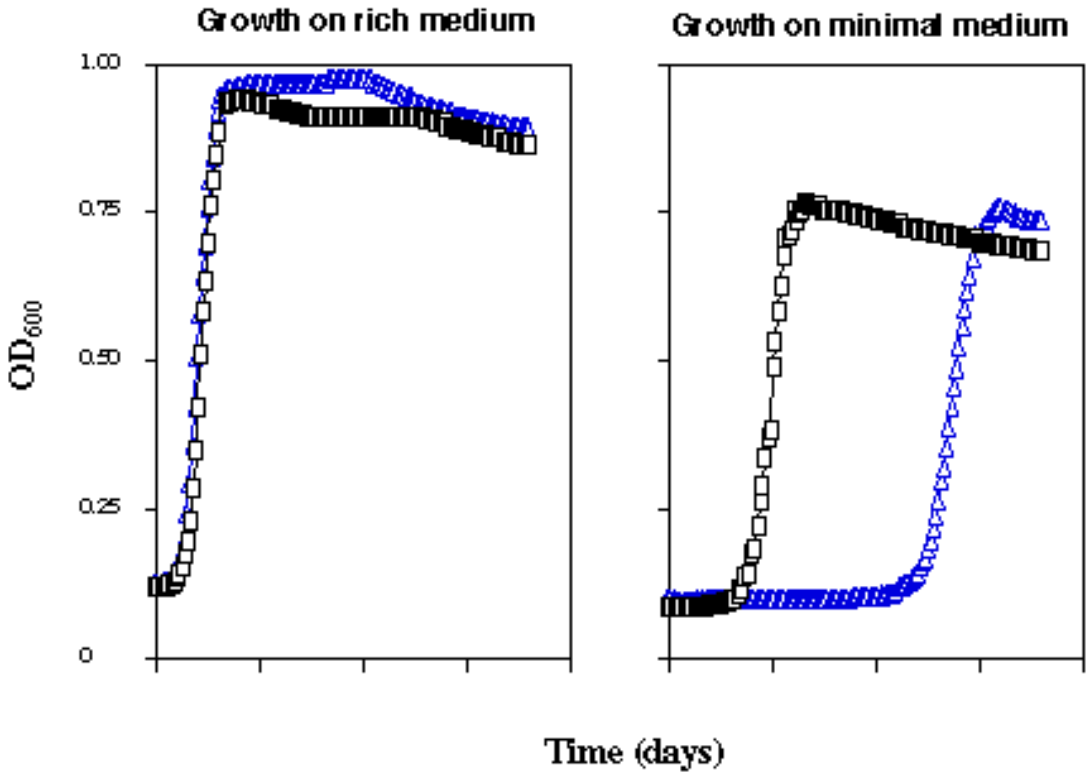
(5) **1.** Strains with a *mutD* mutation have a mutator phenotype. When *mutD* strains are growing fast (e.g., in rich medium) the frequency that mutations accumulate is much higher than when they are growing slowly (e.g., in minimal medium). Why?

ANSWER: More DNA replication occurs during fast growth than during slow growth conditions. During rapid growth, initiation of DNA replication occurs more often, resulting in an increased number of DNA replication forks. (DNA polymerase does not work any faster, there are just more DNA replication occuring simultaneously.) In a mutD strain, the epsilon subunit of DNA Pol III is no longer functional, preventing proof-reading by DNA polymerase and thus resulting in the accumulation of misincorporated nucleotides. However, the rate of misincorporation per nucleotide synthesized is the same under both fast and slow growth conditions.The different mutagenesis frequency between fast and slow growing mutD strains arises from the fact that any error correction in these mutants must occur as a POST-REPLICATIVE process via the mismatch repair system. During fast growth conditions mutD strains have so many more replication forks that the ability of the mismatch repair system to handle all of the error correction is saturated (i.e. a second round of DNA replication occurs before error correction can take place efficiently).

(5) **2.** A mutant strain of *Salmonella* was isolated that grows as well as the wild-type parent on rich medium, but when transferred from rich medium to minimal medium the strain shows a very long lag phase compared to the wild-type parent. In the figure below, growth of the wild-type is shown by squares and the mutant strain is shown by triangles.



How could you determine if the growth that occurred after the long lag phase was due to a second mutation that permitted growth in minimal medium or simply due to a requirement for longer time for adaptation to the minimal medium.

