

1. (a 12 pts.) The sequences of the three stop codons are: 5'UAG^{3'}, 5'UAA^{3'}, and 5'UGA^{3'}. Give the sequences of the codon portion of the four tRNA genes that can be mutated by a transition mutation to generate a nonsense suppressing allele. (A transition is a mutation of a G•C to A•T or an A•T to G•C). For your answer, write out the DNA sequence of the anti-codon portion of the gene for each tRNA (be sure to label the 5' and 3' ends of both DNA strands).

5'CAG3'	5'TGG3'	5'CAA3'	5'CGA3'
3'GTC5'	3'ACC5'	3'GTT5'	3'GCT5'

Consider the following segment from the middle of a gene coding sequence with the translated amino acids indicated below each codon.

5' ... TGG CCC TTG GAT AGC ... 3'
 ... Trp-Pro-Leu-Asp-Ser ...

You isolate a single base pair insertion creating a frameshift mutation. The additional base is underlined and the resulting amino acid sequence is shown.

5' ... TGG CCC TTG GGA TAG C ... 3'
 ... Trp-Pro-Leu-Gly-Stop

(b 8 pts.) Briefly explain why a nonsense suppressing tRNA mutation cannot correct this mutation.

Although a nonsense suppressing tRNA will correct for the stop codon, it will not correct for the shifted reading frame. Therefore, all of the amino acids downstream of the frameshift mutation will be incorrect.

(c 8 pts.) You isolate a mutation in tRNA^{pro} that carries an additional base in the anticodon loop of the tRNA (the normal anticodon sequence is 5' GGG 3' whereas your mutation has the anticodon sequence 5' AGGG 3'). This type of tRNA mutation is known as a frameshift suppressor. Write out the amino acid sequence from the segment of the protein that is produced when the frameshift is suppressed by the tRNA^{pro} frameshift suppressor mutation.

TGG - CCCT - TGG - GAT - AGC
 TRP - PRO - TRP - ASP - SER

2. A LacI^{S} mutation alters the Lac repressor so that it cannot bind to inducer molecules such as IPTG, thus causing repression of the Lac operon even when inducer is present. Starting with an $\text{F}^- \text{LacI}^{\text{S}}$ strain, which makes white colonies on X-gal plates, you isolate a number of revertants that form blue colonies. After mating each revertant to a strain carrying an F' with a wild type Lac operon (including the LacI gene) you find that the revertants are of two types.

(a 8 pts.) Type 1 revertants exhibit constitutive Lac operon expression but show normally regulated expression when they carry $\text{F}' \text{Lac}$. What type of mutation is the most likely cause of the type 1 revertants? Be as specific as you can.

A Lac I^- mutation would be most consistent with the behavior of the type 1 revertants.

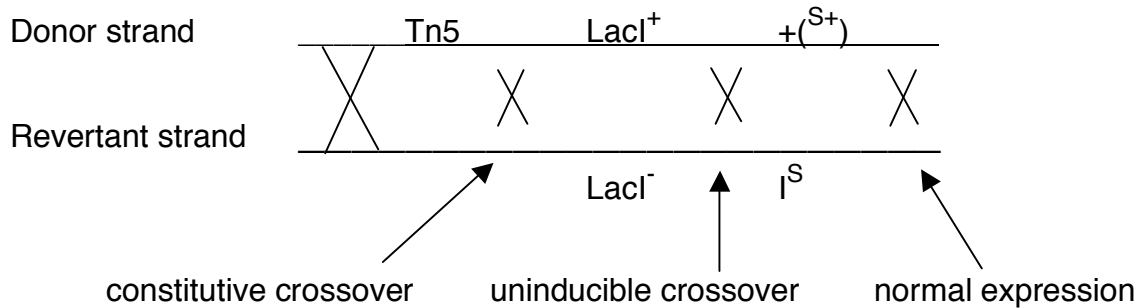
(b 8 pts.) Type 2 revertants exhibit constitutive Lac operon expression even when they carry $\text{F}' \text{Lac}$. What type of mutation is the most likely cause of the type 2 revertants? Be as specific as you can.

A mutation in either the Lac operator (O^c or O^-) or an $\text{I}^{-\text{D}}$ mutation would be most consistent with the behavior of the type 2 revertants.

(c 8 pts.) Which type of revertant would you expect to be the more frequent? Explain your reasoning.

