Regulation of Gene Expression by Nascent Peptides

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Abstract

Translation of proteins from mRNA is highly dependent on the flawless function of the ribosome. Although many alterations can occur in the ribosome inhibiting the function of the ribosome, the focus of the rest of the paper is on the ribosomal exit tunnel. As with anything moving through a space, if something gets in the way then the movement will be inhibited. Take this idea and apply it to the exit tunnel of the ribosome. With an alteration of the A752 nucleotide, the exit tunnel might become smaller and not allow the newly translated protein to exit the ribosome. A quick summary of the process used starts with finding the optimal growth temperature and time of the bacteria containing the mutant nucleotide in the ribosome of the cells. The cells were then lysed to release the protein into solution and the cell debris was discarded. Two dimensional gel electrophoresis was used to characterize the proteins and the wild type and mutant strands were compared. Results conclude that *pyrB* is expressed in the wild type and not expressed in the mutant strains.

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Introduction

A gene is responsible for passing along traits from parent to offspring. It is a sequence of nucleotides along segments of deoxyribonucleic acid (DNA) that code for the synthesis of ribonucleic acid (RNA) [2]. The synthesis of RNA is called transcription. The RNA molecule will then have the same sequence as the gene after transcription. In eukaryote cells newly produced RNA molecules contain introns and exons so the primary transcript (RNA) undergoes a process called splicing to remove the introns. The splicing process is done because only the exons carry information for protein synthesis. In prokaryote cells, such as bacteria, there is no splicing process because they lack of introns. In bacteria transcription generate a mature RNA. The mature product is called messenger RNA (mRNA). The mRNA then undergoes protein synthesis, also known as translation, and yields a sequence of amino acids that defines the protein produced. In Figure 1 below, one can see a schematic of the relationship between genes, transcription and translation.

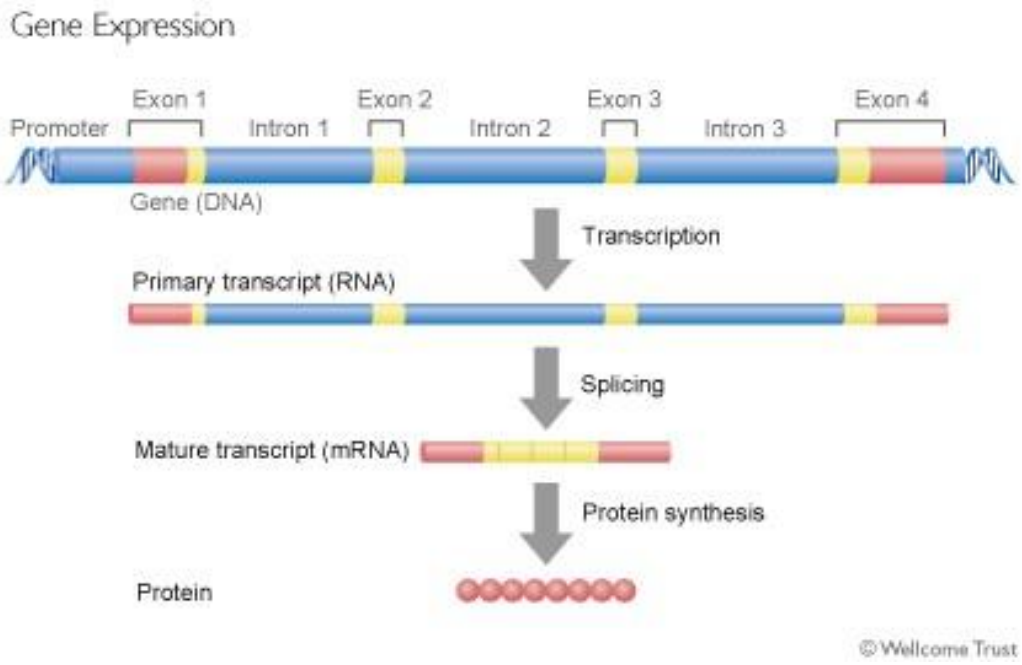

Figure 1: Relationship between Genes, Transcription and Translation [1]

Gene expression can be altered at the transcription and/or translation level.

