VIRAL ONCOPROTEIN TAX & REGULATION OF THE HTLV PROMOTER

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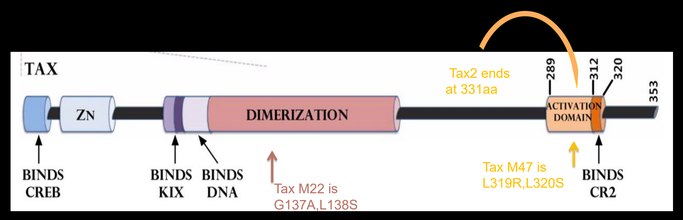
Abstract

Tax, an oncoprotein virally expressed from Human T-cell Leukemia Virus (HTLV), is a transcriptional regulator with incompletely understood functions in host cells. HTLV is a retrovirus of clade deltraretroviradae with two known sub-types (HTLV Type-1 and HTLV Type-2). HTLV-1 is associated with a subset of patients in development of an extremely aggressive blood cancer called Adult T-cell Leukemia/Lymphoma (ATLL) but HTLV-2 is not. It is known that Tax recruits host activator and co-activator proteins to bind to the integrated viral promoter region of HTLV and rapidly activate viral gene expression. Tax coding regions vary in HTLV-1 and type-2 in the predicted transcriptional activation domain. Exploring interaction surfaces of Tax with various host proteins involved may help us understand the basic transcriptional activation mechanisms that are manipulated by the HTLV-1. To investigate this, we accumulated a library of Tax expression plasmids with the Tax wild-type and mutant coding sequences from HTLV-1. The mutants collected have been established to impair transcriptions or cytoplasmic activities of Tax and include in contrast the Tax coding sequence from HTLV-2. Once the mutants were obtained, we established a stock that can be used in the future for downstream expression, purification, and activity binding assays.

Introduction

Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020 (2). Cancer etiology is complex and can be caused by many different things. Viruses have been found to number among that list forming a small but impactful class of oncogenic viruses. Human T-cell Leukemia Virus Type-1 (HTLV-1) is an oncogenic virus known to be the causative agent of Adult T-cell Leukemia (ATL). Of particular interest, is the virally encoded activator protein known as Tax. Tax has been shown to malignantly transform cells in culture absent of all other proteins from HTLV-1. The interactions responsible for this can be broadly categorized into the cytoplasmic effects of Tax or the viral transcription activation capability of Tax. In this project, we have developed and generated a library of Tax mutants affecting cytoplasmic or transcriptional activity of Tax. This library of mutants was generated using midi-preps and sequenced to ensure quality. I have created a library of many mutants of tax including m47, m22, sTax2, K88A, and V89A with midi-preps, and then a restriction digest to build the knowledge of this oncoprotein. Progress is ongoing with expression of a matched set of mutants and experiments comparing the mutant interfaces planned next.



1. Figure 1. Shows the different binding domains involved in HTLV oncoprotein Tax and where different mutant stains are located along the diagrammed primary structure.

Recombinantly expressed HTLV-Tax

Figure 2. Snapgene map which predicts where certain restriction enzymes are and what ones would work with tax to get the best results. Restriction enzymes insert themselves into specific DNA sites. Restriction enzymes can cut and isolate specific genes. Minipreped Tax expression plasmids are sequenced to confirm correctness. Appropriate restriction enzymes can be used to subclone Tax into high efficiency expression constructs.
Figure 3. Is a 2D diagram that shows the domains of Tax such as KIX, Tax A.D., and bZIP. KIX domain is a binding site for transcriptional co-activators and bZIP domain is another binding site, but it connects two DNA regions. Tax A.D. is an activation domain that activates transcriptional or genetic information in the HTLV genome that creates the virus proteins

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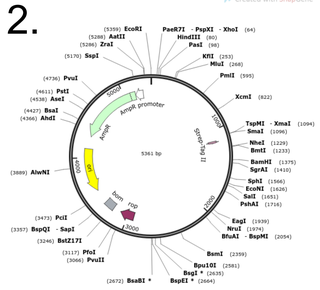

