Utilization of *Escherichia Coli* for the growth of Y Family DNA Polymerase Rev1 and GSTrap column for purification

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Abstract

Rev1 is a Y family DNA polymerase that specializes in translesion DNA synthesis. Rev1 is unique in that it preferentially incorporates dCTP in the growing DNA strand, regardless of the templating base. This is because the template base is evicted from the active site and a template amino acid, arginine 324 (R324) acts as the template for the incoming dCTP. We hypothesize that arginine 324 and the neighboring leucine (L325) facilitate the eviction of the DNA template from the active site. To test this hypothesis, we worked to purify R324G/L325G Rev1 double mutant for the purpose of X-ray crystallographic examination of the protein-DNA-dNTP ternary complex. We transformed Escherichia coli (E. coli) and induced expression of both wild type Rev1 and R324G/L325G Rev1. The bacterial cells were lysed by sonification, and the lysate was purified with a GSTrap column. We were able to successfully isolate the Rev1 enzyme. Further purification and crystallization will be necessary to explore the x-ray crystal structure of R324G/L325G Rev1 protein

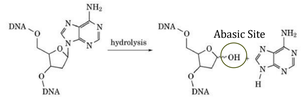
Introduction

One in every fifteen to thirty million nucleotides can be considered a mutation in healthy individuals. A mutation is a change found in the DNA sequence. Some mutations could be minor and have no effect on phenotype, while other mutations could lead to severe phenotypic changes, such as disorders and tumor growth. When there is the absence of a base within the DNA, classical DNA polymerases stall and cannot proceed with DNA replication. To bypass the stalled replication fork, Y-family DNA polymerases are recruited for the purpose of translesion DNA synthesis. Rev1 is a Y family DNA polymerases that replicates through abasic sites and exocyclic guanines by incorporation of dCTP into the growing DNA strand. Rev1 uses an amino acid for its template, rather than the DNA template. In the active site, the template DNA is evicted from the active site and arginine (R324) hydrogen bonds with the incoming dCTP. When the enzyme moves on to the next site, the evicted template base then pairs with the complementary nitrogenous base. We know that arginine 324 participates in the binding of incoming nitrogenous bases in the active site, but it is unknown how the template DNA base is evicted from the active site and stabilized away from the active site while arginine acts as the protein template. Leucine (L325) is a large non-polar amino acid that is located next to arginine 324. We hypothesize that the arginine and leucine side chains play a role in the eviction of the template DNA from the active site of Rev1. To test this hypothesis, we are working with a double mutant (R324G/L325G) of Rev1, where the arginine and leucine have been mutated to a glycine (R324G/L325G). By replacing the arginine and leucine with glycine, the side chains of these two enzymes are effectively removed. If our hypothesis proves correct, R324G/L325G Rev1 would bind DNA with the nitrogenous base in the active site, acting as the template base for nucleotide incorporation. To test this hypothesis, we worked to express and purify R324G/L325G Rev1 for the purpose of x-ray crystallographic analysis of the ternary structure of R324G/L325G Rev1 in complex with template DNA and incoming dNTP. We have grown *E. coli* with wild type and mutant Rev1 and are in the process of purifying the Rev1 protein for crystallography.

DNA Damage

Abasic Sites

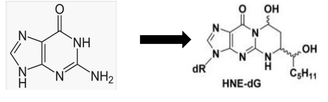
Formed by hydrolysis reaction at physiological pH



Nucleotide Base detached from DNA backbone

Exocyclic Guanine Adducts

Extra rings are added to the nucleotide bases via carcinogens.
This alters the Watson-Crick binding structure and coding of DNA.



Rev1 Sequence Alignment

Double Mutant

Double Mutant	PVNLNNLEAKRIVACDDPDFLTSYFAHSGGHHLSAWKANLKDKFLNENIHKYTKITDKDT
Wild Type	PVNLNNLEAKRIVACDDPDFLTSYFAHSRLHHLSAWKANLKDKFLNENIHKYTKITDKDT