An enzyme called RNA polymerase generates transcription. Transcription goes through a process of initiation, elongation and termination. During the initiation step the RNA polymerase aides in the separation of the DNA double helix into two complimentary single strands. The RNA polymerase then uses the single strand of DNA as instructions to code for strands of ribonucleotides. During the elongation step of transcription, the RNA polymerase extends the strand of ribonucleotides to the new RNA being formed. The elongation process will continue until the RNA polymerase reaches a termination sequence on the single strand DNA. This new strand of RNA will go through a processing step called splicing where the exons are combined into one mature strand of mRNA.

Translation is the process of using the mRNA as the instructions for the ribosome to synthesize proteins. The process of translation occurs in 4 consecutive steps similar to that of transcription. The steps that comprise translation are activation, initiation, elongation and termination. The figure below illustrates translation.

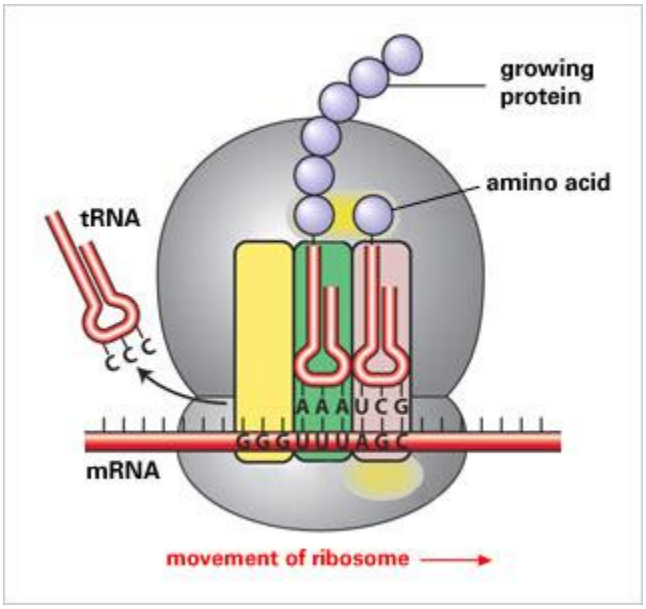

Figure 2: Ribosomal Translation [3]

During the activation step, the transfer RNA (tRNA) attaches to a specific amino acid through a covalent ester bond which will be used in the ribosome after initiation. During the initiation step, the ribosome attaches to the mRNA strand. Elongation occurs when the ribosome moves down the mRNA strand, decoding the mRNA information and producing a polypeptide known as the nascent peptide. Once the ribosome reaches a stop codon on the mRNA strand, the nascent peptide will be detached from the peptidyl transferase center, the ribosome active site, and leave the ribosome. The three stop codons present in bacterial cells are UAA, UAG and UGA.

The ribosome itself is composed of two main subunits. Ribosomal subunits are described by their rate of sedimentation in a centrifuge referred to as their Svedberg (s) values. In eukaryotic cells, the ribosome has a Svedberg value of 80s, composed of a 40s subunit and a 60s subunit. In prokaryotic cells, the ribosome has a Svedberg value of 70s, composed of a 30s subunit and a 50s subunit. Algebraically, the Svedberg values of the two subunits do not add up to the Svedberg value of the entire ribosome. This is due to the fact that sedimentation is dependent on shape and size, and not independently on molecular weight [4]. *Escherichia coli* (*E.* coli) is a bacterium and therefore is a prokaryotic cell. The figure below shows an illustration of a prokaryotic ribosome during translation.

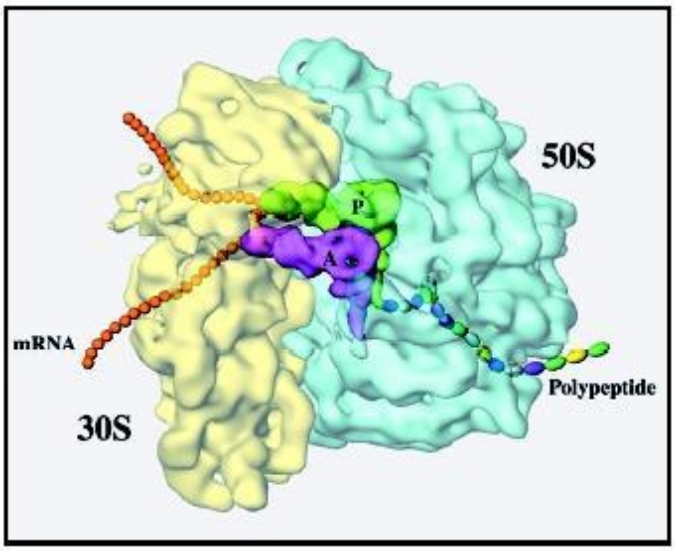

Figure 3: Prokaryotic Ribosome Subunits [5]

