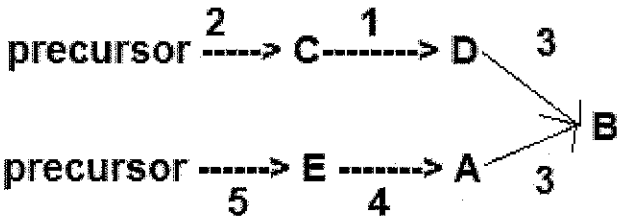
Biol 3F09

Genetics Test Corrections
Question 2

250e Gen.

2. Since supplement B rescues all the mutants, it is obviously at the end of the pathway. After looking at the data, we see that it is not a simple linear pathway because the number of +'s don't steadily decrease for all the mutants. As in, supplement A and D both rescue 2 mutants, but both rescue 2 different mutants – the same is true for supplements C and E. This indicates that it is a branched pathway.
Since we know from class that the supplements in the beginning of the pathway tend to rescue less mutants than those at the end of the pathway, C and E are the beginning of their respective pathways. The same is true for A and D.
Notice that supplement E and A's rescues overlap, showing that they are in the same pathway. Also the rescues of supplement C and D overlap, showing that they are in the same pathway.

Using this analysis, we can come up with the following pathway:



The numbers indicate the specific mutants.

ok, +2pts

b) The compounds that would need to be added for the double mutant would be supplement D, supplement A and supplement B. This is because if there was a double mutant of 1 and 4, it would mean that the Neurospora crassa cannot synthesize the supplement D, A and B. Supplement D, A and B are needed for it to get rescued.

My original answer for part b was incorrect because I put supplements C and E when I should have put supplements D and A. Supplements C and E are wrong because these are not the supplements that rescue mutants 1 and 4. This means that 1 and 4 can synthesize C and E, but they cannot synthesize D and A and B. Therefore D, A and B are needed to be added to the minimal medium to allow a mutant of 1 and 4 to grow, not C and E.

[redacted]
Genetics Test Corrections
Question 4

4) A band of the expected size was drawn for each of the six genotypes. The following is a description of how each band was obtained:

a) This is the wild type genotype because it has a functional repressor, operator and a functional lacZ (leads to formation of Beta-galactosidase) and lacY (leads to formation of Permease). Therefore the band size here on the gel would show **5kb when induced**. When there is **no induction by lactose, there will be no mRNA produced** because the repressor will bind to the operator and stop transcription of lac operon will halt.

b) Here there is a defective repressor – a repressor that cannot bind to the operator, which means that with or without induction mRNA will be produced (constitutive). Also another mutation this genotype has is that it has non-functional lacZ and lacY genes; however it is stated in the problem that this does not decrease the length of the mRNA, it just makes a non-functional enzyme. Given this information, we can conclude that either **it is induced or not induced, the operon will make a 5kb mRNA strand** because of the mutated repressor.

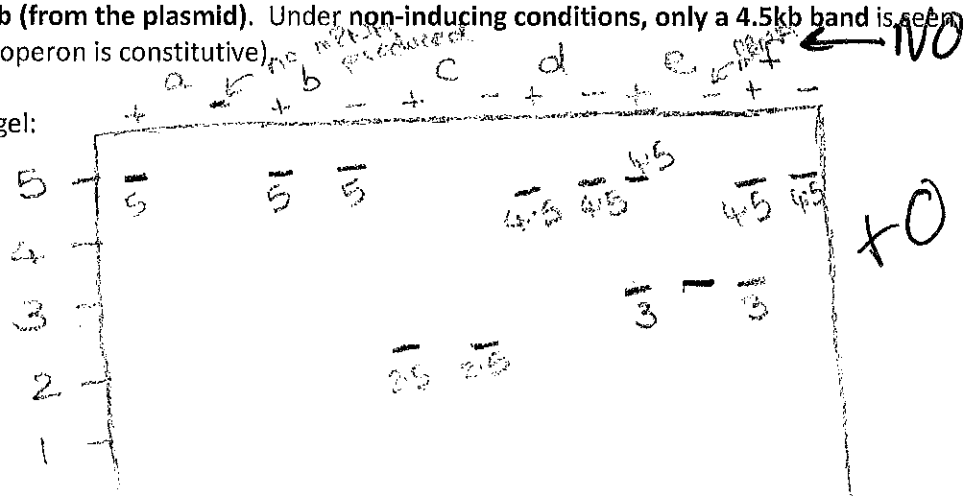
c) In this genotype there is a mutated repressor (constitutive) and a lacZ and lacY deletion. Since there is a mutated constitutive repressor, an mRNA will be produced that is shortened with or without an inducer. Since a lacZ deletion shortens the mRNA by 2kb and a lacY deletion shortens the mRNA by .5, the overall **mRNA band will be 2.5kb for both induced and not induced.**

