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1. Introduction

Release factor 2 (RF2) is a key structural component found in the ribosomes of all prokaryotic organisms As RNA passes through the ribosome it is translated into a peptide chain which passes through an exit tunnel in the ribosome. When either the UAA or UGA stop codon is present, RF2 recognizes these codons and initiates ribosomal stalling of translation. It is believed that RF2 undergoes conformational changes in the presence of the aforementioned stop codons which causes the peptidyl transferase center to cleave the peptide bonds of the RNA; resulting in ribosomal stalling.

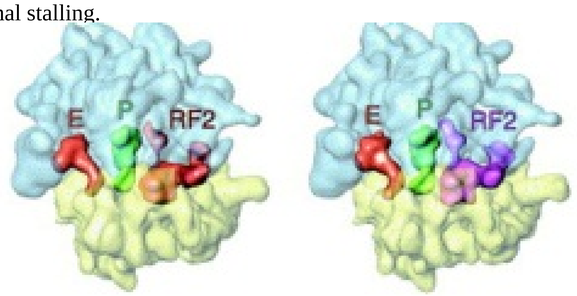


Fig. 1 Two different views of the 70S ribosome of Escherichia coli showing RF2, the tRNA peptidyl site (P) and, the tRNA exit site (E).

The tna operon is a determining factor in transcription antitermination and it is is catalyzed by tryptophan. Therefore; in the presence of tryptophan, the tna operon will prevent the ribosome from stalling. By mutating amino acid residues of release factor 2 and observing the enzymatic activity of these mutated cells in different concentrations of tryptophan it was possible to gain a better understanding of the important sites involved in the expression of the *tna* operon in *Escherichia coli*. The amino acid residues that were mutated are present on either the inside of the petide exit tunnel or the decoding region that recognizes stop codons.

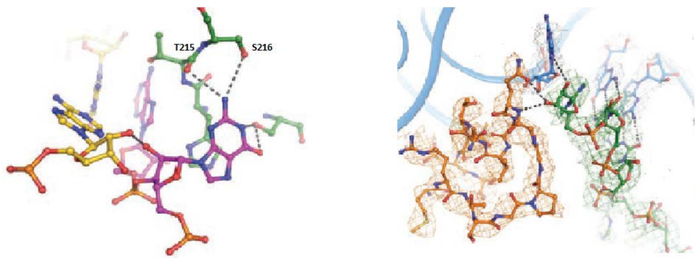


Fig 2. (Left) The sites T215 and S216 are part of the decoding region that recognizes release factors UAA and UGA. (Right) A section of RF2, in orange, reacts with the tRNA, in green, at the P site.

2. Method

The first step of this experiment was to design oligonucleotides to be transformed and create mutant E.coli colonies to be later tested for enzyme activity influenced by tryptophan.

ATG TTT GAA ATT AAC CCG GTG AAT AAC CGC ATT CAG GAC CTC ACG GAA CGC ACC AAC GTT CTT AGG GGG TAT CTT GAC TAC GAT GCT AAG AAA GAG CGT CTG GAA GAA GTA AAC GCC GAG CTG GAA CAG CCG GAT GTC TGG AAC GAA CCT GAG CGC GCG CAG GCG CTG GGA AAA GAG CGT TCA TCG CTC GAA GCG ATC GTC GAT ACG CTT GAT CAA ATG ACT CAG GGG CTG GAC GAC GTT TCC GGG CTG CTG GAA CTG GCG GTA GAA GCT GAC GAC GAA GAG ACG TTT AAC GAA GCC GTC GCG GAA CTG AAT ACG CTG GAA GAG AAG TTG GCG CAG TTG GAA TTC CGC CGG ATG TTC TCC GGC GAG TAC GAT AGC GCC GAT TGC TAT CTC GAT ATT CAG GCC GGT TCC GGT GGT ACT GAA GCG CAG GAC TGG GCC AGC ATG TTG CTG CGT ATG TAT CTG CGT TGG GCG GAA GCG CGC GGC TTC AAG ACA GAA GTC ATT GAA GAA TCG GAA GGG GAA GTC GCC GGT ATT AAG TCC GCG ACT ATC AAA ATC TCC GGC GAA TAT GCC TAT GGC TGG CTG CGC ACT GAA ACC GGC GTA CAT CGT CTG GTG CGT AAA AGC CCG TTT GAC TCC GGC GGT CGC CGT CAT **ACC(GCG) TCG(GCG)** TTT AGC TCC GCG TTT GTG TAC CCG GAA GTG GAC GAC GAT ATC GAT ATC GAC ATT AAC CCG GCG GAT CTG CGT ATC GAC GTC TAT **CGC(GCG)** GCG TCT GGC GCA GGC GGT **CAG(GCC)** CAC GTT **AAC(GCG)** CGT ACG GAA TCC GCC GTG CGT ATC ACC CAT ATT CCA ACC GGA ATC GTG ACG CAG TGC CAG AAC GAC CGT TCG CAG CAC AAA AAC AAA GAC CAG GCC ATG AAG CAG ATG AAA GCG AAG CTT TAT GAA CTG GAG ATG CAG AAA AAG AAC GCT GAA AAA CAG GCG ATG GAA GAC ACT AAG TCC GAT ATC GGC TGG GGA AGC CAG ATT CGT TCC TAT GTC CTG GAC GAT TCC CGC ATT AAA GAC CTG CGT ACT GGG GTG GAA ACC CGC AAT ACA CAG GCG GTA CTG GAC GGT AGT CTG GAT CAA TTT ATC GAA GCA AGT TTG AAA GCA GGG CTA TGA

