

Question 1, continued

d) One of these two mutants is fully functional, while the other is not. Which mutant peptide do you predict is functional and which one is not? Why?

Question 2

You are fascinated by CHWDWN, and decide to continue your research over the summer. A graduate student in your lab has developed a collection of strains of bacteria containing different mutant tRNAs.

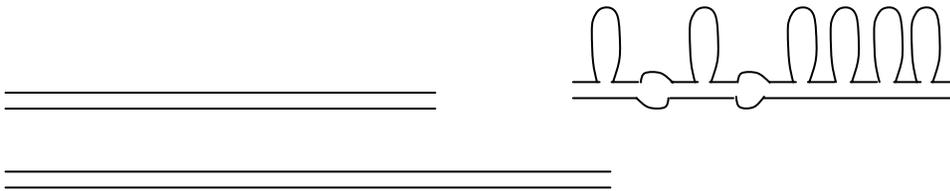
- a) In wild-type cells, what is the anticodon on the tRNA charged with trp? Indicate 5' and 3'.
- b) In strain X, the 5' nucleotide of the anticodon on the trp tRNA is changed to a G, and no wild-type trp tRNA is present.
 - i. Would you expect CHWDWN polypeptide production in X to be affected? If yes, explain how it would be affected. If no, explain why not.
 - ii. What proteins other than CHWDWN would you expect to be affected? Why?
 - iii. Would you expect strain X to grow on media containing yummose as the only carbon source? If yes, how strong would you expect that growth to be with respect to the wild-type strain? If no, explain why you expect no growth.
- c) In strain Z, the tRNA with the anticodon for trp found in wild-type cells is actually charged with amino acid gln, and no wild-type trp tRNA is present.
 - i. Would you expect CHWDWN polypeptide production in Z to be affected? If yes, explain how it would be affected. If no, explain why not.
 - ii. What proteins other than CHWDWN would you expect to be affected? Why?

Question 2, continued

iii. Would you expect strain Z to grow on media containing yumbose as the only carbon source? If yes, how strong would you expect that growth to be with respect to the wild-type strain? If no, explain why you expect no growth.

- d) You find that the protein sequence of CHWDWN is highly conserved (~80%) in humans. Excited, you acquire DNA fragments encoding bacteria and human CHWDWN proteins. You
1. combine both samples into one test tube
 2. briefly treat the sample in the test tube with heat
 3. let the sample cool
 4. examine the contents of the test tube in electron microscope.

You find that you have three types of complexes in your sample:



You reason that two of the types are the original double stranded bacteria and human DNA, and that the third was made when a strand of the human DNA base paired with a strand of bacteria DNA.

On the figure above, identify each complex. For the bacteria-human hybrid, indicate which strand is bacterial, and which is human.

Briefly justify your choices.

7.014 Solution Set 4

Question 1

Shown below is a fragment of the sequence of a hypothetical bacterial gene. This gene encodes production of CHWDWN, protein essential for metabolizing sugar yummoose. The transcription begins (and includes) the G/C base pair in bold and proceeds to the right.

5' ...TTCGAG**G**CTCTCGTCGTCGAGATACGCGATGATATTAGTGGTAATATGGGGATGCACT...3'
3' ...AAGCT**C**GAGAGCAGCAGCTCTATGCGCTACTATAATCACCATTATAACCCCTACGTGA...5'

- a) Give the sequence of the mRNA transcribed from this gene and indicate the 5' and 3' ends of the mRNA.

5'-GCUCUCGUCGUCGAGAUACGCGAUGAUUUAGUGGUAAUAUGGGGAUGCACU...3'

- b) Give the sequence of the peptide that will be translated from this mRNA. Label the amino and carboxy termini of the peptide.

N-Met-Ile-Leu-Val-Val-Ile-Trp-Gly-Cys-Thr...-C

- c) You study two different mutants, Mutant A and Mutant B.

- i) In the DNA sequence for Mutant A, you find the insertion of a G/C base pair between positions 22 and 23 (position of insertion is indicated by an arrow):

5' ...TTCGAG**G**CTCTCGTCGTCGAGATACGCGATGATATTAGTGGTAATATGGGGATGCACT...3'
3' ...AAGCT**C**GAGAGCAGCAGCTCTATGCGCTACTATAATCACCATTATAACCCCTACGTGA...5'



Give the sequence of the new peptide produced by mutant A. Label the amino and carboxy termini of the peptide.

N-Met-Thr-Arg-C

- ii) In the DNA sequence for Mutant B, two consecutive G/C base pairs are inserted between positions 22 and 23 (position of insertion is indicated by an arrow on the figure above).

Give the sequence of the new peptide produced by mutant B. Label the amino and carboxy termini of the peptide.

N-Met-Asp-Ala-Met-Ile-Leu-Val-Val-Ile-Trp-Gly-Cys-Thr...-C

Question 1, continued

d) One of these two mutants is fully functional, while the other is not. Which mutant peptide do you predict is functional and which one is not? Why?