d) In this genotype, there is a mutated operator (therefore making the operon constitutive) with mutated structural genes. The mutated genes are mutated in such a way that lacZ still forms an enzyme, but a non-functional one, but the lacY gene is a deletion – the mRNA is shortened by .5. Therefore under **inducing and non-inducing conditions, a band of 4.5kb is seen on the gel.**

e) In this genotype, there is not only the regular gene, but also a plasmid. The trick here is that, the regular gene will make one mRNA strand and the plasmid will make another mRNA strand. Given this information, the regular gene has a normal repressor and operator with mutated lacZ and lacY genes. The mutated Z gene forms a non-functional enzyme, while the mutated Y gene is a deletion of .5. For the plasmid, there is a constitutive operator with a deleted lacZ and a non-functional lacY gene. Therefore **under induced conditions, we should see two bands – one of 4.5kb and another of 3kb** because the regular gene and the plasmid's gene, both form their own mRNA. For the **non-inducing conditions, no mRNA will be produced by the regular gene,** but an 3kb mRNA will be produced by the plasmid's operon.

f) There is also a plasmid in this genotype. The cell's operon has a constitutive operator (therefore an mRNA will always be made) and it has a structural gene deletion in the lacY gene. The plasmid, however has a normal repressor and operator, but with a deletion in the lacZ gene. Therefore in **inducing conditions, two bands are produced on the gel – 4.5kb (from the cell's operon) and 3kb (from the plasmid).** Under **non-inducing conditions, only a 4.5kb band** is seen (since the cell's operon is constitutive).

Here is a picture of the gel:



[redacted]

Genetics Test Corrections
Question 6

6) If an insertion of 4bp takes place in the eleventh of 14 total exons, it would mean that a frameshift mutation would result. A frameshift mutation is a mutation where the reading frame of the mRNA changes and this can led to drastically different mRNA. When the insertion takes place, new (probably incorrect) amino acids would be made from the point of the insertion. So when transcription occurs, there will be no stop codon in the originally intended spot, although there is a chance that there might be a stop codon after the 14th exon due to the insertion; in any case, the strand will be longer than intended because of the 4bp insertion.

70

induction of premature stop codon - "nonsense mediated decay"

My original answer was incorrect because the problem never states anything remotely close to a 5'methylguanine cap or a poly-A-tail. Though, those mutations can lead to mRNA instability (by making it susceptible to degradation), the problem only talks about a 4bp insertion and therefore the logical conclusion (after the above thought process) would be that the insertion caused the mRNA to have an increased length and therefore made it unstable. A 5' methylguanine cap or poly-A-tail, really has nothing to do with this problem.

[redacted]
Genetics Test Corrections
Question 7

7) In this question, if there is a mutated dam gene, it would mean that the DNA does not get properly methylated (a methyl group is not added to the N^6 position of adenine). How would this lead to a higher mutation rate? If there was a mismatch repair mechanism after a DNA strand was replicated, the mechanism would not be able to differentiate between the newly formed strand and the old strand because the old strand would not be methylated. The mismatch repair mechanism discriminates between the new and old strand by assessing the degree of methylation of each strand – in a normal replication, the newly formed strand has no methylation (temporarily); using this idea, the mismatch repair mechanism is able to use the old strand as the template to repair the new strand. +2

If there is a mismatch and the degree of methylation of the new and old strand is almost the same, then the mechanism will not be able to identify which one is the correct strand and therefore it will randomly excise either one of the two bases. Therefore there is a high mutation rate when the dam gene (which methylates the old DNA) is mutated. ✓

My original answer was incorrect because tautomeric shifts occur when there is a single proton shift in the molecule. Here we are given that a methyl group is not attached to the molecule, not that a proton has shifted, therefore my original answer was incorrect. O.K.

