Project title: A study of *tnaC*'s role in the universal expression regulation of RF2-dependent regulons

Project Description: L-Tryptophan (L-Trp), one of the amino acids essential for bacterial survivability, is metabolized under strict regulation in order to optimize the use of energy in bacterial cells. *Escherichia coli* (*E. coli*), a pathogenic bacterium, expresses a genetic unit named *tna* operon to utilize free L-Trp in the environment as a source of energy. Conversely, *E. coli* can also synthesize L-Trp as another genetic unit called *trp* operon is expressed to produce appropriate enzymes. Due to their opposite roles, these two genetic units are coordinated such that only one of them can be expressed based on the corresponding L-Trp cellular concentrations. If present in high cellular concentration, L-Trp reduce the expression of the *trp* operon while increase that of the *tna* operon. However, the regulation by L-Trp is still under more investigation, as it is important to thoroughly understand the mechanisms that help this pathogenic bacterium to survive environmental changes.

The *tna* operon consists of three genes: a regulatory gene (*tnaC*) positioned upstream from two genes encoding for enzymes that play major roles in breaking down L-Trp. In the presence of high L-Trp cellular concentrations, L-Trp will interact with the machinery that synthesizes proteins, the ribosome, and interrupt the synthesis of the TnaC peptide, enabling the expression of the downstream genes. **During this regulatory process, the peptide chain release factor 2 (RF2), a protein that aids the protein synthesis termination, is thought to bind the jammed ribosome and become sequestered there**. On the other hand, the *trp* operon, comprising of five genes, is regulated by several mechanisms. All of these mechanisms sense the absence of L-Trp increasing the expression of the enzymes responsible for producing L-Trp. Fascinatingly, *trp* operon's *trpB* and *trpA* genes are regulated independently from the whole operon such that the translation of the *trpB* gene is required for that of the *trpA* gene. **The proper synthesis termination of *trpB*'s aided by RF2 is essential to initiate *trpA*'s synthesis; thus, the activity of RF2 plays a major role in the expression of *trpB/A* and the *trp* operon's activity.**

It is hypothesized that if RF2 is sequestered within the ribosomes jammed at the tnaC gene under high cellular concentration of L-Trp, the free cellular concentration of RF2 will be reduced, and the expression of trpB/A complex will be diminished, leading to a decrease in the synthesis of L-Trp. Based on this postulation, this project is proposed to investigate the role of *tnaC* and L-Trp in regulating the expression of *trpB* and *trpA*. A gene construct made by fusing the green fluorescent protein (GFP)'s genetic information with that of *trpA* will allow facilitated detection of the *trpB/A* complex's expression. To control the expression of the gene construct, a cellular stable analog of L-Trp, 1-L-MT, will be employed. Under high concentration of 1–L-MT, the fluorescence produced by *trpA*-GFP construct is expected to be reduced, due to a lack of free RF2 leading to an inhibition in the translation termination of *trpB*, as compared to that in the analog's lower concentration. **This will suggest that L-Trp, through regulating *tnaC*'s expression, controls the amount of free RF2 in the cells and, ultimately, the expression of other genes.** Discovering new avenues of gene regulation during bacterial survival could be potentially useful in developing strategies that alter their survivability of pathogenic bacteria as desired.

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