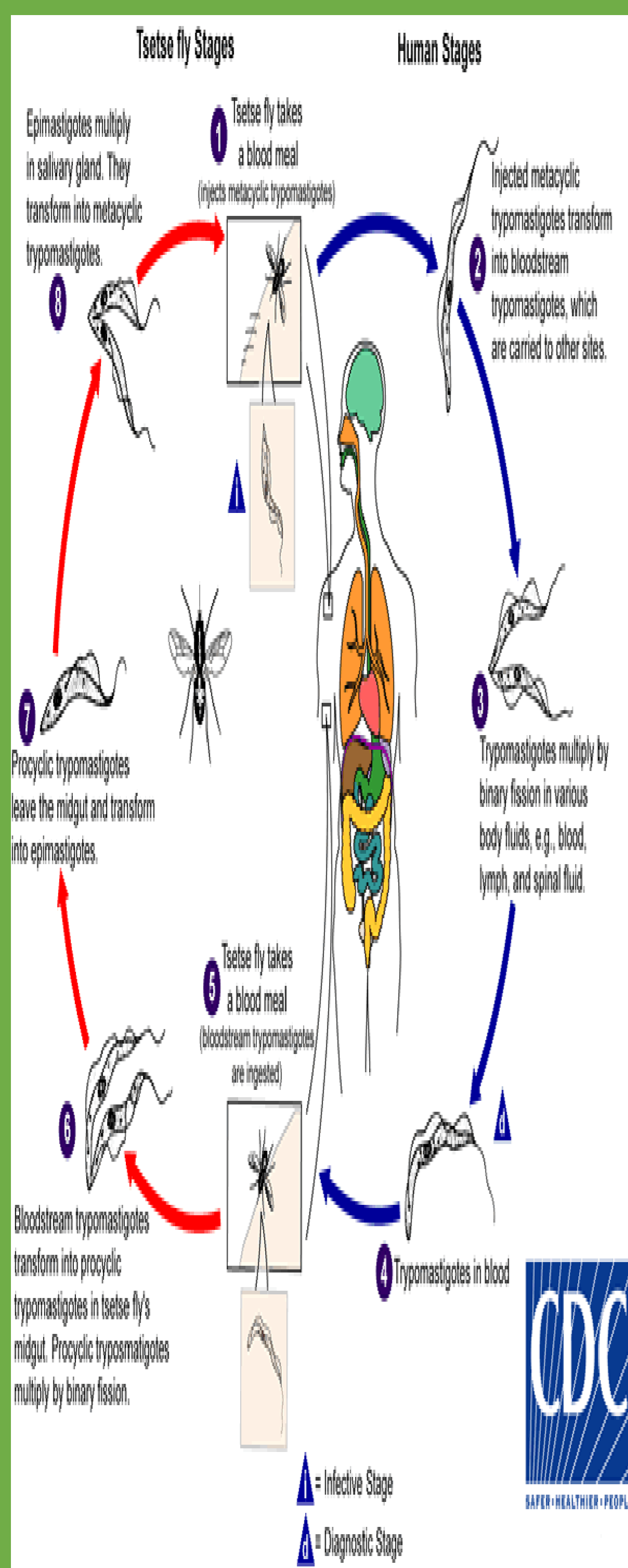


Figure 1. The different stages of *Trypanosoma brucei* life cycle. Image adapted from (Cdc.gov, 2018).

Methods

The gene for *C21ORF2* was overexpressed 24 hours by transfecting *T. brucei* with a plasmid that generated more copies of the gene *C21ORF2*. The plasmid was switched once tetracycline was added to the media (known as induced cells). In order to preserve the cells and cease all the metabolic mechanism, *T. brucei* cells were fixed with freshly prepared 2.5% glutaraldehyde and then washed in buffer. The cells were prepared into wells, following buy critical point drier. Finally coating with a gold sputter, thus the cells could be observed using scanning electron microscopic (SEM).

Results

Using SEM was possible to observe that most cells the phenotype of induced 24 hours post induction presented similar phenotype of the non- induced cells. However, some induced cells presented abnormal shapes: detached flagellum (A-D), rounded shapes (E-F) and problem in cell division (G-J) (Fig 2).