[redacted]

Genetics Test Corrections
Question 8

+1

8) The role of the GC boxes and the CAAT boxes is to help initiate transcription by binding transcription factors (that help to bind RNA polymerase to the promoter). If these sequences are deleted, transcription would decrease dramatically. The phenotypic effect of these sequences is that expression of phenylalanine hydroxylase would decrease dramatically and so that less phenylalanine would be metabolized in a person's body and so the phenylalanine would accumulate in the body and lead to possible mental retardation, seizures, learning difficulties, etc.

My original answer was incorrect because I failed to answer the second part of the question – the phenotypic effects of deleting the GC and CAAT boxes. Phenylalanine would not be metabolized and led to severe disorders caused by an excess of phenylalanine in the person's body. – can just say person would have PKU

OK.

[redacted]
Genetics test corrections
Question 9

9. This question tries to find out which mutation caused molecular changes in the DNA.
Mutant 1: The mutation here was a base substitution and this therefore led to a missense mutation in the overall protein. A missense mutation is one in which one amino acid is replaced by another, incorrect, amino acid. The effect on the translation of the polypeptide is that serine is made instead of proline, therefore there is increased expression of serine. Here probably, the first nucleotide in the codon was replaced from a "C" to a "U", therefore changing the codon to read from proline to serine. This is a transition mutation. ✓

Mutant 2: This mutation is due to base substitution, which led to a nonsense mutation and led to premature mRNA termination of translation. A nonsense mutation is one where an original amino acid is replaced by a STOP codon, therefore leading to premature mRNA termination – the effect on the translation of this polypeptide. Probably of the argenine codon (CGA) was replaced by a "U", therefore coding UGA. This is a transition mutation. ✓

Mutant 3: This is an addition mutation where one nucleotide was inserted into the mRNA and this led to a frameshift mutation, where the codons are read in the wrong frame and so the entire amino acid sequence downstream of the mutation is disrupted and very different from the normal polypeptide – the effect on the translation of this polypeptide. ✓

Mutant 4: This is a deletion mutation and therefore leads to a frameshift mutation. Because the reading frame is changed, the amino acids arginine and leucine are missing in this mutant's polypeptide...though Glutamic acid and Glycine are still made due to the degeneracy of the genetic code. ✓

Mutant 5: This is an addition mutation where three nucleotides were added together (therefore 2 leucines are present). This leads to increased expression of leucine. ✓

My original answers were incorrect because I didn't fully answer the questions. For example, I wrote "frameshift mutation" in my original paper without writing whether or not it is an addition mutation or a deletion mutation..since these mutations lead to a frameshift mutation. Also I didn't fully explain the effects on translation of the mutated polypeptide.

+3

[redacted]
Genetics Test Corrections
Question 13

13) GTP is used for initiation of translation of mature mRNA in the cytoplasm. GTP is used by initiation factors (they enhance binding of the initiation complex) to form the initiation complex. Without this initiation complex, the translation of the polypeptide would not start because the AUG codon would be unable to bind to the small subunit.

GTP is also used during the elongation phase where it is used by the elongation factors to help move the charged or uncharged tRNA from the A site to the P site to the E site.

GTP is also used during the termination phase where GTP-dependent release factors help to cleave the polypeptide chain from the tRNA with the STOP codon – they do this via GTP.

Therefore the step of protein synthesis that would be blocked if GTP was omitted would be translation.

My original answer was incorrect because GTP is not used at all during transcription, it is however used in all three phases of translation.

OK. +1.5 pts

3 F09

BIOL 2500 FALL 2009

EXAMINATION 3

NAME [redacted]

PLEASE MAKE SURE THAT YOU WRITE YOUR NAME ON THE TOP OF EVERY PAGE THIS PAGE IS RESERVED FOR GRADING. THERE ARE 13 QUESTIONS AND 9 PAGES. YOU MAY USE THE REVERSE SIDE OF EACH PAGE AS SCRATCH PAPER. A GENETIC CODE TABLE IS PROVIDED IN THE BACK OF YOUR EXAM.