ANSWER: A simple approach is to take an aliquot from the culture that grew in minimal medium after the long lag phase and to subculture the bacteria in rich medium. After the culture grew in rich medium, subculture into minimal medium. If the original growth in minimal medium was due to a mutation, the strain should begin growing soon after subculture. In contrast, if the original growth in minimal medium was due to adaptation, it should require the strain just as long to adapt upon subculture from rich to minimal medium as it took following the first subculture.

(5) **3.** Strain FC40 is unable to grow on lactose as a sole carbon source (Lac^-). A Luria-Delbruck fluctuation test was done by plating 10^8 cells from 10 independent cultures (#1-10) onto minimal lactose plates, and at the same time plating 10^8 cells from a single culture (#11) onto 10 minimal lactose plates. After a long incubation, the following results were obtained.

Independent cultures (Culture #)	Number of Colonies	Culture 11 (Plate #)	Number of Colonies
1	22	1	17

2	18	2	24
3	19	3	23
4	24	4	26
5	20	5	20
6	23	6	22
7	21	7	21
8	22	8	24
9	21	9	20
10	17	10	19

Based upon these results, would you conclude that the mutation to Lac^+ is random or adaptive? [Explain your answer.]

ANSWER: The variance between the number of Lac^+ colonies from individual cultures equals that seen with multiple samples of a single culture. These results suggest that the phenotype is not likely to be due to a random, spontaneous mutation. (A random mutation which would show much greater variance between individual cultures.) The simplest interpretation of these results is that the mutation to Lac^+ is an example of "adaptive mutagenesis".

Note - your answer must describe the comparison of the variance between cultures #1-10 vs the multiple platings of culture #11. When given a problem like this, you need to evaluate the data given, do not simply say that it must be random mutagenesis because I said in lecture that mutagenesis is random!

(10) **4.** Resistance to the toxic proline analog Azetidine-2-carboxylic acid can occur in two ways: (i) specific missense mutations in the *proB* gene (the first step in proline biosynthesis) which make it insensitive to feedback inhibition; or (ii) mutations that inactivate the *putP* gene (the permease which transports proline into the cell).

a. Which class of mutants would you expect to be more common and why?

ANSWER: The *putP* mutations would be most common because a null mutation anywhere within the gene could inactivate the protein. Only a limited number of sites in *proB* would be likely to produce a functional protein, which is insensitive to feedback inhibition.

b. Indicate whether each of the two types of mutation is dominant or recessive to the wild-type allele of that gene. [Explain your logic.]

ANSWER: A *putP* null mutation is a simple loss of function mutation, which

would be recessive to the wild-type allele. That is, *putP*$^-$ /*putP*$^+$ would be able to transport both proline and Azetidine-2-carboxylic acid into the cell, resulting in sensitivity to the proline analog.

A *proB* mutant insensitive to feedback inhibition ("*proB**") would be dominant to wild-type. That is, *proB** /*proB*$^+$ would have one copy of the gene which is insensitive to feedback inhibition, allowing accumulation of a high intracellular proline concentration and thus resulting in resistance to the proline analog.

(10) **5.** NADP is an essential cofactor for many cellular processes. Because it is not transported, exogenous NADP cannot supplement mutants unable to synthesize intracellular NADP. Five independent mutations were obtained that affect the synthesis of NADP. The properties of the mutations are described in the table below (where + indicates growth on rich medium, - indicates that no growth on rich medium, and -/+ indicates weak growth on rich medium).

Mutation	**Growth temperature**			
	30°C	**42°C**	**30 → 42°C**	**42 → 30°C**
nad$^+$	+	+	+	+
nad-1	+	–		
nad-2	+	–		
nad-4	–	+		
nad-7	–	+		
nad-1 nad-2	–	–	–	–
nad-1 nad-4	–	–	–	+
nad-1 nad-7	–	–	+	–

a. Note the properties of *nad-1*, *nad-2*, *nad-4*, and *nad-7* in the above Table. Indicate both whether the mutant has a conditional phenotype (temperature sensitive, cold sensitive, null) and whether the allele is likely to be due to a missense, nonsense, frameshift, deletion, or insertion mutation? Briefly explain your answers.

ANSWER:

nad-1 Ts, missense
nad-2 Ts, missense
nad-4 Cs, missense
nad-7 Cs, missense

All of these mutations are probably missense because Ts and Cs mutations usually arise due to amino acid substitutions that destabilize the resulting protein.

b. Interpret the results for each pair of double mutants shown in the Table. If you are not able to determine the order of some of the gene products from the data given, indicate which gene products fall into this category.

