Problem Set 5 April 5 due April 12

1. Two Class II tRNA synthetases (RS) from *S. aureus* have been purified to homogeneity and characterized kinetically. One catalyzes the charging of phenylalanine and the other catalyzes the charging of tyrosine. Each synthase was studied with amino acids F and Y and the results are shown in Table 1.

Table 1: Kinetic data on Y-RS and F-RS with Y, F substrates

substrate		Y-RS	F-RS
Y	k_{cat}	$100\ s^{-1}$	$20\ s^{-1}$
Y	K_m	$10^{-6}M$	$10^{-3}M$
F	k_{cat}	$1\ s^{-1}$	$100\ s^{-1}$
F	K_m	$10^{-3}M$	$10^{-6}M$

Given the kinetic data in Table 1, what conclusions can you draw about the mechanism of fidelity of these Class II RSs? Show your quantitative reasoning.

2. EF-G is involved in the translocation of mRNA during the elongation process in polypeptide biosynthesis on the ribosome. EF-G (a protein) is a structural mimic of EF-Tu•GTP•aatRNA (see handout from class). The translocation process has been studied by methods similar to those discussed in class to understand the function of EF-Tu. Stopped-flow fluorescence using fluorescently labeled charged tRNAs, and studies with two antibiotics (viomycin and thiostrepton) have been especially effective in mechanistic deconvolution and are described below. Viomycin functions by inhibiting translocation of the mRNA, but has no effect on GTP hydrolysis. Thiostrepton inhibits both GTP hydrolysis and translocation.

The model for the role of EF-G prior to the studies described below has been similar to that proposed for EF-Tu (that is, EF-G was thought to be a GTP dependent switch). Specifically, EF-G•GTP was proposed to bind to the pre-translocation ribosome and induce a conformational change that allows mRNA translocation and tRNA movement. Subsequent to the translocation, EF-G•GTP hydrolyzes and generates EF-G•GDP that dissociates from the ribosome. The following studies were undertaken to test this model.

The results of stopped flow fluorescence studies are shown in Figure 1. Ribosomes that were loaded with fluorescently labeled FMet-F-tRNA^F in the A site (Fmet is formylmethionine and exists as the dipeptide, Fmet-Phe attached to the 3'-end C in FMet-F-tRNA^F) and tRNA^{FMet} (uncharged) in the P site were placed in one syringe. The contents of this syringe were rapidly mixed with EF-G•GTP in the second syringe (saturating conditions) in experiment 1. In a second set of experiments (experiment 2) viomycin was placed in syringe containing the EF-G•GTP and in a third set of experiments thiostrepton was placed in the syringe containing EF-G•GTP (experiment 3). The conditions in all three experiments are identical with the exception of the presence or absence of antibiotics. As revealed in Figure 1a and expanded time scale 1c, the rapid rate of fluorescence decrease was essentially the same in all three experiments. Furthermore, results from several additional experiments in which GTP was replaced with non-hydrolyzable GTP analogs, revealed that the rapid drop in fluorescence was not affected by this substitution (Figure 1b).

(Image Removed)

In a second set of experiments (Figure 2a), GTPase rates were measured using [γ-^{32}P]-GTP and a rapid chemical quench apparatus. Pi (inorganic phosphate) release was monitored. The experiment was carried out with no antibiotics (closed circles) with viomycin (open circles) and with no EF-G (open triangles) under conditions identical to those described in Figure 1. The reactions were rapidly quenched in acid. The fluorescence data obtained in Figure 1a and the GTPase data from Figure 2a in the absence of any antibiotics are shown in Figure 2b.

(Image Removed)

Questions: i. Draw a cartoon of the pre-loaded ribosomes placed into syringe one in experiment 1. Given your knowledge of the elongation process explain which step in the elongation process this pre-loaded state is meant to mimic.

ii. Design an experiment to convince yourself that the loading of the ribosome is in fact appropriate for studying the translocation process. [Hint, think about the use of another antibiotic]

iii. Given the role of the antibiotics described above (viomycin, thiostepton), provide a hypothesis for the increase in fluorescence observed in experiment 1 and not in experiments 2 and 3 of Figure 1, following the initial rapid decrease in fluorescence observed in all three experiments.

iv. What do the data in Figure 2a tell you?

v. What do the data and the fit to the data in Figure 2b tell you?

vi. Do these data agree with the postulated role for EF-G in the translocation process? Propose a model for the role of EF-G based on these data. Explain how the data support your model.