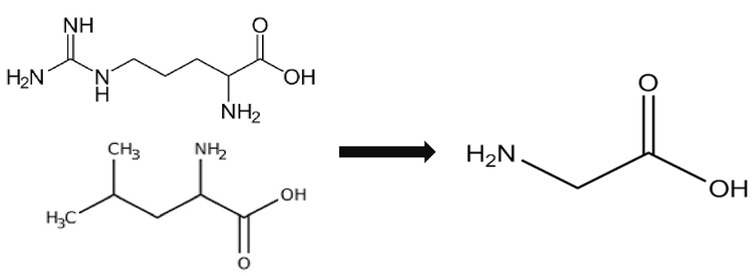


Figure 2. Arginine (top left) has a side chain that consist of a three-hydrocarbon straight chain, and guanidinium group. Because of the structure of the side chain, Arginine is categorized as a basic amino acid. Leucine (top right) has a side chain that is made up of an isobutyl group. Because of the structure of the sidechain, Leucine is considered a nonpolar amino acid. Glycine (left) has a side chain is a single hydrogen, and the amino acid is nonpolar. Mutating the arginine and leucine to a glycine should change the mechanism of Rev1

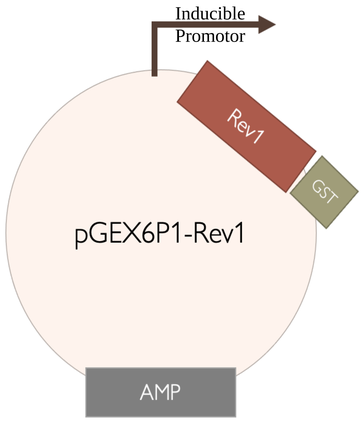
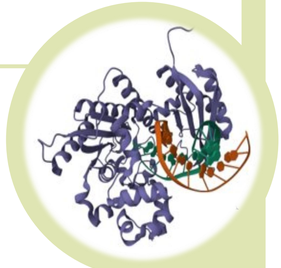


Figure 2. A model of the plasmid containing the Rev1 double mutant (R324G/L325G). ITPG is used start transcription of the Rev1 (red). The GST tag (green) aids in the purification process using a GSTrap column. Ampicillin (grey) allows for the gene to be antibiotic resistance and allows for the selection of *E. coli* colonies to grow that have taken up the plasmid through transformation.



Materials and Methods

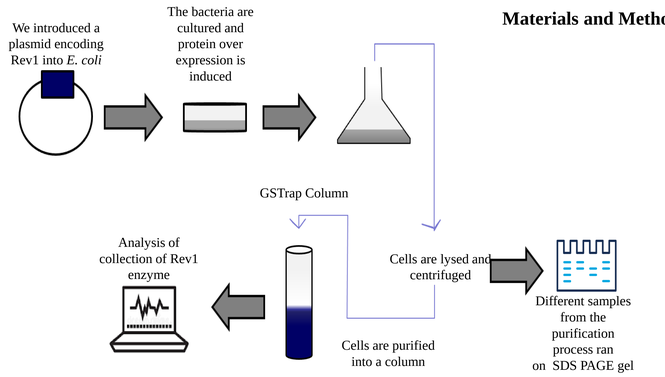


Figure 3. pGEX6P1-Rev1 plasmid was transformed into *E. coli*. The bacteria was then plated and incubated on ampicillin, chloramphenicol, and LB plates to select for *E. coli* that took up the plasmid. We inoculated 1.5 L flasks for overexpression of protein. After removing the LB, we lysed the cells using sonification and centrifuged the bacteria cells. We took samples from the pellets and lysate before and after sonification and analyzed the protein content on an SDS PAGE gel (Figure 4). We, column purified the lysate through a GSTrap column to pull out Rev1 (Figure 5).

SDS PAGE Gel

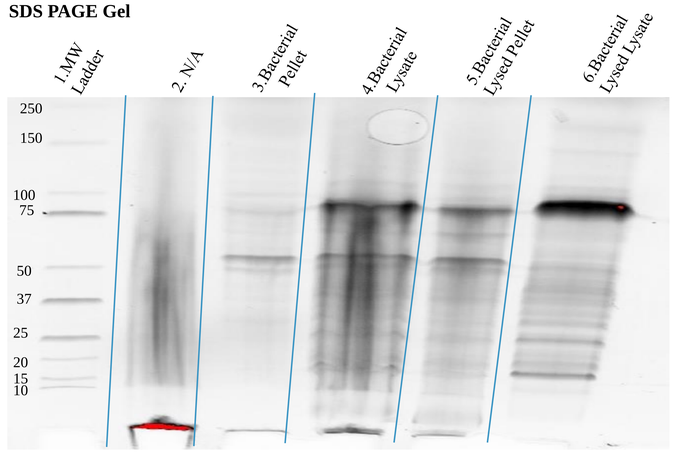


Figure 4. Gels were ran throughout the purification protocol. We used gels from Bio-Rad Laboratories (Hercules, California) and a Tris/glycine/SDS buffer in The Criterion Cell. Samples were mixed with Laemmli. Samples incubated for five minutes at room temp and then for three minutes at 95°C. Samples were centrifuged for three minutes then loaded into wells gels in the electrophoresis machine.