Peptide A is truncated after three amino acids, so it is likely the one that is not functional. Peptide B has three extra amino acids on the amino terminus with respect to the wild-type peptide. These amino acids are not likely to affect the overall fold of the peptide. Thus, it is likely that peptide B is fully functional.

Question 2

You are fascinated by CHWDWN, and decide to continue your research over the summer.

A graduate student in your lab has developed a collection of strains of bacteria containing different mutant tRNAs.

a) In wild-type cells, what is the anticodon on the tRNA charged with trp? Indicate 5' and 3'.
5'-CCA-3' or 3'-ACC-5'

b) In strain X, the 5' nucleotide of the anticodon on the trp tRNA is changed to a G, and no wild-type trp tRNA is present.

i. Would you expect CHWDWN polypeptide production in X to be affected? If yes, explain how it would be affected. If no, explain why not.

The anticodon now reads 5'-GCA-3'. Because of the mutant anticodon, in strain X the tRNA charged with amino acid trp pairs with the codon 5'-UGC-3'. Moreover, no wild-type trp tRNA exists in strain X. This would cause translation of CHWDWN to terminate after the sixth codon, since no tRNA will be available to translate codon 7, UGG.

Based on the gene sequence in Question 1, CHWDWN protein contains at least one trp codon.

ii. What proteins other than CHWDWN would you expect to be affected? Why?

The codon 5'-UGC-3' normally encodes cys. Thus, whenever the codon UGC appears in frame in the coding sequence of a gene in strain X, part of the time amino acid trp will be inserted instead of cys. In addition, as illustrated in part i above, any time the codon for trp is present in frame in a coding sequence of a gene in strain X, translation will be terminated. So the proteins that would be affected are all those that have codons UGC or UGG in their coding sequences.

iii. Would you expect strain X to grow on media containing yummose as the only carbon source? If yes, how strong would you expect that growth to be with respect to the wild-type strain? If no, explain why you expect no growth.

In order to grow on yummose as the only carbon source, strain X would need to have a functional CHWDWN protein. Since translation of CHWDWN in strain X terminates after only six amino acids, it is very unlikely that strain X could grow on yummose as the only carbon source.

c) In strain Z, the tRNA with the anticodon for trp found in wild-type cells is actually charged with amino acid gln, and no wild-type trp tRNA is present.

i. Would you expect CHWDWN polypeptide production in Z to be affected? If yes, explain how it would be affected. If no, explain why not.

Since the amino acid that a trp tRNA is charged with changes in strain Z, the seventh amino acid of the CHWDWN protein will now be gln, instead of trp.

ii. What proteins other than CHWDWN would you expect to be affected? Why?

Any protein that in a wild-type strain contains amino acid trp will, in strain Z, contain amino acid gln in its place.

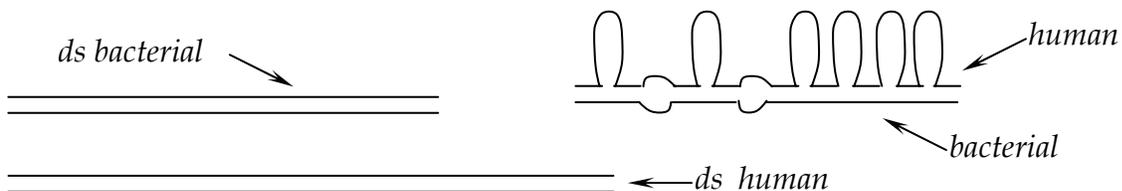
Question 2, continued

- iii. Would you expect strain Z to grow on media containing yummose as the only carbon source? If yes, how strong would you expect that growth to be with respect to the wild-type strain? If no, explain why you expect no growth.

In order to grow on yummose as the only carbon source, strain Z would need to have a functional CHWDWN protein. At least in position 7 of the protein, trp has been substituted by gln. If the codon for trp is contained anywhere else in the gene, the amino acid substitution will occur in that position as well. Gln is a smaller amino acid than trp is, and it is also polar, while trp is hydrophobic. Thus, is it likely that substitution will have some impact on the protein, but it is unclear whether it will render the protein unable to perform its function.

- d) You find that the protein sequence of CHWDWN is highly conserved (~80%) in humans. Excited, you acquire DNA fragments encoding bacteria and human CHWDWN proteins. You
1. combine both samples into one test tube
 2. briefly treat the sample in the test tube with heat
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You find that you have three types of complexes in your sample:



You reason that two of the types are the original double stranded bacteria and human DNA, and that the third was made when a strand of the human DNA base paired with a strand of bacteria DNA.

On the figure above, identify each complex. For the bacteria-human hybrid, indicate which strand is bacterial, and which is human.

Briefly justify your choices.

Human DNA for the same protein is likely to be significantly longer than a bacterial gene encoding homologous bacterial protein because the human gene is likely to have introns. Those are represented by the large loops in the human-bacterial hybrid in the figure above. Furthermore, because the protein sequence is not 100% identical between the human and bacterial versions, some portions of the human and bacterial genes are likely to not be similar enough to base pair (even with mismatches). Those regions are represented by the small bulges that occur on both the human and bacterial strands in the hybrid figure above.