

Bio 213 GENETICS (Fall 2000)

Problem Set 4

Questions related to pathways

- * P4.1. Make up an analogy in the macroscopic world to the phenylalanine degradation pathway (shown on p.316). The analogy should have correlates to enzymes, to substrates, to the flow of metabolites down a pathway, and to the results of loss of enzyme by mutation.

Questions relating to the coding problem

- P4.2. Suppose that mRNA was translated through a doublet code (two adjacent bases specify one amino acid). How many different amino acids could this code specify?
- P4.3. Suppose that the fourth base (C) of the RNA sequence shown below were mutated. How many amino acids might be affected if the code were nonoverlapping triplet? How many if the code were overlapping triplet?

GAGCGUGCGAACC

- P4.4. Crick et al began their study by mutagenizing wild-type T4 with proflavin, to produce a mutant T4 strain called FC0¹, which contained a one-base deletion in one of the *rII* genes.² Suppose the deletion was in the *rIIA* gene in a position shown below:



- 4a. What would you expect to be the phenotype of the T4 mutant FC0? Write it in the table to the right.

- 4b. Considering that mutation is rare and even after exposure to proflavin, most T4 phage would be unaffected, how do you think the mutant was detected in the first place?

T4 mutant FC0 was exposed to proflavin and the phage were plated on *E. coli* K(lambda). The vast majority of the phage was unaffected by the mutagen, but some suffered mutation in the *rII* region. Some normal-sized plaques arose.

- 4c. What kind of T4 do you think was responsible for the plaques observed?

Phenotypes of T4 and mutant derivatives

T4 strain	Plaques on <i>E. coli</i> B	Plaques on <i>E. coli</i> K(lambda)
wild-type	normal	normal
FC0		
FC0/1 FC0/9 FC0/14	normal	normal
FC1 FC9 FC14		
FC1/9 FC1/14 FC9/14		
FC1/9/14		

T4 was isolated from three of the plaques and named FC0/1, FC0/9, and FC0/14.

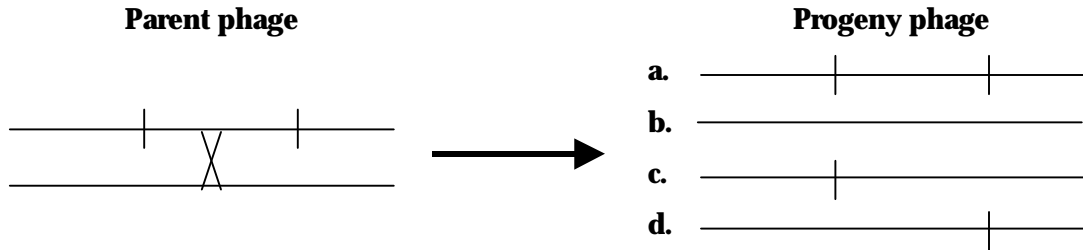
- 4d. Presuming that these phage have two mutations, the original one found in FC0 and another, draw possible positions of the second mutations in the three strains. Use the

¹ Any guesses as to why the strain was called FC0?

² Actually they didn't know whether the mutation was an insertion or a deletion. I'll call it a deletion to simplify the discussion.

format shown at the beginning of this problem. Call the mutations 1, 9, and 14 and label them + or - (indicating insertion or deletion).

Each of the three mutant T4 strains (FC0/1, FC0/9, and FC0/14) were mixed with wild-type T4, and the mixture was used to infect *E. coli* B. When two phage infect a single cell, it is possible for genetic recombination to occur:



Most of the progeny (**a** and **b**) are genetically the same as the parent, but in rare cases recombination occurs and the progeny (**c** and **d**). Both large and normal-sized plaques were obtained in the cross between wild-type T4 and the three mutant T4 strains.

4e. Which kind of plaques were in the majority? From what kind of progeny phage did the minority size plaques arise? The T4 phage isolated from the minority size plaques were called FC1, FC9, or FC14 (depending on which of the three crosses produced them). Fill in the appropriate line in the table on the preceding page, and draw a map of the *rII* region for each strain (using the format shown at the beginning of the problem)

The three new T4 phage, FC1, FC9, and FC14, were mixed in each of the three possible pairwise combinations and used to infect *E. coli* B, and phage were identified (by a complicated procedure I won't go into here) that were recombinants, combining the mutations of the two parent strains. These were called FC1/9, FC1/14, and FC9/14, depending on the cross. In a like fashion, a triple T4 mutant was obtained: FC1/9/14.

4f. Draw a map of the *rII* region for each of the double mutants and triple mutant. Fill in the appropriate lines of the table on the preceding page.

Note: This would have been the answer to the question Daniela Falter asked in class if I didn't appeal to lack of time.

