2018

Screen for interacting factors for Trypanosoma brucei telomere protein RAP1

Annelise Radzin
Cleveland State University

Elizabeth Beran
Cleveland State University

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Annelise Radzin, Elizabeth Beran and Bibo Li
Center for Gene Regulation in Health and Disease, Dept. of Biological, Geological, and Environmental Sciences, College of Science, Cleveland State University, Cleveland OH

Abstract

Trypanosoma brucei is a protozoan parasite that causes human African Trypanosomiasis, also known as sleeping sickness. The parasite is transmitted through the bite of the tsetse fly and is able to evade the host's immune system by changing its major surface antigen, variant surface glycoproteins (*VSG*s). VSG expression sites are located near the *T. brucei* telomeres and we have previously shown that the telomere protein, *Tb*RAP1, is essential for regulating VSG silencing. In order to better understand how this protein silences *VSG*s, we aim to identify proteins that interact with *Tb*RAP1. Here we have identified 10 candidates from one yeast 2-hybrid screen using the *Tb*RAP1 – aa426 – 761 fragment as bait.

Introduction

Trypanosoma brucei

Trypanosoma brucei is a protozoan parasite that causes sleeping sickness in humans and nagana in cattle. The parasite is transmitted to the mammalian host through the bite of the tsetse fly. Once the parasite enters the host's blood stream, it differentiates into a bloodstream form and expresses a highly antigenic variable surface glycoprotein (VSG) coat. The host's immune system produces antibodies that recognize the specific VSG coat being expressed, and a large number of the parasites are eradicated. The remaining parasites, however, change the VSG coat being expressed and escape the host's immune response. This phenomenon is called antigenic variation. The parasite numbers rise until the host produces new antibodies that recognize the new VSG coat, thus eradicating a large number of parasites, except for those that switched their surface antigen coat again. This pattern continues until the host's immune system can no longer fight off the parasite, and it crosses the blood-brain barrier. This infects the central nervous system of the host, causing more debilitating effects and eventually leading to fatality.

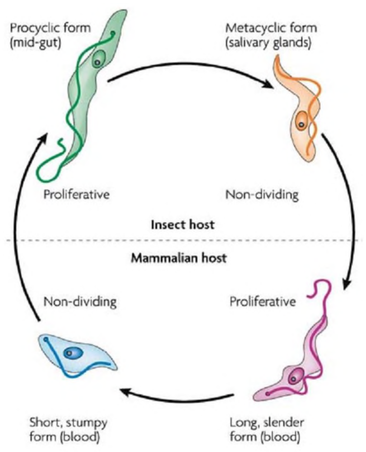

Fig 1. Life cycle of *T. brucei*

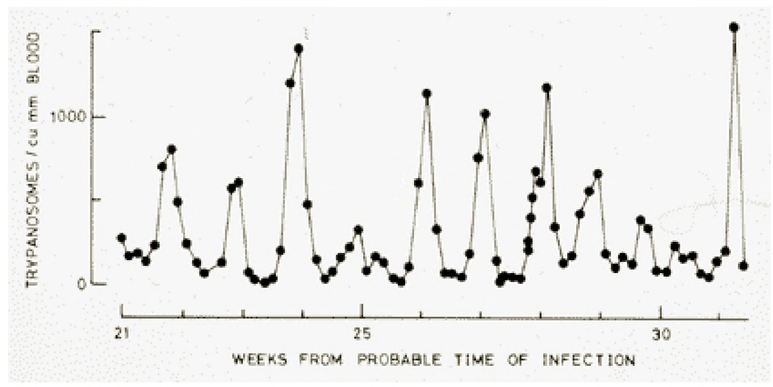

Fig 2. Periodical parasetemia of a typical *T. brucei* infection

Telomeres

Telomeres are nucleoprotein complexes found at the ends of linear chromosomes that often contain TG-rich simple repeats. Telomeres consist of short repetitive sequences and associated proteins. They protect the genome from events such as nucleolytic degredation and spontaneous DNA recombination. *VSG*s are expressed from subtelomeric loci in a strictly monoallelic fashion, and there are more than 2500 *VSG* genes as well as pseudogenes in the genome of *T. brucei*. The *VSG*s located at subtelomeric loci can only be expressed from roughly 20 similar VSG Expression Sites. The unique property of these Expression Sites is that only one of them is transcribed at a time while the rest remain silent. It is because of this expression that telomere functions are extremely important in antigenic variation.