ANSWER:

nad-1 nad-2 Cannot interpret gene order because both mutations are Ts
nad-1 nad-4 Mutation *nad-4* (Cs) must act before *nad-1* (Ts)
nad-1 nad-7 Mutation *nad-1* (Ts) must act before *nad-7* (Cs)

(15) **6.** Mutations in the *proA*, *proB*, or *proC* gene are proline auxotrophs. Revertants of *proB* and *proC* mutants were isolated.

a. How would you select for revertants?

ANSWER: Demand growth on minimal medium without proline (i.e. select for prototrophy)

b. Some intragenic suppressors of the *proC* mutants were found to be temperature sensitive. Suggest a likely explanation for this result.

ANSWER: The temperature sensitive properties of some intragenic suppressors is a consequence of the second amino acid substitution in the protein (i.e. a second missense mutation) that affects protein folding and stability.

c. Intergenic suppressors were found that restore prototrophy of a deletion mutant that removes the *proB* gene. Suggest a likely mechanism for this suppression.

ANSWER: The results indicate that in the absence of ProB another pathway can substitute for this gene product to bypass the ProB reaction (i.e. the intergenic suppression is due to a bypass suppressor).

(5) **7.** An amber mutation in phage T4 can grow on strains carrying *sup-1* but not on strains containing *sup-2*, even though both *sup-1* and *sup-2* are amber suppressors. Suggest an expanation for this result.

ANSWER: *sup-1* and *sup-2* are both amber suppressors -- due to a mutation in the gene encoding a tRNA, which allows recognition of the UAG codon. However these two mutations affect two different tRNA genes such that, although both mutant tRNAs recognize amber codons, they insert different amino acids (because they are charged with the amino acid that charges each of the two different wild-type tRNAs). For example, *sup-1* might be a mutated tRNA-leu

gene whereas *sup-2* might be a mutated tRNA-tyr gene. Thus, the suppression in *sup-1* would insert either leucine and the suppression in *sup-2* would insert a tyrosine at the position in the protein corresponding to the amber codon. If the inserted amino acid is not similar in size and/or charge to the amino acid at that position in the wild type protein, the resulting amino acid substitution may interfere with the structure and function of the resulting protein.

(15) 8. The *pdx* gene products are required for the biosynthesis of pyridoxine (vitamin B6) in *E. coli*. Nonsense mutations in the *pdxJ* gene result in very slow growth due to polarity on the *dpj* gene, which is located downstream of *pdxJ* within the same operon. Suppressor mutations were obtained that decreased activity of a protease encoded by the *lon* gene.

a. How would an amber mutation prevent expression of a downstream gene? [Your answer should clearly explain the mechanism.]

ANSWER: Polarity is failure to express downstream genes in an operon due to a mutation located upstream. Nonsense mutations result in polarity because they cause translation termination, which allows Rho to bind to the untranslated mRNA ("naked mRNA"), resulting in transcription termination.

b. How might *lon* mutants suppress the low expression of *dpj*?

ANSWER: The concentration of Dpj is decreased due to polarity, however a low level of Dpj is made. A *lon* mutation could decrease the degradation of Dpj allowing the accumulation of a higher intracellular concentration of this gene product (i.e. this would be an overproduction suppressor).

c. What other types of suppressors would you expect to find that might overcome the *pdxJ* polarity?

ANSWER: Multiple classes of suppressors are possible. One class of suppressor you might expect would be due to a mutation that inactivates *rho* – absence of Rho factor would prevent Rho-dependant polarity and potentially allow higher level expression of Dpg. Another class of suppressor you might expect is a deletion that removes the DNA between the nonsense codon and the end of the gene, thereby preventing Rho-dependant. Another class of suppressor you might expect is a nonsense suppressor (tRNA) that misreads the nonsense codon, thereby preventing polarity.

(15) **9**. Phage P22 infects *Salmonella* and lyses the infected bacteria, producing a turbid plaque. Six phage P22 mutants were isolated that produce clear plaques. Complementation tests were done

with the P22 clear-plaque mutants by co-infecting a *sup^o* strain of *Salmonella* Typhimurium with two mutant phage. The results are shown below (− indicates clear plaques and + indicates turbid plaques).

	P22-1	P22-2	P22-3	P22-4	P22-5	P22-6	P22 wild-type
P22-1	−	−	−	+	−	−	+
P22-2		−	−	+	+	−	+
P22-3			−	+	+	−	+
P22-4				−	−	−	+
P22-5					−	−	−
P22-6						−	+

a. What do the complementation results of the P22 mutants vs P22 wild-type indicate about each of the mutants?

ANSWER: P22-1, P22-2, P22-3, P22-4, and P22-6 are recessive to P22 wild-type. P22-5 is dominant over P22 wild-type.