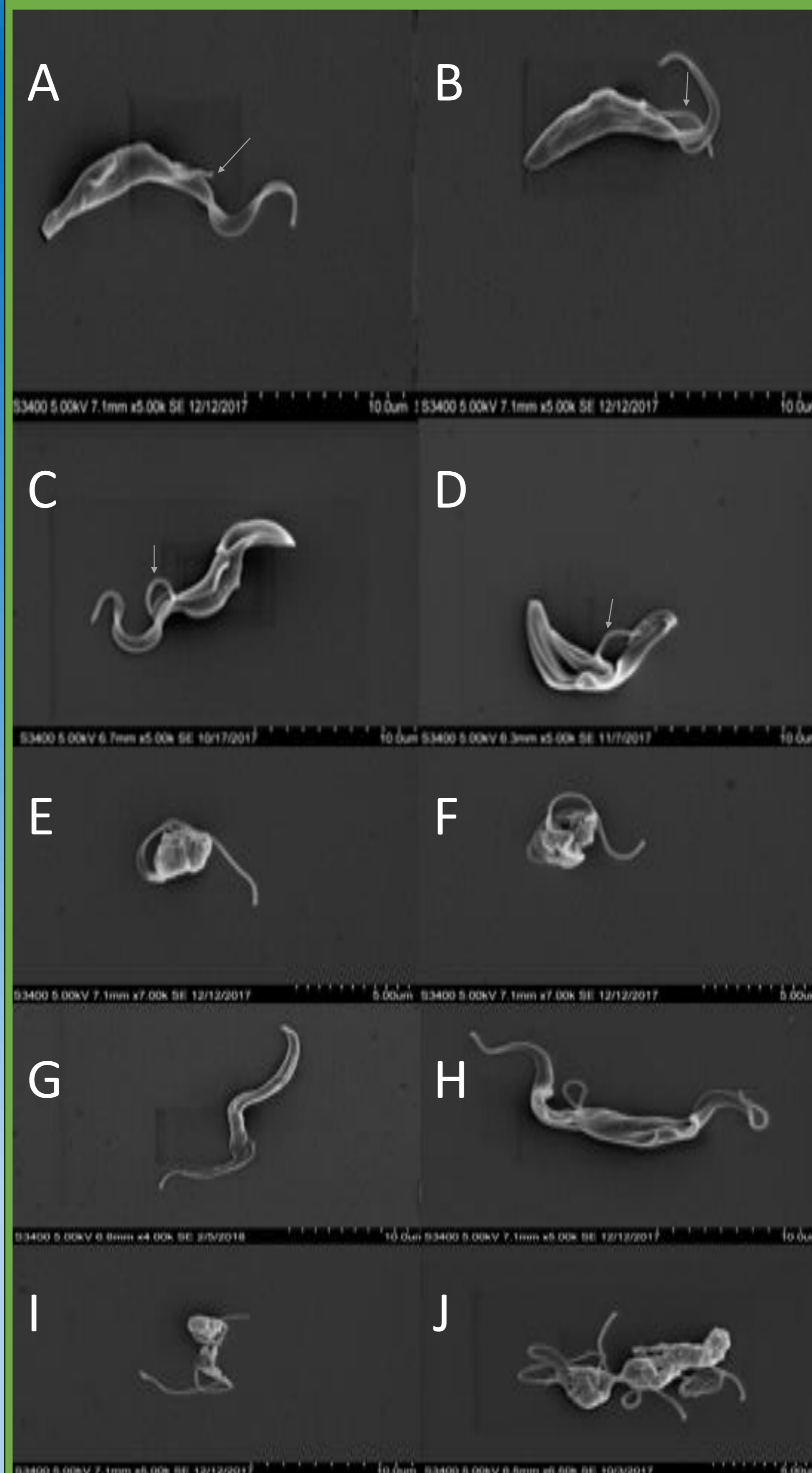


Figure 2. Images of 24-h *C21ORF2* RNAi-induced cells affecting the cells shape and flagella. Bars: (A,B,C,D,G,H and I) 10 μ m; (E,F and J) 5 μ m.

Discussion

Sleeping sickness caused by *T. brucei* still spreads resistance to drugs by the parasite and to insecticides by the tsetse vector (Corbel and Henry, 2011). Moreover, only four drugs with severe side effects are used for chemotherapy of sleeping sickness (Nkemgu-Njinkeng et al., 2002). Thus, a better understanding how the *C21ORF2* affects the *T. brucei* life cycle may lead to the potential target of finding a new drug. *C21ORF2* RNAi produces dramatic changes in cell form, one of the main advantages of gene expression induction is having the capacity to reproduce stable transfectants suppressing toxic gene; whereas ordinary gene expression may fail (Marchetti et al., 2004).

Recent studies show variable effects of the integration of *C21ORF2* and also emphasise its importance. The diverse outcomes of cells phenotypes may be partly explained by the effect of the *C21ORF2* (Wang et al., 2016). Evidence showed that *C21ORF2* is related to skeletal ciliopathies and abnormalities of ciliogenesis such as Jeune syndrome, axial SMD and retinal dystrophy (McInerney-Leo et al., 2017). The cells shape varied radically from the original trypomastigote shapes, this could be confirmed by SEM of these cells.

Conclusion

In conclusion, to find out the role of *C21ORF2* in flagellum biogenesis in *Trypanosoma brucei*, 24 hours post-induction was done; where the gene for *C21ORF2* had been overexpressed using the tetracycline-inducible system. Therefore, by using SEM it was possible to observe the effect of *C21ORF2* gene, which resulted in three different phenotypes: detached flagellum, cells with rounded shape and cells with division problem. However, this process was done only 24 hours post- induction, and further research is required to ensure that gene *C21ORF2* with longer period of post- induction hours may affect the phenotype of the cell even more. Inducing cells with this gene may alter the *T. brucei* cells leading to a potential therapeutic target for new treatment.