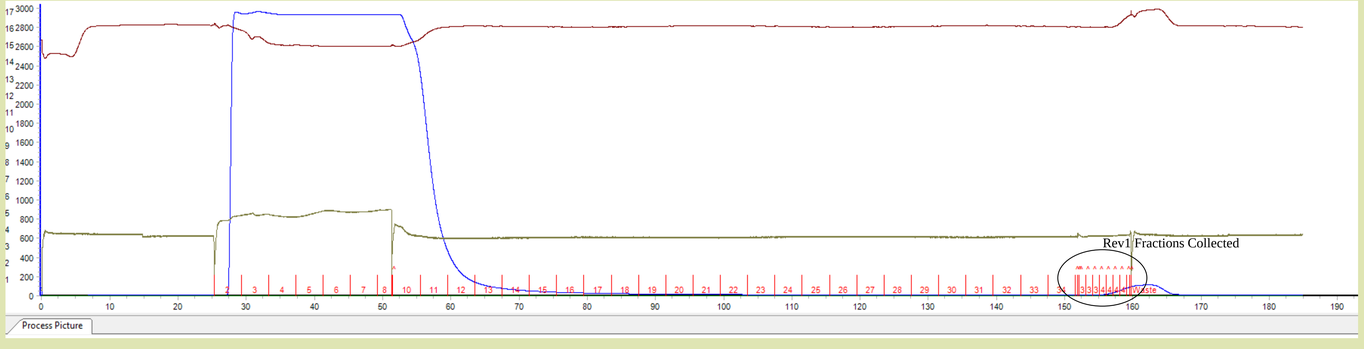


Figure 5. Fast protein liquid chromatography (FPLC) is a medium pressure chromatography utilizing a pump to control the speed at which the mobile phase passes through the stationary phase. The stationary phase remains fixed in the column while the mobile phase carries components of the mixture through the medium. The column used was a GSTrap column that pulls Rev1 out of the lysate. The GSTrap column has a high affinity for GST-tagged proteins. Within the pGEX6P1 plasmid, there is a GST sequence that when transcribed/translated, is attached to the Rev1 protein for easy detection and purification. Shown in the graph (left) we ran a lysis buffer over the column then an elution buffer to collect Rev1 into tubes. Rev1 was collected in a few tubes towards the ends (circle).

Discussion/Future Direction

Our goal is to complete the rest of our purification process. We will run the Buffer exchange GTS-Rev1 with Rev1 low salt buffer. This will be done at 4°C. We will run over 5mL resource S cation exchange column with the Rev1 low salt buffer. After that, we will elute with GST-Rev1 high salt buffer. Once that is done, we will run factions on gels to ensure that GST-Rev1 has been eluted. We will then move on to buffer exchange GST-Rev1 low salt buff at 4°C. After we have run through the buffers, we will cleave the GST-tag using Precision Protease at 4°C for 2-4 hours. From here, we will have one of two options. We will either incubate Rev1 with 3mL of glutathione resin to capture the GST-tag and precision protease. The other option would be to re-run the cleaved Rev1 over the Resource S column. Our last thing to do would be to put the protein through Gel filtration. To do this, we will concentrate OGG1 to 2mL. After that we will run over S200 GF with GF/Storage buffer. In the end, we will combine fractions containing purified Rev1.

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References

1. Hernández C., et al. *PLoS ONE*, 2013, Vol. 8, no. 2,
2. Leake, M. C. CRC Press /Taylor & Francis Group, 2017.
3. "Biotin Surfaces." *Biotin Surfaces | MicroSurfaces, Inc.*, 2015,
4. Weaver, T.M., Freudenthal, B.D., *Proc.Natl.Acad.Sci*, 2020
5. Weaver TM, et al. Proc Natl Acad Sci U S A, 2020
6. Chatterjee N., et al. Epub,