Title

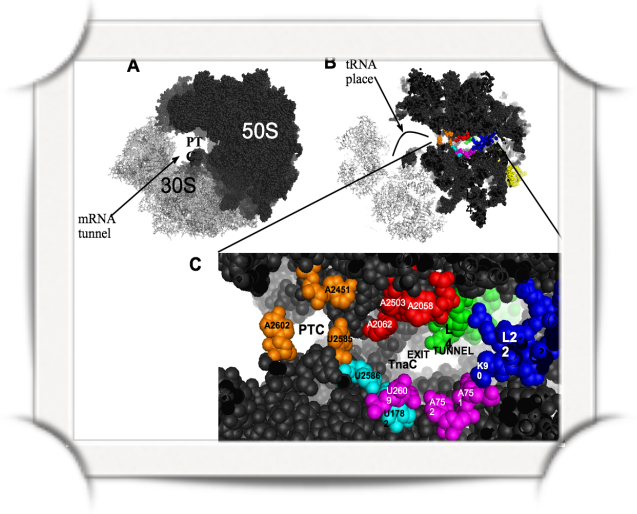
Isolation of L4 ribo-protein mutants that affect the expression of tna operon of Escherichia coli by Soniykha Dhevi Murukan.

Research

Research Experience for Undergraduate Students, Summer 2012, University of Alabama in Huntsville.

Introduction

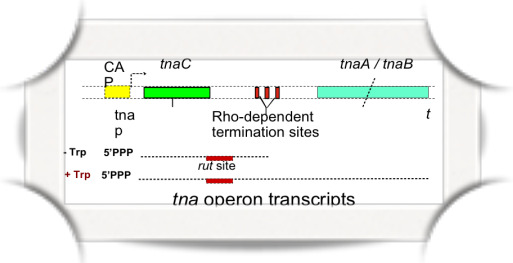
The ribosome consist of two subunits, 30S and the 70S as it can be seen in **Fig 1**. A tunnel exist in the ribosome that carries the nascent peptide chain. The L4 ribo-protein is made of amino acids in which some are proven to be hydrophobic and therefore creating a pocket of hydrophobic shelter for the Tryptophan residue in the tunnel. The isolation of L4 ribo-protein mutants that affect the expression of tna operon of Escherichia coli was carried out.



(Fig 1)

Figure 1 shows an image of Escherichia coli's ribosome, part A shows the two subunits that make up the ribosome and also the mRNA tunnel that's present. Part B shows an image of a sagittal cut ribosome which shows the ribo-proteins and the tunnel. Part C shows the L4 ribo-protein (green), the exit tunnel and other ribo-proteins.

The tryptophanase (tna) operon plays a crucial role in translation; it consist of tnaC , followed by the Rho factor, tnaA and tnaB as shown in **Fig 2**. The expression of the tna operon is dependent on tnaP, also known as the transcriptional promoter. The tna operon's expression is regulated by tryptophan, thus allowing transcription to take place.



(Fig 2)

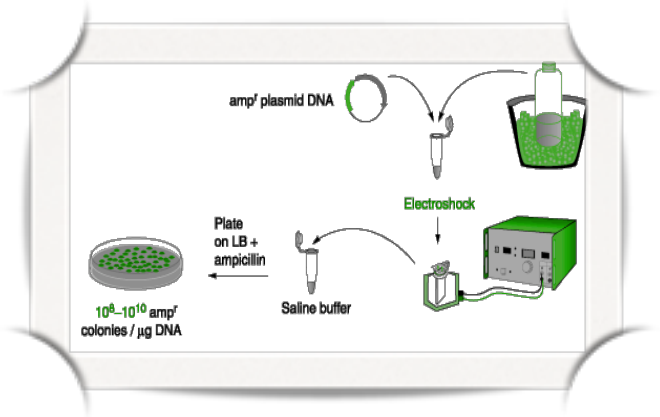
Figure 2 shows an image of the tna operon, in the absence of Tryptophan (-Trp) transcription is terminated at the Rho factor. The rut site interacts with the Rho factor thus enabling termination to take place. In the presence of Tryptophan (+Trp) transcription successfully takes place and reaches tnaA and tnaB, later it will be terminated when it reaches the (t) sequence on the operon.

In order to perform the L4 ribo-protein's mutagenesis, the Escherichia coli's 50S ribosomal L4 subunit proteins sequence was analyzed. Oligo pieces were created to perform mutation, its affect on the tna operon's expression was later analyzed.