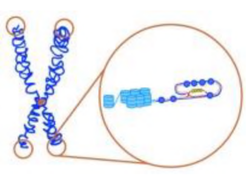

Fig 3. Telomere Structure

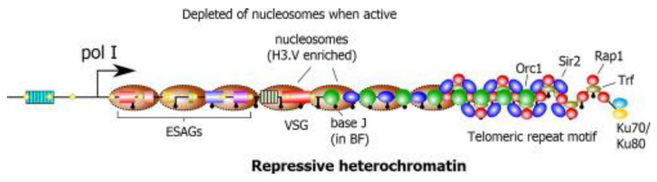

Fig 4. Subtelomeric *VSG* expression sites

Telomere Structure of *T. brucei*

In *T. brucei*, telomere maintenance is mediated primarily by an enzyme called telomerase. This enzyme contains a protein subunit, TERT, and an RNA subunit, TR. We have previously identified TbTRF as a duplex telomere DNA binding factor in *T. brucei* which interacts with *Tb*RAP1, another protein localized at the telomere. *Tb*RAP1 is an essential protein for both normal cell proliferation and for silencing the Expression Sites linked with *VSG*s. It has the strongest repression affect on genes that are located closest to the telomeres. Although it is known that *Tb*RAP1 is needed for *VSG* silencing, its underlying mechanism is unknown. In this study, we attempt to identify *Tb*RAP1 interacting factors through a yeast 2-hybrid screen using the *Tb*RAP1-aa-426-761 fragment as bait and a normalized *T. brucei* cDNA library as prey. Work from our lab suggests that the *Tb*RAP1 aa426 – 761 region appears to play an important role in *VSG* silencing. Therefore, we intend to investigate more about the function of this *Tb*RAP1 fragment, including its interacting factors. This information will hopefully shed some light on how *Tb*RAP1 carries out *VSG* silencing.

Yeast 2 Hybrid

Yeast 2 hybrid is a method used to test the interaction between two proteins through the binding of a transcription factor to an upstream activating sequence. The transcription factor is divided into the DNA binding domain and an activating domain. The DNA binding domain binds to an upstream activating sequence and the activating domain activates transcription. Binding the transcription factor to the upstream activating sequence then activates a downstream reporter gene. The transcription regulator LexA contains the DNA binding domain and the transcription regulator Gal4 contains the activating domain. If the protein fused to LexA interacts with the protein fused to Gal4, the reporter genes, LacZ and *HIS*3, will be transcribed when LexA binds to the LexA binding site. LacZ will then produce an enzyme called β-galactosidase. When 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) is added, it is cleaved by β-galactosidase. One of the byproducts is 5-bromo-4-chloro-3-hydroxyindole, which then oxidizes to 5,5'-dibromo-4,4'-dichloro-indigo, producing an intense blue color. The *HIS*3 gene encodes an enzyme that allows the yeast to synthesize histidine, so the yeast cells are able to grow on a medium lacking histidine.

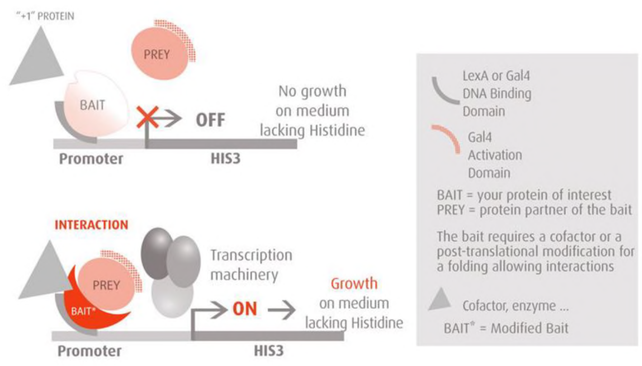

Fig 5. Principle of Yeast 2 hybrid Assay

Methods and Results

Yeast Transformation

The protein pBTM116-TbRAP1-aa462-761 was first transformed into L41 cells, and selected for on agar plates lacking tryptophan. Then, the L41/pBTM-116-TbRAP1- aa426 – 761 cells and *T. brucei* library were introduced into yeast cells through yeast transformation. A total of 300,000 primary transformants were obtained and plated onto agar plates lacking histidine, tryptophan and leucine. This step selects for those that express the *HIS*3 gene. The plates were incubated at 30°C for three days, and then the resulting yeast colonies were counted. A significant amount of bacteria was found on the plates, indicating contamination, and a total of 95 yeast colonies were obtained. The yeast colonies were then re-plated on agar plates lacking tryptophan and leucine to be saved as a stock for future tests.