Problems related to cracking the code

P4.5. The author of your text did a bad thing. He made up the data shown in the table on p.331, which is OK, but didn't say so, which is not. Let me show you some of the actual data from the random RNA polymer experiments designed to assign amino acids to triplet codons. RNA was made using ATP and CTP in the ratio of 5:1 or 1:5. The resulting random RNA polymers were translated in vitro, yielding the results shown to the right. From these results, deduce as much as you can about which triplet codons encode which amino acids.

Amino acid	Incorporation of radioactivity directed by random polymer*	
	A:C=5:1	A:C=1:5
asparagine	1097	71
glutamine	1078	70
histidine	294	315
lysine	4555	14
proline	328	1342
threonine	1206	279

*The differences in incorporation of radioactivity between the two experiments is not significant. Each experiment should be considered separately. data is from Speyer et al (1966) Cold Spring Harbor Symposium of Quantitative Biology 31:559-567

- * P4.6. If you've seen the movie ET, you'll remember the scene where they've got ET on the operating table and you hear some excited molecular biologist calling from the next room, "He's got DNA!". Suppose they didn't cut away to the kid and his alien but instead followed the story of real interest: What kind of DNA does he got? Is his code the same as ours? From the results obtained by noted molecular exobiologist, Martian Nirenberg, what can you conclude about the coding scheme ET-like creatures use. (underlines indicate the repeating unit). Note that polypeptide synthesis can initiate anywhere within the synthetic RNA.

Synthetic RNA Polypeptide(s) synthesized*		Synthetic RNA Polypeptide(s) synthesized*	
<u>A</u> AAA...	<u>Lys</u> LysLys...	<u>AGAGAG</u> ...	<u>Arg</u> ArgArg...
<u>U</u> UUU...	<u>Phe</u> PhePhe...	<u>ACAACAACA</u> ...	<u>ThrGlnAsn</u> ThrGlnAsn...
<u>C</u> CCC...	<u>Pro</u> ProPro...	<u>AUGAUGAUG</u> ...	MetTrp
<u>G</u> GGG...	none	<u>AUAUAUAUA</u> ...	<u>IleTyrAsn</u> IleTyrAsn...
<u>ACACAC</u> ...	<u>Thr</u> ThrThr... and <u>His</u> HisHis...	<u>AUCAUCAUC</u> ...	<u>IleSerHis</u> IleSerHis
<u>AUAUAU</u> ...	<u>Ile</u> IleIle... and <u>Tyr</u> TyrTyr...		
*Underlined portion of the sequence is repeated indefinitely			

Questions relating to Translation (Using the genetic code)

- * P4.7. List the changes that can be produced by a single basepair mutation in the AGA codon encoding arginine and label each silent, conservative, hydrophobic-to-hydrophilic, hydrophilic-to-hydrophobic, or other.

P4.8. Starting from the first conventional start codon, translate the RNA strand given below:

GAAGCAUGUCCGAGCAAUGAGCCGA

P4.9. Sickle cell anemia results from a mutation in the sixth amino acid of β -globin protein from glutamic acid to valine (see Fig. 17-1, p.456 in text). What base change produced this mutation?

P4.10. It is unfortunately quite common for humans or computers to make errors in determining the sequence of nucleic acids. It is particularly common for a string of G's or C's (e.g., CCCCC) to be read erroneously as one too few or one too many. Suppose that you have sequenced some DNA and you are certain that the sequence listed below contains the translational start of a large protein (greater than 300 amino acids), beginning with the normal start codon.

GGGGAGGATAGCCATGCCAGCCCCCTAATTAGGGGGAGTTTCTCTGCAAAA

4.9a. What should convince you that there is an error in your sequence?

4.9b. Presuming that there is only one error, a deletion or insertion of a single base, which do you suppose it is and where?

- * P4.11. Hemophilia A is an X-linked disease associated with the absence of an essential blood clotting factor, factor VIII (if you don't have any idea what an X-linked trait is, don't worry about it). Factor VIII is encoded by the gene called *FACTOR8*. This gene was cloned from several individuals -- some affected, some not -- and sequenced. A portion of each sequence

that you're sure contains the beginning of the gene (i.e., the start codon) was compared with the same portion of the wild-type sequence, as shown below. Each sequence contains only one mutation, shown emphasized.

Wild-type 5'-GGAGTTGAGTCATGGACTCTAAGCAGCGATCCACAAAG...

Individual **a** 5'-GGAGTT**T**AGTCATGGACTCTAAGCAGCGATCCACAAAG...

Individual **b** 5'-GGAGTTGAGTCAT**T**GACTCTAAGCAGCGATCCACAAAG...