In the figure above, the small 30s subunit is colored yellow and the larger 50s subunit is colored blue. The nascent polypeptide is exiting through the ribosomal exit tunnel. Within the 50s subunit there is a 23S subunit consisting of 2,900 nucleotides. The 23s subunit is located in close proximity to the exit tunnel. The research of this thesis was conducted on the A752

nucleotide within the 23S subunit. The nucleotide is theoretically an adenine. The mutation under research changes an adenine by a thymine at the 752 position (A752T) in the 23S subunit.

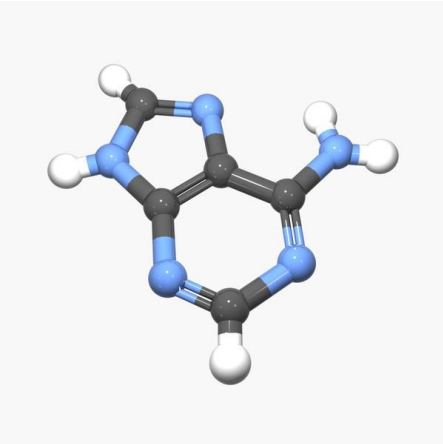

Figure 4: Ball and Stick Model of Adenine [6]

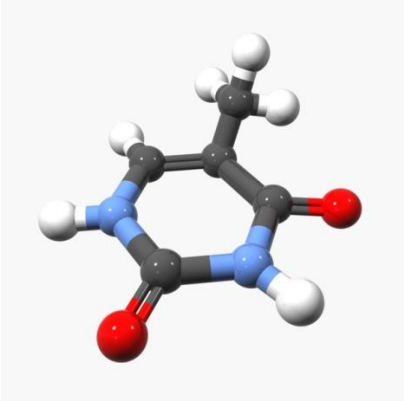

Figure 5: Ball and Stick Model of Thymine [6]

The figures above show the difference in the adenine and thymine bases. A chromosome in *E. coli* contains close to 5 million base pairs that make close to 4,400 genes. Of the 4,400 genes, around 4,300 are translated. Therefore, the nascent peptide exit tunnel must be universal. This shows the large impact of altering one base pair close to the exit tunnel.

Materials and Methods

A. Growing Colonies in a Rich Media Plate

When growing cultures, the environment should be sterile due to the possibility of growing unwanted bacterial strains. The loop should be sterilized by placing it in a flame until it becomes red. The loop should then be cooled in the new media before collecting a bacteria colony. From here, the loop was used to transfer one colony from the media to the new rich

media plate and placed in a small line on the media. The loop should be sterilized again and cooled. The loop was then placed in the new rich media plate and a perpendicular line from the initial placed colony. The loop should be sterilized again and cooled. The loop was then placed in the new rich media plate and "Z" shaped lines were made into the media spreading out the colony.

B. Growth Conditions and Growth Curves

E. coli MG1655 Δ7 cells with plasmids containing wild type ribosomal rRNA operon and plasmids containing the A752T mutant insertion on the 23S rRNA gene of the operon was used to create the growth curves [7]. The cells were grown on 10 milliliter of LB rich media containing 10 microliters of Amp (100 micrograms per milliliter). The cell growth was monitored every 15 minutes using a Klett-colorimeter. The data was plotted using Microsoft Excel with the Klett units on the Y-axis and the time in minutes on the X-axis. The bacterial strains were monitored until they reached a growth plateau. At this point the cells were discarded.