Page 2 10 (max 10)

Page 3 18 (max 22) +2

Page 4 10 (max 12) +0

Page 5 8 (max 12) –

Page 6 2 (max 8) +3

Page 7 9 (max 15) +3

Page 8 12 (max 12)

Page 9 6 (max 9) +1.5

TOTAL: 75

84.5

10

Name: [redacted]

1. The assignment of amino acids to particular codons was done by several techniques, including analysis of proteins synthesized using cell-free translation of RNA copolymers of random sequence and RNA copolymers of known sequence. (10 pts).

a. What amino acids would be expected and in what ratios if the copolymer translated were a repeating copolymer of the dinucleotide GU?

b. What amino acids would be expected and in what ratios if a mixed copolymer consisting of 20% guanosine and 80% uracil in random sequence were translated?

a- GUGUGU = expected codons: GUG, UGU

= expected amino acids: Val, Cysteine (Valine) ✓

ratio is 1:1, for every valine amino acid, there is a cysteine amino acid.

b- GUUUU~~[illegible]~~ ~~expected codons:~~

(1/5) G & (4/5) U

.8% = GGG = $(1/5)^3 = \frac{1}{125} = .8\%$

12.8% = UGU, ~~[illegible]~~, UUG, GUU = $(4/5)^2(1/5) = \frac{16}{25}(\frac{1}{5}) =$

3.2% = GUG, GGU, UGG = $(\frac{1}{5})^2(\frac{4}{5}) = \frac{4}{125} = 3.2\%$ $\frac{16}{125} = 12.8\%$

51.2% = UUU = $(\frac{4}{5})^3 = \frac{64}{125} = 51.2\%$

~~[illegible]~~4% glycine (Gly), 12.8% Cysteine (Cys), 12.8% leucine (leu),

~~[illegible]~~16% Valine (val), ~~[illegible]~~ 3.2% Tryptophan (trp),

sixteen percent

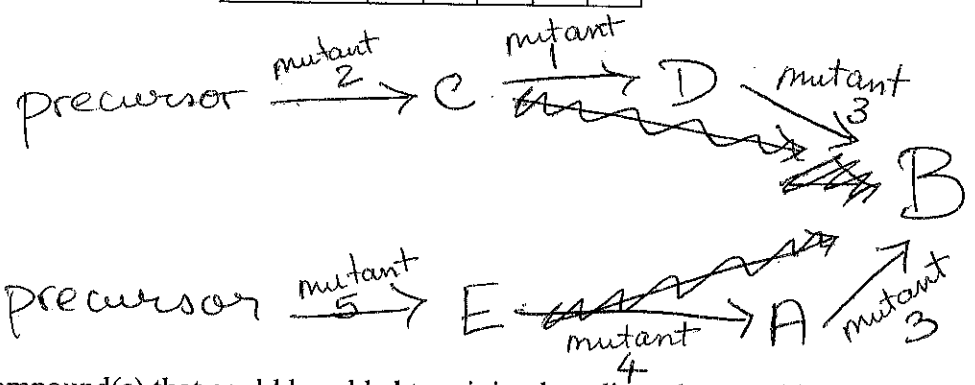
51.2% Phenalalanine (Phe)

Name: [redacted] 18

2. Auxotrophic mutants 1-5 with defects in a biosynthetic pathway were isolated from the bread mold *Neurospora crassa* and tested for their ability to grow on certain supplements. Using the data provided in the table below, diagram a biochemical pathway consistent with these data (10 pts).

Mutant	A	B	C	D	E
1	-	+	-	+	-
2	-	+	+	+	-
3	-	+	-	-	-
4	+	+	-	-	-
5	+	+	-	-	+

C → E → A → D → B



10

b. List the compound(s) that could be added to minimal medium that would allow a double mutant of 1 and 4 to grow (4 points).

The compounds that can be added are C and E or D & A

3. The sequence below represents the first 20 bases of an *E. coli* mRNA. Draw the double-stranded DNA that encoded this mRNA. Label the template and non-template strands and 5' and 3' ends. Draw an arrow over the template strand that shows the direction in which RNA polymerase moved to synthesize the mRNA. (8 points)

5' AUCGGACCAUUCGCGUCUUGG... 3'

3' TAGCCTGGTAAGCGCA

8

RNA polymerase →

template → 3' TAGCCTGGTAAGCGCAGAACC 5'

5' ATCGGACCATTCGCGTCTTGG 3' ← non-template

Name: [redacted]