Fig 3. Amino acid sequence of Salmonella Typhimurium RF2. Mutated codons are bolded while the replacement codon is in parentheses.

The next step was the mutagenesis of E. coli cells.. Oligonucleotides of Salmonella typhimurium were created by MWG operon and then transformed with the QuikChange Lightning Mutagneisis kit into chemically competent E. coli cells. Salmonella RF2 was used instead of E.coli RF2 because overexpression of E. coli RF2 is lethal for the cells. After these cell colonies were grown, the plasmids were extracted and tranformed into electrocompetent SVS1144 strain E. coli cells. These cells contain a lacZ reporter gene associated with the *tna* operon. This gene allowed testing of enzymatic activity by a reaction in which O-nitrophenol-galactose was degraded by β-galactosidase into O-nitrophenol and galactose. Optical testing of the concentration of O-nitrophenol allowed for calculation of *tna* activity of mutants when compared to a wild type control. These calculation were found in Miller Units by the following equation:

$$Miller\ Units = 1000\ x\ [(OD_{420} - 1.75\ x\ OD_{550})]/(T\ x\ V\ x\ OD_{600})$$

Where, T= time in minutes

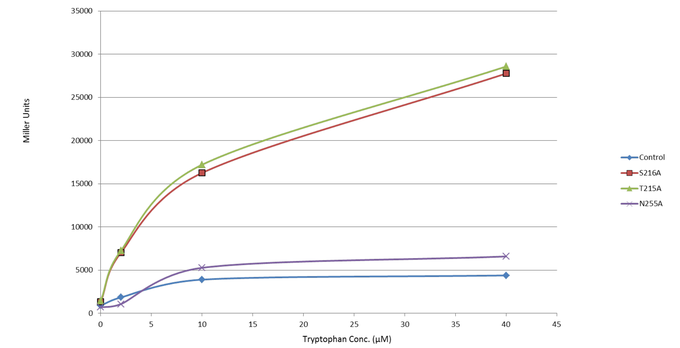
V=mLs of culture used in assay

3. Results

This table indicates whether or not each mutation increased enzymatic activity of the *tna* operon at the various concentrations of free floating tryptophan that were tested.

Trp. Conc.	N255A	T215A	S216A
0	No	Yes	Yes
2	No	Yes	Yes
10	Yes	Yes	Yes
40	Yes	Yes	Yes

3. Results (cont.)



The above graph shows the level of tryptophan induced enzymatic activity by the *tna* operon in the E. coli colonies containing a mutation as well as a wild type control colony.

4. Conclusions

Experimental results from the β-galactosidase assay indicate that changing the aparagine at the 255 position of RF2 (N255) into an alanine slightly elevates the activity of the *tna* operon in 10 μM and 40μM concentrations of tryptophan. However, this same mutation causes a decrease in operon activity at 0μM and 2 μM concentrations. Mutating the threonine and serine residues, located at the 215 and 216 positions respectively (T215 and S216), to alanine caused a drastic increase in operon activity in all concentrations of tryptophan. These results indicate that mutations to amino acid residues located in the peptide exit tunnel, such as N255, can cause increased tryptophan induced enzymatic activity in high levels of free floating tryptophan. Mutations to decoding region amino acid residues, such as T215 and S216, seem to cause a dramatic increase in tryptophan induced enzymatic activity of the *tna* operon in virtually any concentration of free floating tryptophan.

Acknowledgments

This research was funded by the RCEU program with funds provided the the Presidents/Provosts office, funds provided by the Vice President for Research, funds provided by the Chemistry Department through their patent account, and external funding from the Alabama Space Grant Consortium

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