

Trypanosoma brucei PEX 13s YG-rich and SH3 regions: expression and purification

Kelty Fletcher; Meredith Morris PhD

University of South Carolina School of Medicine Greenville; Eukaryotic Pathogens Innovation Center: Clemson University

Abstract

Trypanosoma brucei is a Kinetoplastid parasite that causes Human African trypanosomiasis, better known as African Sleeping Sickness. These parasites contain vital peroxisome-like organelles called glycosomes that house multiple metabolic pathways. Proteins are targeted to glycosomes by the soluble receptors, PEX 5 and PEX 7 that bind PEX 13 and PEX 14 contained in the docking complex. PEX5 and PEX7, each detect a peroxisome targeting sequence (PTS sequence) on proteins destined for the glycosomes and interact with the docking complex. Kinetoplastid parasites have two PEX13s, PEX13.1 and PEX13.2, both containing a N-terminal YG-rich region that interacts with PEX5 and PEX7. PEX13.1 differs from PEX13.2 in that it contains a C-terminal SH3 region. The PEX13.1 YG region has conserved serine residues while the PEX13.2 YG region has conserved leucine residues that we believe play a role in the binding affinity of the PEX5 and PEX7 interactions. These unique regions have potential as prospective drug targets. We have transformed the gene construct for the PEX13.1 YG region, PEX 13.2 YG region, and PEX13.1 SH3 region into M15 strain of *E. coli* for protein purification. In this work, we explored several expression and purification conditions but were unable to find conditions that supported protein expression. We have transformed expression constructs into a different strain of bacteria, BL21, and future studies will focus on assessing whether proteins are expressed in these cells. Once purified, these recombinant proteins will be employed in future experiments to study the binding affinity with PEX 5 and PEX 7.

Objectives

- Expression & purification of PEX13s
- Better understand structure and function of PEX13s
 - Potential as drug target
- Study interaction between PEX13s with PEX5 & PEX7 (Image 1)

Methods

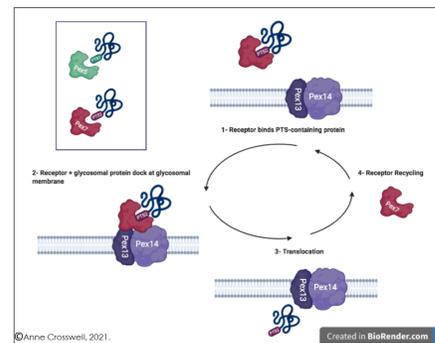
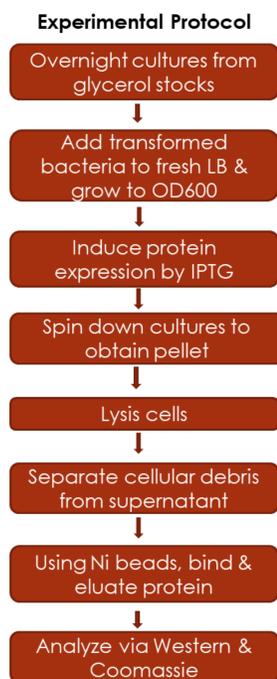


Image 1. Schematic of glycolytic protein transport to glycosome.

- Following the general experimental protocol to the left, we grew up previously transformed M15 strain *E. coli* to assess for expression of the proteins of interest.
- Samples run through SDS-PAGE and analyzed by Western Blot, probing for protein of interest as well as Coomassie stain, displaying unspecific total protein in each sample.

Results

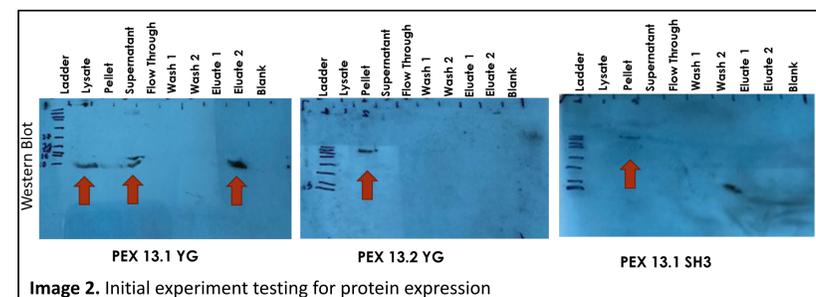


Image 2. Initial experiment testing for protein expression

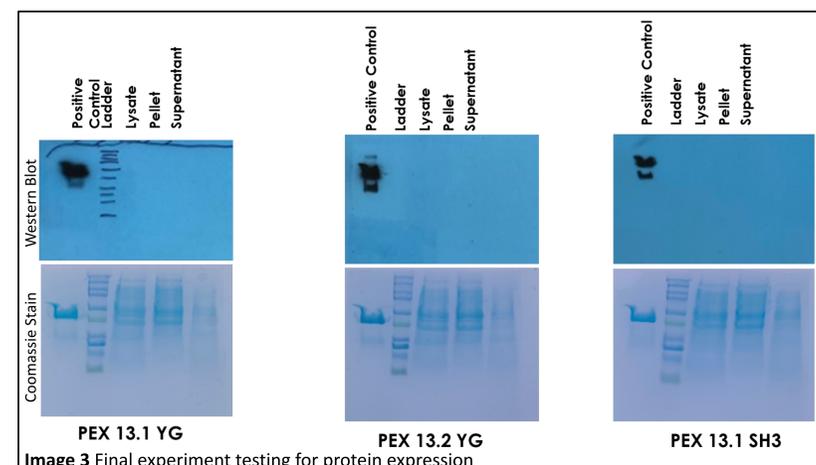


Image 3 Final experiment testing for protein expression

Discussion

The initial experiment provided promising results, as seen in image 2, analysis by Western blot showed protein in the lysate, supernatant, and eluate. However, in following experiments the results were not reproducible. In general, our experiments resulted in insufficient expression of the proteins of interest. Before coming to this deduction, we explored other conditions to optimize the experimental protocol. We adjusted the lysis method as well as extended the incubation period in the buffer. We shortened the protocol to three samples to determine the location of the proteins of interest. In our final experiment we found no expression of desired protein (image 3). Moving forward, we have transformed expression constructs into a different strain of bacteria, BL21, to implement in future studies to test for protein expression. Once we confirm expression and purify PEX13.1 YG, 13.2 YG, and 13.1 SH3, we will employ the products to study the binding affinity with PEX 5 and PEX 7.

Clinical Significance

- To implement these studies to develop safer, more effective treatment therapies and better outcomes

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