4. A Northern blot experiment is carried out with mRNA from *E. coli* using a DNA probe corresponding to the *lacA* gene. Wildtype lac mRNA is approximately 5 kb in length. Two kinds of *lacZ* and *lacY* mutations are studied. The *lacZ*⁻ and *lacY*⁻ mutations are simple nucleotide substitution mutants that encode inactive proteins. The *lacZ** and *lacY** mutations are deletions, The *lacZ** deletion is missing 2kb of *lacZ* coding sequence, *lacY** is missing 0.5 kb of *lacY* coding sequence, and neither allele produces a polypeptide product. The mRNA from the following six genotypes is analyzed by Northern blot after growth either in the presence (+) or absence (-) of lactose. For each genotype, draw a band of the expected size for the *lac* mRNA on the Northern blot under inducing (+) and non-inducing (-) conditions. (12 points)

constitutive wildtype

a. $lacI^+ O^+ Z^+ Y^+$ 2.5 lacZ⁻, lacY⁻ inactive

b. $lacI^- O^+ Z^- Y^-$ 5 2.5 wild type: 5kb

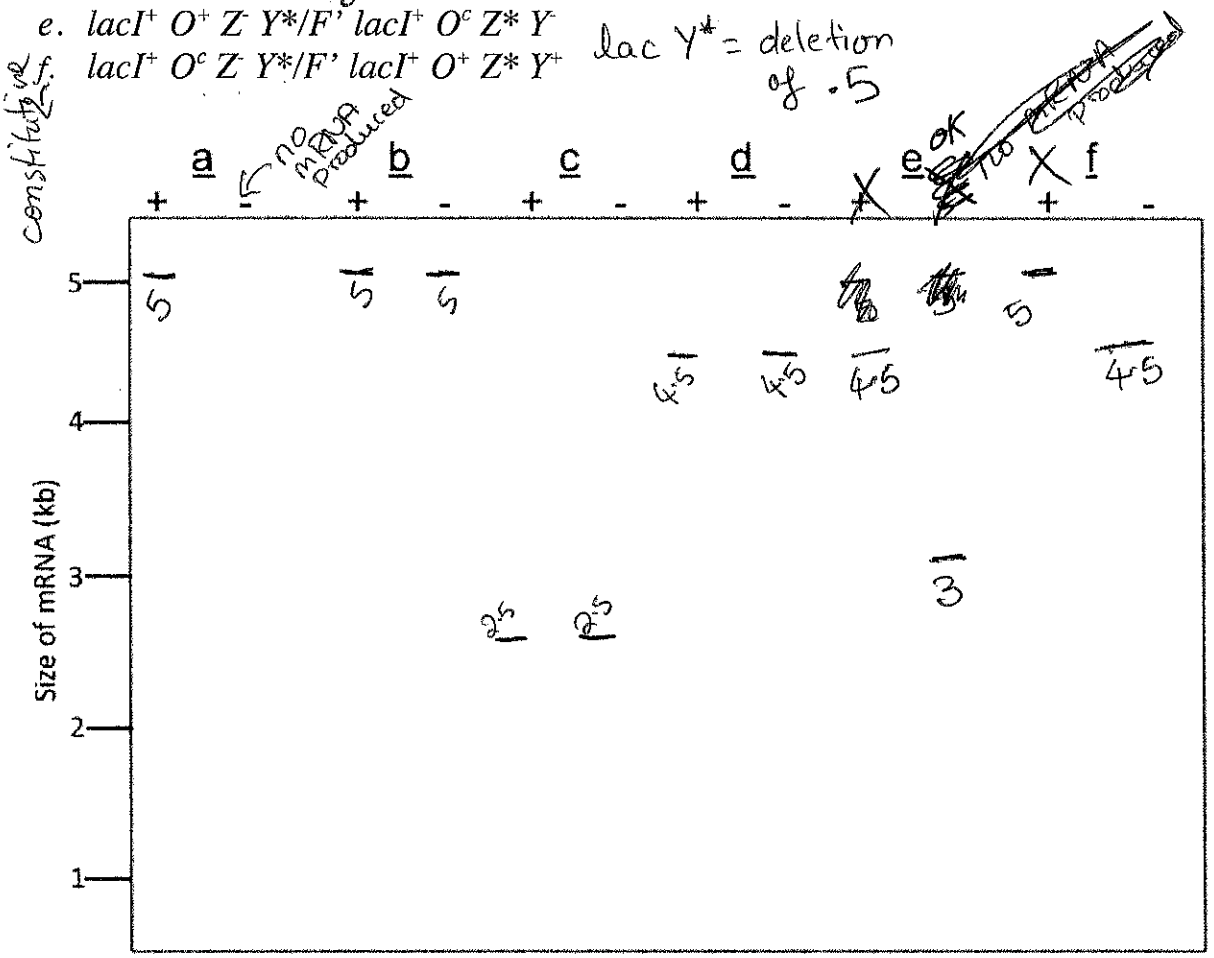
c. $lacI^- O^+ Z^* Y^*$ lacZ* = deletion of 2Kb