Method

In order to perform the mutagenesis, firstly the wild type plasmid (pNHL4-A160V) was transformed by using a chemically competent cell. It was later plated on an ampicillin plate, after a couple of days the bacteria was plated again by streaking to reduce the concentration of the bacteria. One colony from this plate was later cultured with LB solution. Once the plasmid was transformed it was later extracted by using QIAprep Spin Miniprep Kit.

Now that the plasmid is extracted, the mutagenesis can now be performed. The oligos received by Operon was diluted along with the original plasmid and PCR was performed by using QuikChange Lightning. The mutated plasmids were later transformed using a chemically competent cell as previously done. It was later plated again. Later the bacteria was cultured by growing in a 37°C water bath for approximately 17 hours. The mutated plasmids were then extracted. After performing all four mutation, the expression of the tna operon was analyzed by the B=Galactosidase Assay. In order to perform the assay the mutated plasmids were transformed using an electro competent cell (SVS 1144). This cell has a reporter gene known as lac Z, this gene is known to express the β-Galactosidase Assay. The plasmids were transformed via electroporation, shown in **Fig 3** and later the plasmid was plated.



(Fig 3)

Figure 3 shows an image of the electroporation process. The mutated plasmid, along with the electro competent cell were mixed in a micro tube and later transferred into an electro cuvette and shocked.

Electroporation allows the plasmid transformation to be done faster because of the use of the electro competent (SVS1144) cells compared to the chemical competent cell transformation. The use of electric in this transformation speeds the transformation.

Once the cuvette in placed in the electroporator, the electric field therefore allows the ions present in the solution to move the its respective charge. Thus allowing the plasmid to be incorporated along with the cell and transforming it.

Since the mutated plasmids were transformed the β-Galatosidase Assay was carried out to check for each of the mutations enzyme activity. Each transformed mutagenesis was plated on an ampicillin plate, after a couple of days visible colonies will be present. Each mutation's bacteria colony was allowed to grow overnight in M9 minimal media. Different concentration of L-Tryptophan was added into bacteria cells and was allowed to grow for 7 hours. In order to start the reaction the appropriate amount of cells, z buffer, chloroform and SDS was added. Finally the reaction was started by adding ONPG and stopped by adding sodium carbonate, when a sufficient yellow color was present in the reaction tube. The time it took to run the reaction was recorded. The optical density of each reaction was measured at 420nm and 550nm. A graph was plotted for all mutation including the control by using the expression shown in (**Eq1)** 1000 x [OD420 −(1.75 x OD550)] / (T x V x OD600)). **Equation 1** shows an expression that was used to plot the graph to analyze the enzyme activity. The optical density of the reaction was measured at 420nm and 550nm, the optical density of the cell was measured at 600nm. The (T) in the expression is the time it took to run the reactions and (V) is the volume of cells used in the reactions.

Results

Text 1 shows a portion of the Escherichia coli's L4 sequence and highlighted in bold are the regions that were mutated. All the chosen regions are hydrophobic and its mutation which was Aspartic Acid (Asp/D) is hydrophilic. The tryptophan residue is hydrophobic and by mutating the regions shown in **Text 1** to hydrophilic, any changes in the expression of the tna operon can be identified.

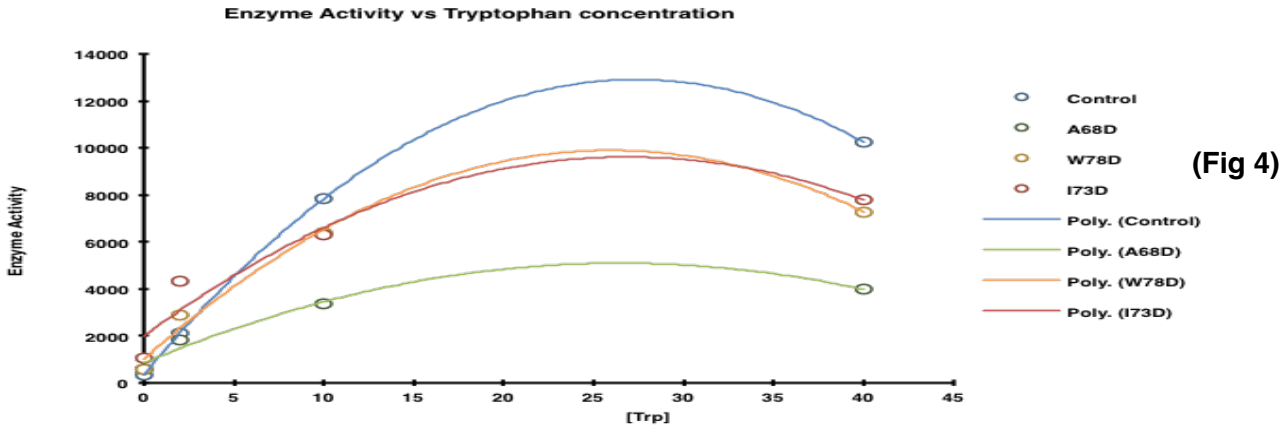
121 CAG GGT ACT CGT GCT CAG AAB ACT CGT GCT GAA GTC ACT GGT TCC GGT AAA AAA CCG **TGG(60 Trp)** 181 CGC CAG AAA GGC ACC GGC CGT **GCG(68Ala)** CGT TCT GGT TCT **ATC(73Ile)** AAG AGC CCG ATC **TGG(78Trp)** CGT TCT 241 GGT GGC GTG ACC TTT GCT CGT CCG CAG GAC CAC AGT CAA.