Individual **c** 5'-GGAGTTGAGTCATGGACTCT**T**AGCAGCGATCCACAAAG...

Individual **d** 5'-GGAGTTGAGTCATGGACTCTAAGCAGC**T**ATCCACAAAG...

Individual **e** 5'-GGAGTTGAGTCATGGACTCTAAGCAGCGATCCACT**T**AAG...

For each individual, choose from the list below to describe what you predict would be the severity of the phenotype, and give the reason for your choice.

- A. Severe hemophilia
- B. Mild hemophilia
- C. No hemophilia

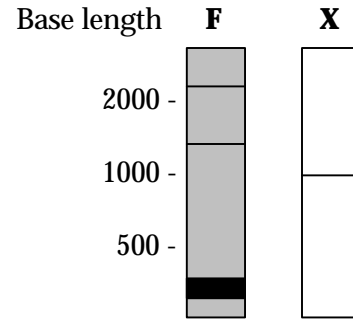
Questions relating to Translation (tRNA and anticodons)

P4.12. Complete the following table:

DNA double helix					A	G					A	
				T			G			T		
mRNA transcribed	5'			A					U			
Appropriate tRNA anticodon						U		G				5'
Amino acids incorporated into protein		met										

- * P4.13. You isolate total RNA from *Clostridium botulinum* (the pathogenic agent of botulism) and run it out on a gel under conditions (somewhat different from those in your lab) such that the RNA is fully extended (i.e., the RNA migrates solely on the basis of its length). You stain the gel so that the RNA is visible under fluorescent light and see the gel shown below (F). Then you blot the gel, which means that you cause the RNA to be transferred to filter paper (see text for details) and probe it with a radioactive fragment of DNA containing the gene encoding botulinum toxin (therefore the probe will bind only to RNA carrying the sequence for the toxin). The blot is autoradiographed (exposed to X-ray film) shown as **X**.

- 4.13a. Why don't the two bands seen by fluorescence appear by autoradiography?
- 4.13b. What are the two bands seen by fluorescence?
- 4.13c. If the botulinum toxin gene encodes a protein 300 amino acids long, what fraction of its mRNA is devoted to that gene? (Show work)



P4.14. What would have been your conclusion if Astrachan et al had observed no drop in the amount of radioactive RNA after chasing the radioactive phosphate with nonradioactive phosphate?

P4.15. Suppose you want to test the notion that tRNA's are dumb intermediaries, connecting the anticodon to the appropriate amino acid. Your strategy is the opposite of that of Chapeville et al. You intend to alter not the amino acid attached to a tRNA but rather the anticodon. Accordingly, you mutate the gene encoding tRNA^{Cys}, changing the anticodon by one base to 5'-GCU-3', and clone this gene in *E. coli*. Then, as did Chapeville before you, you extract tRNAs from the *E. coli*, add amino acids, including radioactive cysteine, and use the mixture in an in vitro translation system. You run the experiment four times, each time with a different artificial RNA polymers:³

- A. poly (U,G) B. poly (A,U,G) C. poly (A,C,G) D. poly (C,U,G)

15a. Which of the four polymers directed the incorporation of a significant amount of radioactive cysteine?

15b. No one was ever fool enough to do this experiment. Why not?

- * P4.16. Belozersky and Spirin published a listing of nucleic acid base compositions from a large number of bacteria [(1958) *Nature* 182:111-112]. Part of the list is shown below. What striking feature is evident in the comparison of DNA and RNA compositions? Given our present knowledge of RNA, how do you account for their findings?

Species	DNA Base Composition					RNA Base Composition				
	Bases (moles per cent)				G+C	Bases (moles per cent)				G+C
	G	A	C	T	A+T	G	A	C	U	A+U
<i>Proteus vulgaris</i>	19.8	30.1	20.7	29.4	0.68	31.0	26.3	24.0	18.7	1.22
<i>Escherichia coli</i>	26.0	23.9	26.2	23.9	1.09	30.7	26.0	24.1	19.2	1.21
<i>Erwinia carotovora</i>	27.1	23.3	26.9	22.7	1.17	29.5	26.5	23.7	20.3	1.14
<i>Mycobacterium vadosum</i>	29.2	20.7	28.5	21.6	1.37	31.7	23.8	23.5	21.0	1.23
<i>Pseudomonas aeruginosa</i>	33.0	16.8	34.0	16.2	2.03	31.6	25.1	23.8	19.5	1.24

**REMEMBER TO ANSWER THE FOUR QUESTIONS
SHOWN ON THE THIRD PAGE OF LAB 6.**

➡ BRING YOUR ANSWERS TO THE FIRST EXAM ←

³ poly (A,U,G), for example, might produce GUGAAGUGGAUGAGUAGGUAUUG...