C. Protein Extraction

The cell growth was stopped by the addition of 50 microliters of sodium azide into the final culture volume of 10 milliliters. The test tubes were then centrifuged so that the cells would fall out of solution. The supernatant was decanted off and the cells were resuspended with 50 microliters of lysis buffer. The lysis buffer consisted of 10 millimolar Tris with a pH of 8.0, 8 molar urea, 5 millimolar magnesium acetate and 4% weight per volume CHAPS. A Fisher Scientific sonicator was used to lyse the cells in ice cold conditions with a 1 second pulse and a lag time of 3 seconds. This process went on for 1 minute. The sonication process was conducted

3 times total to ensure all cells were lysed. The sample was then centrifuged so that the cell debris would pellet out and the desired proteins would stay in solution. The supernatant was then used in further experimentation.

D. Two-Dimensional Gel Electrophoresis

First, the proteins were separated by isoelectric focusing. A buffer containing 8 molar urea, 2 milligrams/milliliter of dithiothreitol (DTT), 4% weight per volume CHAPS and 1% volume per volume Pharmalyte was added to the protein sample. 10 milliliters of the buffer and sample were used. The gel used in the first dimension of separation contains 1 milliliter of CHAPS-Nonidet P 40 detergent solution, 9.2 molar urea, 4% acrylamide and ampholyte of pH range of 3-10 and 5-7. A BIO-RAD PROTEAN II 2-D cell was used with a constant voltage of 200V for 2 hours, then 500V for 2 more hours and finally 600V for 16 hours. The top buffer consisted of 30 millimolar sodium hydroxide solution and the bottom buffer consisted of 10 millimolar phosphoric acid solution. After running the gels, they were removed from the tubes and soaked in 3 milliliter of transfer buffer. The transfer buffer consists of 10% SDS, 0.02 milligrams of Xylene Cyanol and 0.5 molar Tris-HCl pH 6.8. The gel tubes from the first separation were resolved on 12% SDS-PAGE. The tube was placed horizontally on top and sealed by 1% SDS and 1% Agarose in 0.5 molar Tris-HCl pH 6.8. The gels were run at constant current of 40 milliamperes per 20 centimeters for 5 hours.

Results

The results below were obtained from the experimental procedure listed above.