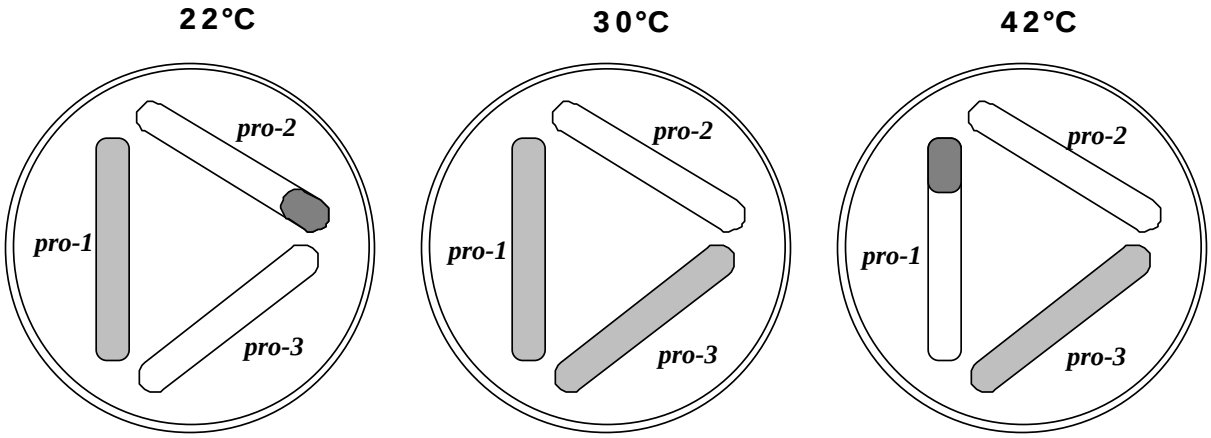
b. How many complementation groups do these mutants represent? Explain your logic.

ANSWER: There are two complementation groups. P22-1, P22-2, and P22-3 are one group; P22-4 is a second group; P22-5 has unusual complementation behavior that makes it difficult to place into a group; P22-6 cannot complement any of the mutants making it unable to place this mutation into one of the two complementation groups.

c. Mutants P22-1, P22-2, P22-3, P22-4, and P22-5 produce clear plaques on both a *sup^o* strain and a *supF* strain of *Salmonella*. In contrast, mutant P22-6 produces clear plaques on a *sup^o* strain but produce turbid plaques on a *supF* strain of *Salmonella*. Suggest an explanation for the complementation behavior of P22-6 based upon this result.

ANSWER: P22-1, P22-2, P22-3, P22-4, and P22-5 are not amber mutants, but P22-6 is an amber mutant. Amber mutants are often polar on downstream genes, so the observed phenotype of P22-6 is likely to be due to rho-dependant polarity (i.e. P22-6 is a *cis*-acting mutation).

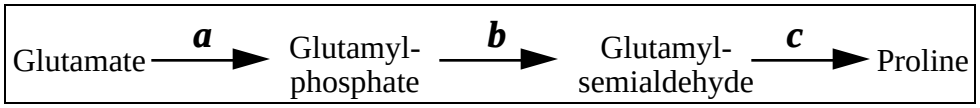
(15) **10.** Three different proline auxotrophs were streaked on minimal plates without proline and incubated at the following temperatures for two days. (The shaded areas indicate growth and white areas indicate where cells were streaked but did not grow.)



a. Describe the nature of the Pro$^-$ mutation in each strain (i.e., is it a null mutation, a temperature sensitive mutation, a cold sensitive mutation, etc)

ANSWER: *pro-1* causes a temperature sensitive phenotype; *pro-2* causes a non-conditional, null phenotype; *pro-3* causes a cold sensitive phenotype.

b. Label the arrows in the following pathway to indicate the enzymatic step affected in each mutant.



ANSWER: a = *pro-1* → b = *pro-2* → c = *pro-3*

c. Why could *pro-3* crossfeed *pro-2* at 22°C but not at 30°C or 42°C?

ANSWER: At 22°C, the cold sensitive phenotype of *pro-3* prevents the conversion of glutamyl-semialdehyde (GSA) into proline. Thus, GSA accumulates and is able to crossfeed the *pro-2* mutant which is blocked in an earlier step in the pathway. In contrast, at the permissive temperature (30°C), the *pro-3* gene product is functional and so GSA is converted to proline in the *pro-3* mutant strain. Since GSA is rapidly converted into proline, no precursors are accumulated. Proline does not crossfeed because it is rapidly into protein and is thus not accumulated.