Screen for interacting factors for Trypanosoma brucei telomere protein RAP1

Annelise Radzin  
*Cleveland State University*

Elizabeth Beran  
*Cleveland State University*

Follow this and additional works at: https://engagedscholarship.csuohio.edu/u_poster_2018

Part of the *Life Sciences Commons*

How does access to this work benefit you? Let us know!

**Recommended Citation**


https://engagedscholarship.csuohio.edu/u_poster_2018/19

This Book is brought to you for free and open access by the Undergraduate Research Posters at EngagedScholarship@CSU. It has been accepted for inclusion in Undergraduate Research Posters 2018 by an authorized administrator of EngagedScholarship@CSU. For more information, please contact library.es@csuohio.edu.
Screen for interacting factors for *Trypanosoma brucei* telomere protein RAP1

Anneline Radzin, Elizabeth Beran and Bibo Li
Center for Gene Regulation in Health and Disease, Dept. of Biological, Geological, and Environmental Sciences, College of Medicine, 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 (VSGs). 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 VSGs, 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* 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 bloodstream, 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 coat, leading to 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.

**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 genetic domain from nucleolytic degradation and spontaneous DNA recombination. VSGs are expressed from subtelomeric loci in a strictly monothetic fashion, and there are more than 2300 VSG genes as well as pseudogenes in the genome of *T. brucei*.

The subtelomeric loci can be expressed from the circular whatman 300,000 similar to protein 10 transformants. In *T. brucei* the telomeric sequence is linked to the telomere, which interacts with TB-RAP1, another protein located at the telomere. TB-RAP1 is an essential protein for both normal cell proliferation and for silencing the Expression Sites linked with VSGs. It has the potential to activate the subtelomeric loci 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 aa426–761 fragment as bait and a normalized 7. 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 Leu4 contains the DNA binding domain and the transcription regulator G4 contains the activating domain. When the protein fused to Leu4 interacts with the protein fused to G4, the regulator genes, La2c and HIS3, will be transcribed when Leu4 binds to the Leu4 binding site. Lac2 will then produce an enzyme called β-galactosidase. When S-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) is added, it is cleaved by β-galactosidase. One of the byproducts is S-bromo-4-chloro-3-hydroxysindole, which then oxidizes to 5,5’-dibromo-4,4’-dichloro-indigo generating an intense blue color. The HIS3 gene encodes an enzyme that allows the yeast to synthesize histidine, so the yeast cells are able to grow on a medium lacking histidine.

**Methods and Results**

**Yeast Transformation**

The protein pBTTM116-TB-RAP1 aa426-761 was first transformed into L41 cells, and selected for on agar plates lacking tryptophan. Then, the L41/pBTTM116-TB-RAP1 aa426–761 cells and T. brucei L41 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 select those that express the HIS3 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 replated 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 TB-RAP1 – 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 extend the domain of the yeast two-hybrid 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°C incubator for one day. After incubation, the plates were observed, and a circular Whatman paper was placed inside. The Whatman paper was soaked in 2 ml of 2 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 2 Buffer/X-gal solution with the colonies facing up. The plates were taken into a 30°C incubator and observed for 2 hours. If TB-RAP1 and its interacting candidate indeed interact, the Lac2 gene will be expressed, and the maltose, lactose, and X-gal will be cleaved. The β-galactosidase and the yeast cell will turn blue. The times in which the color change occurred was recorded. Only 10 candidates turned blue within one hour and are promising candidates.

**References**


**Acknowledgements**

Thanks to Dr. Bibo Li for providing guidance and the research facilities.

Thanks to the Office of Informational Service for proofreading.

Thanks to the Provost’s Office for providing funds for this research.