Yeast 2 hybrid Filter Lift Assay

Yeast transformants that grow on plates lacking histidine, tryptophan and leucine are very likely to contain both the TbRAP1 – aa426 – 761 fragment and another protein that can interact with this *Tb*RAP1 fragment. In order to make the screen more stringent, we would like to confirm these colonies also activate the transcription of the LacZ reporter gene. The colonies from the plates lacking leucine and tryptophan were transferred to a separate agar plate also lacking tryptophan and leucine through replica plating, and grown in a 30° incubator for one day. Empty petri dishes were obtained and a clean, circular Whatman paper was placed inside. The Whatman paper was soaked in 2 mL of Z Buffer/X-gal solution. Another circular Whatman paper was obtained and gently pressed onto the colonies of the transfer plate. The paper was carefully peeled off of the agar and flash frozen in liquid nitrogen, allowed to thaw and then placed on top of the paper with the Z Buffer/X-gal solution with the colonies facing up. The plates were taken into a 30° incubator and observed for 2 hours. If *Tb*RAP1 and its interacting candidate indeed interact, the LacZ gene will be expressed to make β-galactosidase, and X-gal will be cleaved by β-galactosidase and the yeast cell will turn blue. The times in which the color change occurred was recorded. Only 10 candidates turned blue in this assay, and this can be attributed to the level of contamination seen on the plates from the transformation. Among the 10 candidates, 5 of them turned blue within one hour and are promising candidates.

Acknowledgements

Thanks to Dr. Bibo Li for providing guidance and the research facilities.
Thanks to Hanadi Kishmiri for laboratory assistance.
Thanks to the Provosts' Office for providing funds for this research.

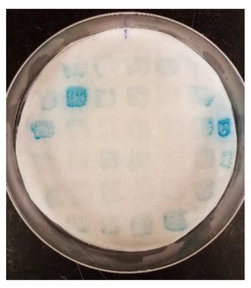

Fig 6. Plate after Yeast 2 Hybrid Filter Lift Assay

Colony	Time (m)
1110	40
1065	40
1023	40
1082	50
1028	60
1078	70
1074	70
1098	80
1075	80
1091	90

Fig 7. Table of reaction times for Yeast 2 Hybrid Screen

Conclusion

In this study, a telomere protein called *Tb*RAP1, which regulates VSG silencing in *Trypanosoma brucei*, was investigated. One yeast 2-hybrid screening was performed using *Tb*RAP1-aa426-761 and GAD fusion protein from the *T. brucei* library in order to identify proteins that interact with *Tb*RAP1. By observing which proteins interact with *Tb*RAP1, its functions can be better understood. During this initial screen 10 candidates were identified. We will follow up with the first five promising candidates and identify their sequences in order to identify their gene names. Subsequently, the interaction between these proteins and *Tb*RAP1 in *T. brucei* cells will be further confirmed by co-IP, IF analysis and yeast 2-hybrid analyses, etc. In addition, more yeast 2-hybrid screens will be performed using various fragments of *Tb*RAP1 as bait in order to identify more potential candidates.

References

1 Cross G, Dreesen O, Li B. Telomere structure and shortening in telomerase-deficient *Trypanosoma brucei*. Nucleic Acids Research. 2005; 33: 4536 – 4543
2 Espinal A, Figueiredo L, Li B, Okubo E, Yang X. Rap1 is essential for silencing telomeric variant surface glycoprotein genes in *Trypanosoma brucei*. Cell. 2009; 137: 99 – 109
3 Shammas M. Telomeres, lifestyle, cancer and aging. Current Opinion in Clinical Nutrition and Metabolic Care. 2001; 14: 28 – 34