Lac I^- mutations should occur more frequently than a operator mutations (O^c or O^-). This is due to the greater number of nucleotides in the LacI coding region presenting a larger target for mutagens than do the fewer nucleotides that comprise the operator. Lac I^- mutations are more frequent than $\text{I}^{-\text{D}}$ mutations. There are generally fewer ways in which mutagens can cause dominant mutations.

(d 15 pts.) You have a Tn5 insertion that is linked to the $LacI^S$ mutation. You grow P1 on an otherwise wild-type strain that carries the Tn5 insertion and then use this lysate to infect one of your type 1 revertant strains, selecting for kanamycin resistance (Kan^r). Among 100 Kan^r transductants, you find that 60 show normally regulated Lac expression, 35 are constitutive and 5 are uninducible. Draw a genetic map showing the relative positions of the Tn5 insertion and the $LacI^S$ and Lac revertant mutations. For your answer give the distances between the Tn5 insertion and each of the relevant mutations expressed as a cotransduction frequency. (Assume that quadruple crossovers are too rare to be present in the 100 transductants you analyze).



The Tn5 and $LacI^+$ co-transduction frequency is 65% [$(60 + 5) / 100$].

The Tn5 and Lac^{S^+} co-transduction frequency is 60% [$60 / 100$].

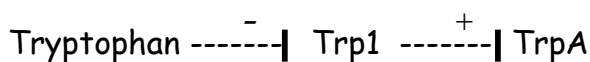
3. You are studying the regulation of synthesis of the amino acid tryptophan in a new bacterial species and you find that the first enzyme in the pathway for tryptophan synthesis (the product of the TrpA gene) is synthesized when there is no tryptophan in the medium, but is not synthesized when tryptophan is present.

(a 9 pts.) You mutagenize the bacteria by generating a collection of random insertions of the transposon Tn5 into the bacterial chromosome. By screening for altered regulation of tryptophan synthesis, you find an insertion mutation, designated Trp1⁻, which gives *uninducible* expression of TrpA even when tryptophan is absent from the medium. Transduction experiments show that the Tn5 insertion is not linked to the TrpA gene. Classify the Trp1⁻ mutation in terms of its likely genetic properties taking into account the type of mutation usually caused by a transposon insertion (explain your reasoning). Propose the type of regulatory function probably encoded by the wild type Trp1 gene. Finally, diagram a model to explain the effects of tryptophan and the wild type Trp1 gene on TrpA expression, assuming a linear pathway.

The Trp1⁻ mutation is most likely *recessive*, as a transposon insertion into the coding region of the Trp1 gene probably would disrupt the normal reading frame and result in a *loss-of-function* mutation.

The Trp1⁻ mutation is *trans-acting*. Transduction analysis showed that TrpA is not linked to Tn5, which is linked to Trp1 (inserted into actually). Thus, any influence of Trp1 on TrpA must occur in *trans*.

The Trp1⁻ mutation yields *uninducible* TrpA expression. This suggests that Trp1 is a *positive* regulator of TrpA.



(b 9 pts.) You isolate a second Tn5 insertion mutation, designated Trp2⁻, which shows *constitutive* TrpA expression even in the presence of tryptophan. The Tn5 insertion in Trp2⁻ is *not* linked either to Trp1⁻ or to TrpA. Diagram the *two* possible models for linear regulatory pathways for TrpA that account for the behavior of the Trp1 and Trp2 genes. For each model include a role for tryptophan.

Trp2 most likely is a negative regulator



(c 6 pts.) Next you combine a Trp1^- with a Trp2^- mutation and find that the double mutant gives *uninducible* *TrpA* expression. Which model from part (b) is consistent with this new observation?

Model 2 is more accurate as the phenotype of the double mutant shows that *Trp1* acts further downstream in the genetic pathway than *Trp2*.

(d 9 pts.) Finally, you mutagenize the wild type *Trp1* gene with a chemical mutagen and find that while most *Trp1* gene mutations cause *uninducible* *TrpA* expression, certain rare alleles give *constitutive* expression. You designate one of these rare constitutive alleles Trp1^* . Using an F' that carries the wild type *Trp1* region of the chromosome, you find that the Trp1^* allele is dominant. Propose a molecular description of the type of mutation Trp1^* might be in the context of your overall model for *TrpA* regulation.

It has been established that *Trp1* is a positive regulator of *TrpA*. Thus, it seems more likely that Trp1^* is a *super-activator*.

A possibility is that the Trp1^* allele has an operator mutation, which results in its constitutive expression.

Another possibility is that the Trp1^* allele carries a coding region mutation such that its gene product cannot interact properly with the *Trp2* gene product.