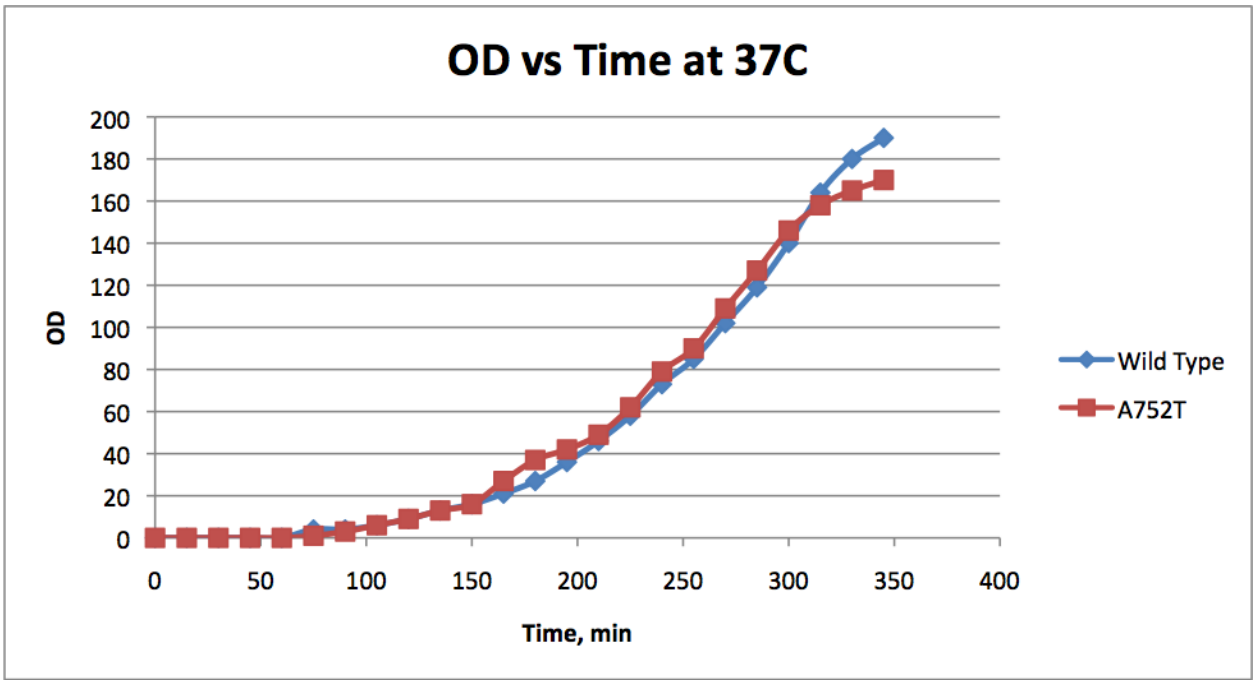

Figure 6: Growth Curve at 37 degrees Celsius

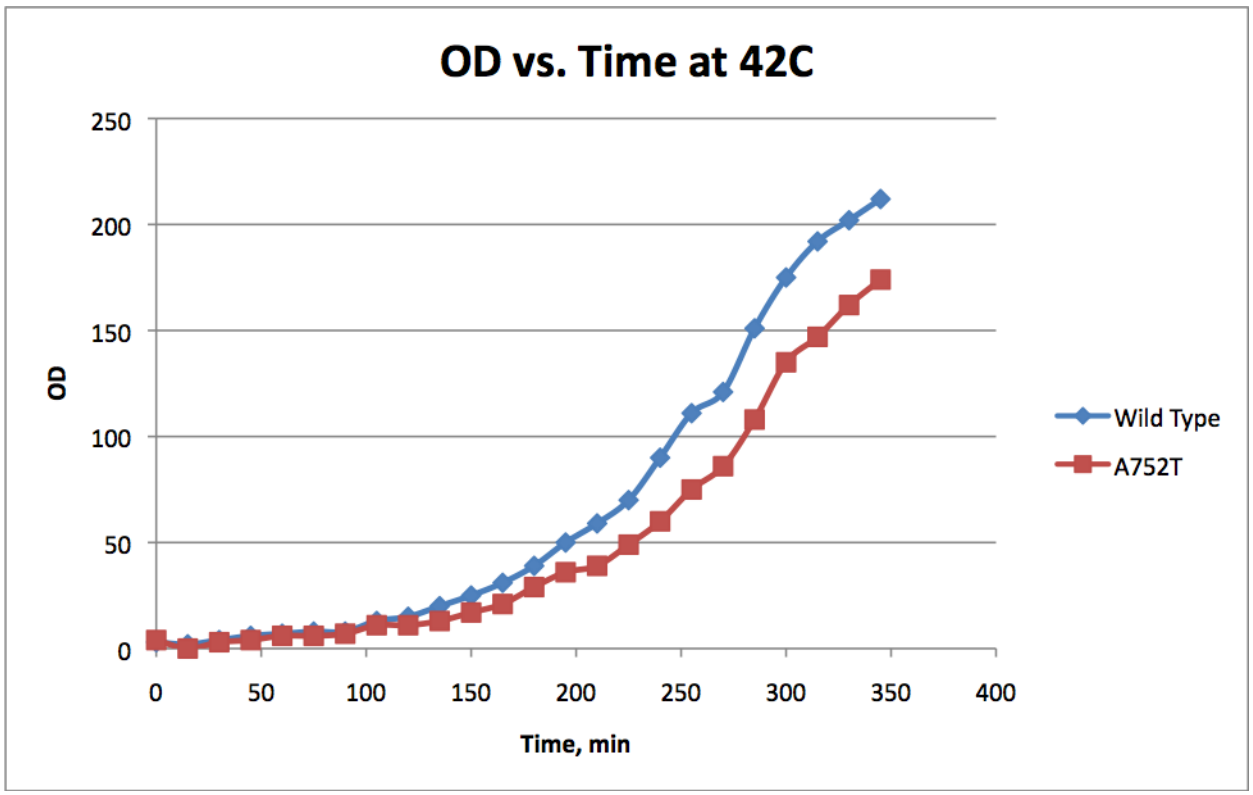

Figure 7: Growth Curve at 42 degrees Celsius

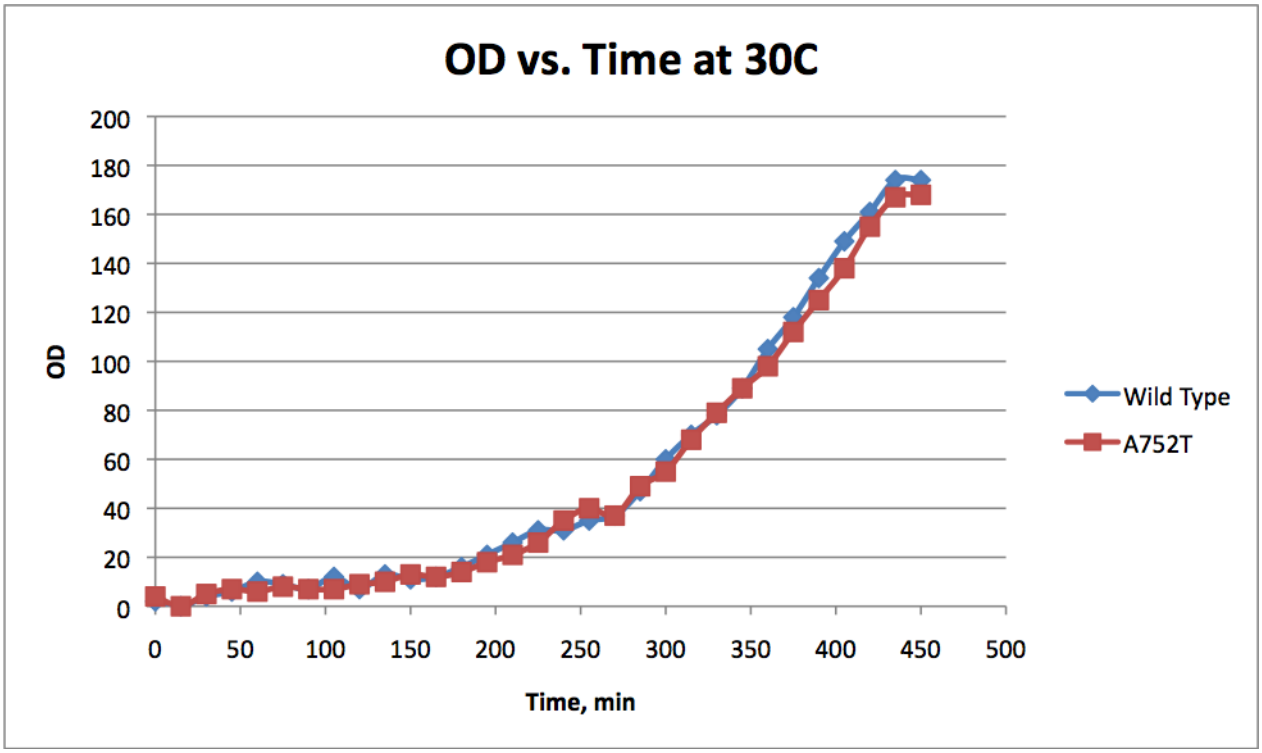

Figure 8: Growth Curve at 30 degrees Celsius

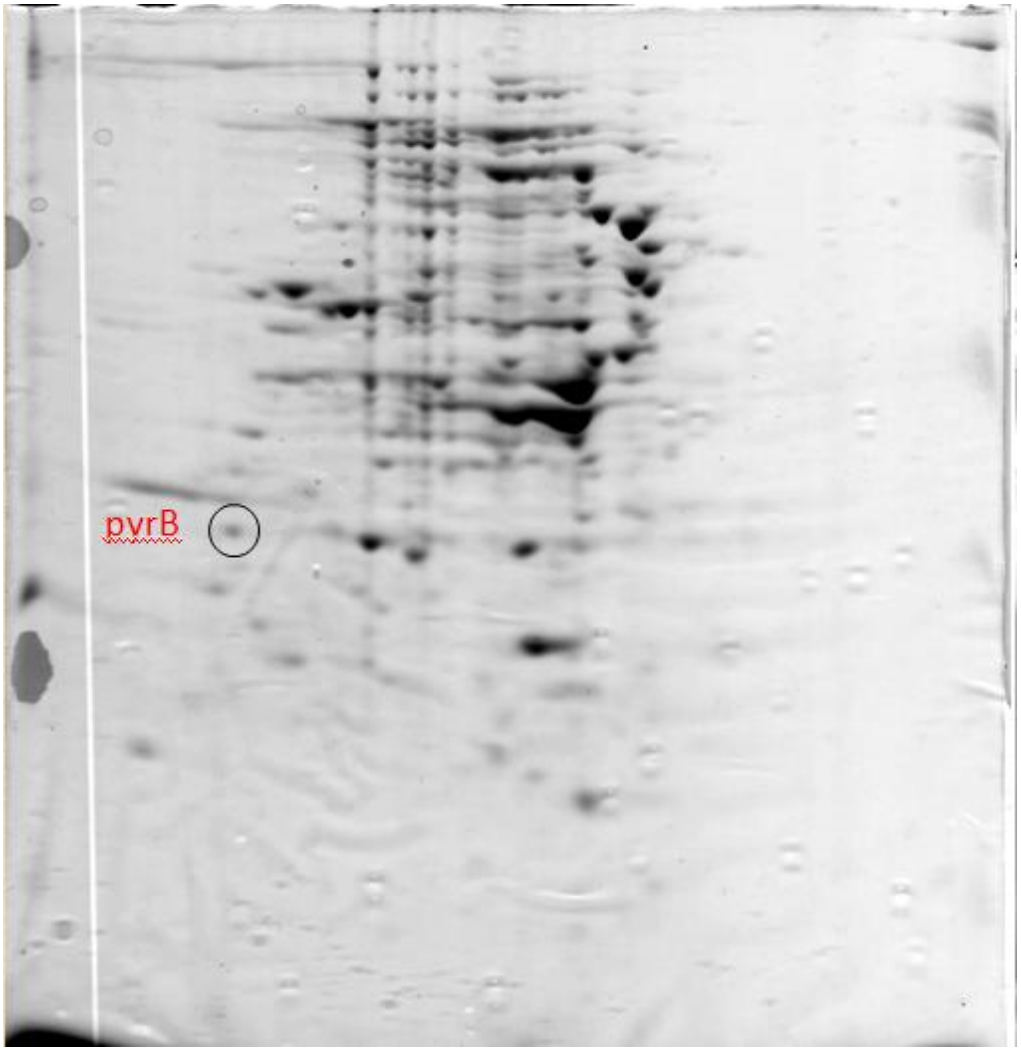

Figure 9: 23S rRNA Wild Type 2D Gel

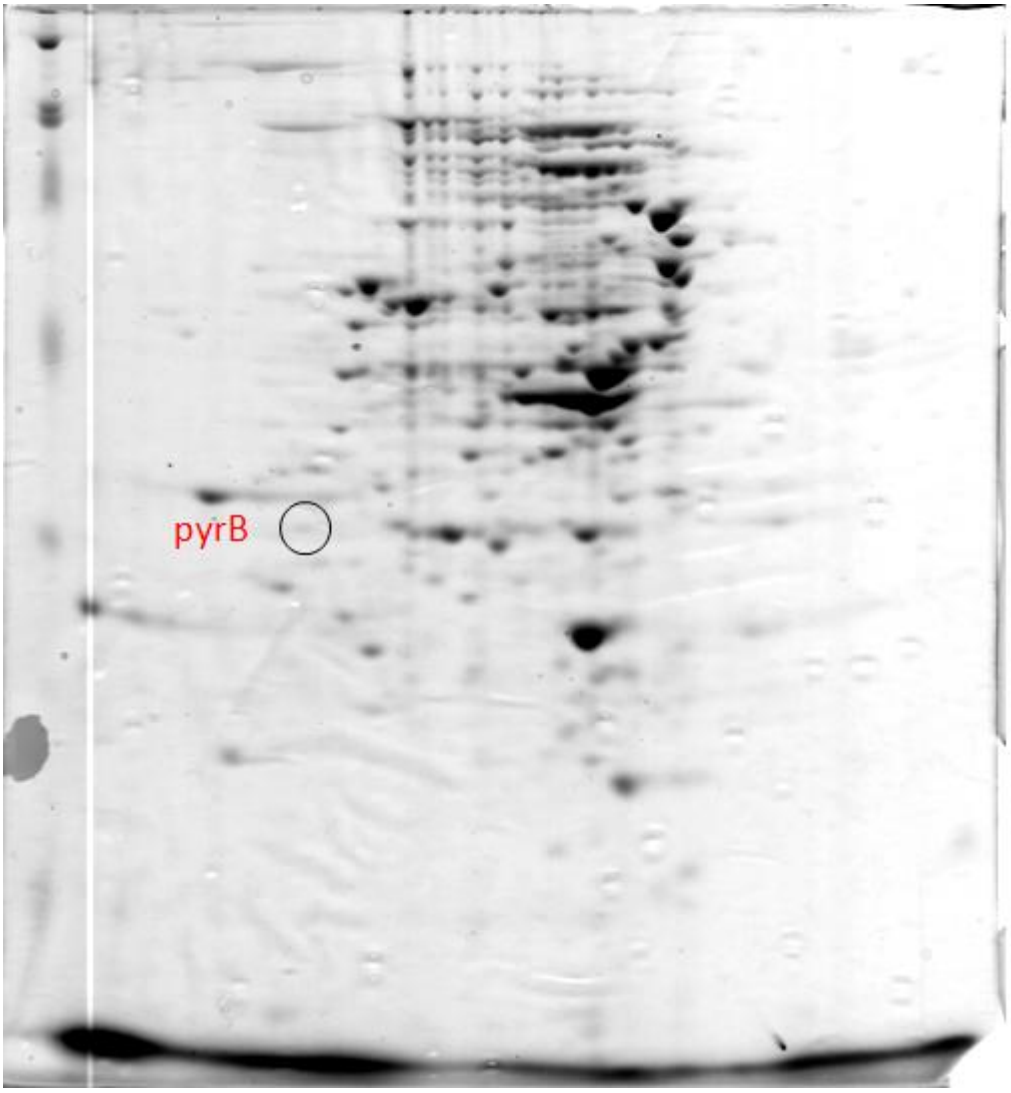

Figure 10: 23S rRNA A752T Mutant 2D Gel

Discussion

Ribosome functions are regulated by some nascent peptides. Comparing the amino acid sequence of mutants and wild type peptides it can be concluded that amino acid sequences of nascent peptides can change the ribosome's function [8]. Peptides interact with the structural operations of the ribosome, essentially with the exit tunnel [8]. The results obtained by the experiments performed in this work have shown that changes in the structure of the exit tunnel