(Text 1)

Text 2 shows each of the mutation that was performed. In parenthesis is the respective substituent (Aspartic Acid/Asp/D).

Trp 60 (W60D) **Ala 68** (A68D) **Ile 73** (I73D) **Trp 78** (W78D) **(Text 2)**

The data obtained from the β-Galactosidase Assay proved that the mutations performed affected the expression of the tna operon. **Figure 4** shows the graph of the enzyme activity of each of the mutations plotted against the tryptophan concentration. **Table 1** shows the data for the enzyme activity obtained by using **Eq 1. Figure 4** shows the graph that was plotted, when compared the polynomial curve of each of the mutation with the curve from the control it was evident that the mutation **A68D** had the highest induction affect on stalling the ribosome. Followed by both **W78D** and **I73D**; both these mutation affected the stalling more than the control but not as much as **A68D.**



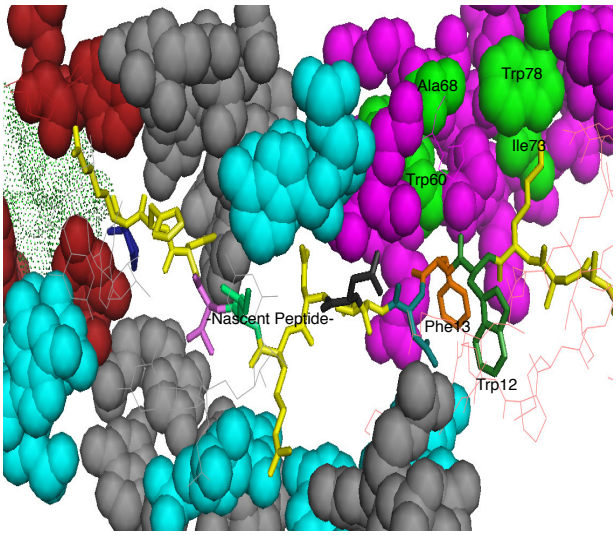
(Fig 4)

[Trp]	Control	[Trp]	A68D	[Trp]	W78D	[Trp]	I73D
0	330.21	0	578.35	0	569.28	0	1034.12
2	2133.76	2	1816.71	2	2886.34	2	4336.51
10	7848.91	10	3364.92	10	6401.28	10	6297.84
40	10252.96	40	3985.41	40	7292.57	40	7816.58

(Tab. 1)

Conclusion

Figure 5 shows the image of the ribo-proteins in the ribosome. The region highlighted in purple is the L4 ribo-protein and the regions highlighted in lime green are the regions that were mutated. As the data obtained from the Assay shows, the mutation **A68D** has the highest induction affect. Shown in chain shape is the nascent peptide that is present in the tunnel of the ribosome. The region colored in orange is Phe13 and the region colored in green is Trp12, both these regions are also hydrophobic.



(Fig 5)

When the mutation has been performed the Trp residue will no longer be able to take shelter in that region, due to the hydrophilic change from hydrophobic. As the data suggest **Ala68Asp** had the highest affect in the expression of the tna operon, the conformational change that Aspartic Acid gave instead of having Alanine at position 68 could not have allowed Tryptophan to hide in that region, therefore having the highest induction affect followed by **Trp78** and **Ile73.**

Acknowledgement

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Reference

1. Cruz Vera, L. R., Gong, M., & Yanofsky, C. (n.d.). Ribosomal features essential for tna operon induction-expression mediate tryptophan interaction with the ribosome .