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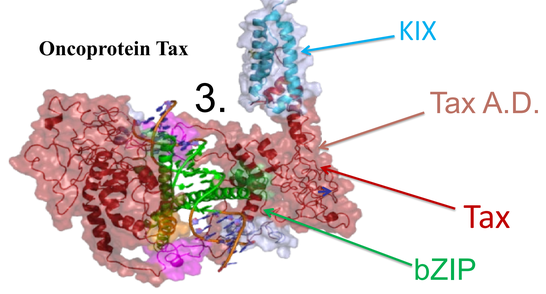

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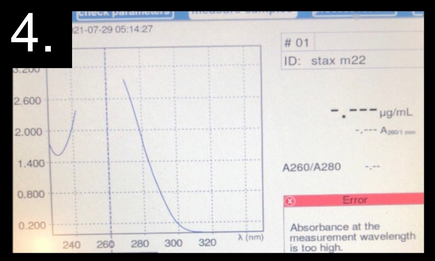

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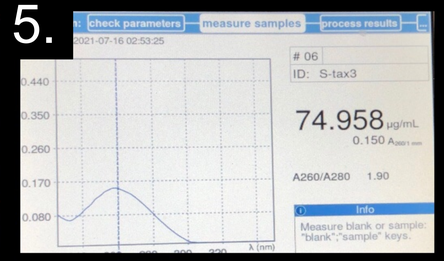

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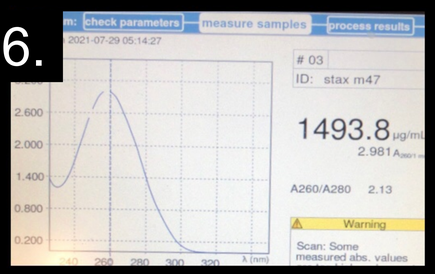

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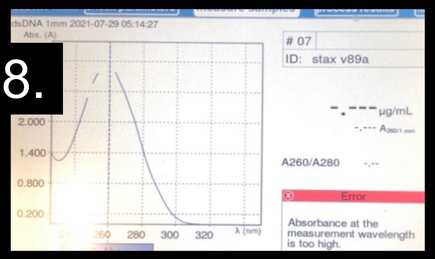


9.

Above are the specs from the midi-preps of S-Tax and S-Tax mutants. Figure 4 portrays the spec of S-Tax mutant m22. Figure 5 is S-Tax. Figure 6 is S-Tax mutant m47. Figure 7 is S-Tax 2. Figure 8 is S-Tax mutant V89A. Figure 9 is S-Tax mutant K88A. Each spec is one of three for each. S-Tax mutants m22, and V89A specs all had an extremely high concentration, to high for the spectrometer to measure. To fix this issue I need to dilute to 10x with medical grade water.

Results

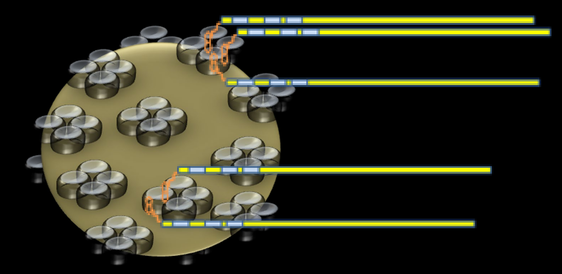
Figure 1 visually explains parts of Tax in a linear way better than figure 3. Figure 3 expresses a 2D model of what we think Tax looks like. Figure 2 is a Snapgene map that shows the restriction enzymes and where they would work best. I used this to help identify which enzymes to use during a restriction enzyme digest to determine what I made after forgetting to add antibiotic to my first midi-prep. Basically, I created a super concentration of S-Tax. Figures 4 through 8 are specs from midi-preps of S-Tax and S-Tax mutants.

Conclusions and Future Directions

I expected to accomplish more. I did not anticipate having to learn lab procedures, safety, or prep for my research project. I am thankful for all of it now because I learned so much, and it taught me important methods needed to proceed.

In the future, I will dilute the mutants, send the mutants for sequencing, and compare them. These mutants can also be used for downstream expression, purification, and activity binding assays. I will start working with magnetic beads to pull apart tax and identify what component makes mutants.

10.



References and Acknowledgements

Citations:
1. “Software for Everyday Molecular Biology.” *SnapGene*, https://www.snapgene.com/.
2. “Cancer.” *World Health Organization*, World Health Organization, 20 Sept. 2021, https://www.who.int/news-room/fact-sheets/detail/cancer.

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