Insights into the Peptide Release Reaction and Translational Stalling by the Ribosome

Key Words: *Peptide Release, Ribosomal Stalling, Gene Regulation, Student Mentoring*

Abstract:

The aim of the proposed research is to gain a mechanistic understanding of peptide release and nascent peptide mediated ribosome stalling by employing both a synthetic and structural approach. This project will **broaden** our understanding of protein synthesis and gene regulation by the ribosome and **promote** teaching and learning in all educational levels through **mentoring** and **collaboration**. This research will be completed with the guidance of Dr. Scott Strobel at Yale University, with all the requested resources and collaborations available to successfully accomplish the following aims.

Background and Significance:

Protein synthesis by the ribosome is a fundamental process found in all life. A set of highly conserved nucleotides located in the active site of the large subunit of the ribosome are responsible for two biologically important reactions: peptide bond formation and release. Termination of protein synthesis occurs when one of three stop codons are recognized in the small ribosomal subunit and decoded by release factor proteins (RFs) [1]. Upon recognition, a highly ordered water molecule nucleophilically attacks the aminoacyl ester linkage of peptidyl-tRNA hydrolyzing the ester bond which links the nascent polypeptide to the peptidyl-tRNA. As seen in **Figure 1a**, it is hypothesized that as the ordered water molecule attacks the ester linkage, the carbonyl carbon proceeds through a tetrahedral transition state containing a developing negative charge, an oxyanion. While termination of translation has been known for some time now[2], it is less studied than elongation and the underlining mechanistic processes are only starting to emerge. Thus transition state characterization and structural studies can help define how the ribosome catalyzes this challenging reaction.

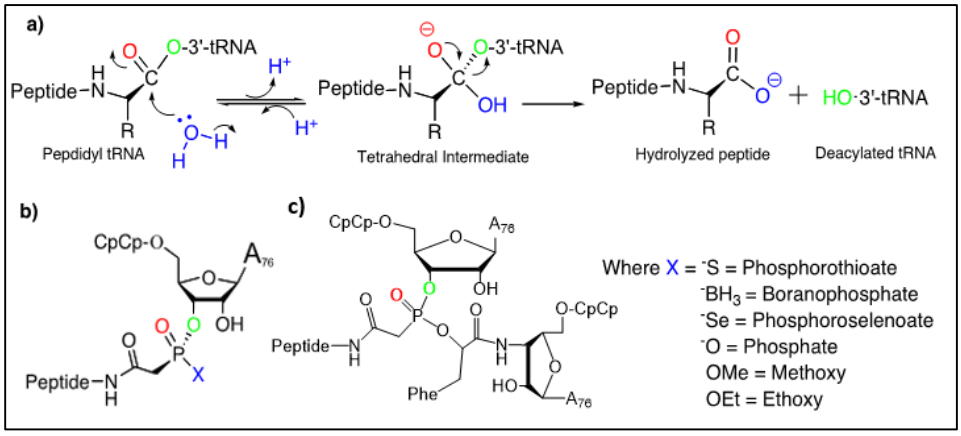

Figure 1. a) General mechanism of peptide release. b) Generic structure of peptide release transition state analogs. c) Generic structure of peptide formation transition state analogs.

In addition to catalyzing the formation and release of polypeptides, the ribosome has also been found to have the ability to monitor the structure of the growing polypeptide during elongation, a process which is poorly understood. Accumulating evidence shows that some nascent peptides result in ribosomal stalling due to specific RNA interactions within the exit tunnel of the ribosome. Many of these have been found to play a role in regulating the expression of genes such as erythromycin resistance in bacteria[3]. Recent cryo-EM reconstructions of the stalled ribosome have suggested that certain interactions within the tunnel are relayed to the peptidyltransferase center (PTC) to arrest translation[4]. However, how this information is communicated to the PTC is essentially unknown. By further understanding the mechanism of ribosome stalling it may yield insights into the events that regulate gene expression from bacteria to humans, which can lead to the rational design of more efficacious drugs.

Specific Aims:

Aim 1: ***To synthesize and measure the binding affinity of a series of transition state analogs.***

In the Strobel lab, I will create a series of peptide release transition state analogs containing functional groups of varying shapes, charge distributions, and hydrogen-bonding potentials and measure their relative affinity for the ribosome using RNA chemical footprinting (**Figure 1b**). With this technique, nucleotides in the 23S rRNA will be probed using dimethyl sulfate as a function of inhibitor concentration using established protocols[5]. All of these inhibitors have the same basic geometry and each is synthesized as a pair of diastereomers that allow both non-bridging oxygens to be characterized independently. Transition state theories predict that enzymes bind the tightest to the transition state of the reactions they catalyze. Therefore, the inhibitors that best complement the electrostatic environment of the active site will bind the tightest, and from changes in the extent of modification of ribosomal residues, the relative affinities will allow us to draw conclusions about the geometry and charge distribution of the active site during release.

Aim 2: ***To gain a structural understanding of peptide release and induced ribosomal stalling.***

Given the implications of the ribosome in peptide release, its role in translational arrest, and its essential yet understudied role in gene regulation, it will be vital to develop a mechanistic understanding of how the ribosome performs all these actions. Using high-resolution crystal structures I will investigate how important structural features of the ribosome, peptidyl tRNA and release factor proteins position a water molecule for optimal attack of the aminoacyl ester linkage of peptidyl-tRNA. I will also elucidate how specific conformations of the nascent polypeptide chain and subtle conformational changes in the ribosome can feedback inhibit the PTC. I will thus collaborate with the Steitz lab at Yale, which is preeminent in ribosomal X-ray crystallography, to get a crystal structure of release factor 2 bound to the ribosome with the best peptide release transition state analog from Aim 1 (**Figure 1b**). Using solid phase synthesis, I will also synthesize transition state analogs of peptide bond formation with an attached polypeptide of known stalling ability that can be tethered into the exit tunnel (**Figure 1c**). By visualizing the peptide-exit tunnel interactions through crystallography together with biochemical and computational data, it is possible to propose a more accurate mechanistic model of nascent polypeptide chain-mediated translational stalling.

Aim 3: ***To promote teaching, mentoring and collaboration in multiple educational levels.***

In collaboration with Dr. Nicolas Carrasco at Quinnipiac University, I am in the unique position to teach and **mentor** possible undergraduate students from both Yale and Quinnipiac who wish to participate in this project by helping them **experience** graduate-level research, and **teaching** them how to communicate their findings at conferences. These students will work toward the synthesis of various oligonucleotide-peptide conjugates in order to further research stalling peptides and investigate the role of various cofactors in the formation of the stable stalled ribosome complex. I will also use this work as a teaching tool during TA sessions at Yale for science and non-science oriented undergraduate classes by teaching students how to think critically and analytically. Additionally, I will become involved in the New Haven Science Fair Mentor Program (NHSFMP) to help elementary school students and teachers become more excited about science. I will facilitate weekly brainstorming sessions meant to teach students to form a hypothesis, develop an experimental approach, and analyze their results. My long term goal is to help create a better science curriculum to show their students how to become future scientists.

References:

1. Weixlbaumer, W. *et al.* (2008) *Science.* 322: 953-956. **2.** Capecchi, MR. *et al.* (1967) *Proc. Natl. Acad. Sci.* 58: 1114-51. **3.** Ramu, H. *et al.* (2011) *Mol. Cell.* 42: 321-330. **4.** Seidelt, B. *et al.* (2009) *Science.* 326: 1412-15 **5.** Parnell, K. *et al.* (2002) *Pro. Natl. Acad. Sci. U. S A.* 99: 11658-1166