affect the expression of some genes in bacteria. The mutation studied for this thesis, A752T in the 23S rRNA of 50S ribosomal subunit, affects the expression of *secM* and *tnaCAB* operon [7-8]. From the growth curves listed in the Results section, curves show that the mutant A752T 23S rRNA grew slower than the wild type only at 42 °C (compare Fig 6 and 8 with Fig 7). These results could be explained if the expression of essential genes is affected at 42 °C. Our proteomic analysis revealed that the expression of the *pyrB* gene was reduced in the mutant strains (compare Fig 10 with Fig 9). *pyrB* gene expresses the aspartate carbamylase enzyme involved in the synthesis of Uridine [9]. Uridine is a very important nucleotide used to produce DNA and RNA [9]. The reduction of expression of *pyrB* would reduce the concentration of uridine and the synthesis of DNA and RNA in the mutant cells slowing down their cell growth as seen in our results. Interestingly, the synthesis of a small nascent peptide is required to regulate the expression of the *pyrB* gene by a process known as attenuation [9]. The usages of ribosome mutants could be beneficial to reveal other genes which expression depends in regulatory nascent peptides that affect the ribosome activity.

Conclusions

The A752T mutant 23S rRNA affects the expression of the *pyrB* geneFurther research on the mutant A752T 23S mRNA of 50S ribosomal subunit will be conducted by future undergraduate, graduate and PhD students under the guidance of Dr. Luis Cruz-Vera. The undergraduate and graduate students will analyze the expression of genes using other relevant mutant ribosomes. Also, they will analyze the expression of genes using different growth conditions. PhD students will study the molecular mechanism involved in the expression of

metE gene, which was reported by a master's student from the same laboratory. Also, they will study how mutations in the ribosome affect the synthesis of the *pyrB* gene.

Acknowledgments

I would like to thank Dr. Luis Cruz-Vera for his guidance and assistance with this project. I am very thankful for the opportunity to become a part of his research laboratory. I also appreciate the helpfulness of everyone working in his laboratory. I would like to thank the Honor's Program for the opportunity to perform hands-on research directly under a expert in the chosen field.

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