d. $lacI^+ O^c Z^- Y^*$ 5 - .5 = 4.5

e. $lacI^+ O^+ Z^- Y^*/F' lacI^+ O^c Z^* Y^-$ lac Y* = deletion of .5

f. $lacI^+ O^c Z^- Y^*/F' lacI^+ O^+ Z^* Y^+$

constitutive



10

Name: [redacted] 8

5. In a bacterium related to *E. coli*, a biosynthetic operon containing genes for the synthesis of the amino acid proline is studied. This operon has a regulatory system analogous to that of the *E. coli Trp* operon, with an aporepressor that binds to proline and a leader sequence in the mRNA that includes five consecutive proline codons. (8 points)

a. Under what conditions would transcription of the proline operon be initiated?

2 It would be initiated if the cell was low on proline (ie. it would use the operon to make proline)

b. Under what conditions would attenuation take place and transcription be halted?

2 Attenuation would take place if there were charged $tRNA^{Pro}$ present, therefore the terminator hairpin would form & transcription would be halted.

c. Under what conditions would transcription continue through to the end of the operon?

2 If there were no charged $tRNA^{Pro}$ present, then the ribosome would "stall" & allow the anti-terminator hairpin to form & ~~end~~ continue transcription.

d. A mutant of this bacterium is isolated that has a defective ribosomal protein which causes translation always to briefly stall whenever a proline codon is encountered. How would this affect attenuation of the proline operon?

2 This would affect attenuation since the ribosome always stalls, it allows the anti terminater hairpin to form & translation would always proceed, w/ no attenuation

6. Tay-Sachs disease is an autosomal recessive disorder that produces deafness, blindness, seizures, and eventually death. The disease results from a defective *HEXA* gene, which encodes the enzyme hexosaminidase A. The function of hexosaminidase A is to degrade GM2 gangliosides. In the absence of hexosaminidase A, gangliosides accumulate in the brain. The most common mutation causing Tay-Sachs disease is an insertion of 4 bp in the eleventh of 14 total exons. Normal transcription of the *HEXA* mRNA occurs in individuals with the Tay-Sachs disease, but the mRNA is unstable. Explain how the 4 bp insertion could cause mRNA instability. (4 points)

The 4bp insertion could cause ~~a~~ unstability by making the mRNA ~~more~~ very susceptible to degradation, ~~&~~ this can occur when the mRNA has a defective / not present 5' methylguanine cap or if it has a defective poly-A-tail in it's 3' end. Missing & either of these can cause mRNA (or defective versions) of stability.

RNA instability

* frameshift mutation

Name: [redacted]

2

7. In *E. coli*, a methyltransferase enzyme encoded by the *dam* gene recognizes the sequence 5'-GATC-3' and attaches a methyl group to the N^6 position of adenine. *E. coli* strains that have the *dam* gene deleted are known to have a higher spontaneous mutation rate than wild-type strains. Explain why. (4 points)

This could lead to higher mutation rates because the adenines are not made correctly (the methyl group is missing), therefore tautomeric shifts could occur, ~~[illegible]~~ therefore O mutations are prevalent.

8. The PAH gene encodes phenylalanine hydroxylase, which is mutated in the disease phenylkenoturia. Upstream of the transcription start site for the PAH gene are DNA sequences including several GC boxes and a CAAT box. What is the role of these sequences? What would be the phenotypic effect if any of these sequences were deleted? (4 points)

The role of these sequences are to help initiate transcription by binding transcription factors (that help to bind RNA polymerase to the promoter). If these sequences are deleted, transcription would decrease dramatically.

2

last part of question

Name: [redacted]

9. A polypeptide has the following amino acid sequence:

Met-Ser-Pro-Arg-Leu-Glu-Gly

The amino acid sequence of this polypeptide was determined for the series of mutants listed below. Classify each mutation by the molecular change in the DNA and by its effect on translation of the polypeptide. (3 points each)

Mutant 1: Met-Ser-Ser-Arg-Leu-Glu-Gly

Mutant 2: Met-Ser-Pro

Mutant 3: Met-Ser-Pro-Asp-Trp-Arg-Asp-Lys

Mutant 4: Met-Ser-Pro-Glu-Gly

Mutant 5: Met-Ser-Pro-Arg-Leu-Leu-Glu-Gly

Mutant 1: There was a base substitution, +2 → ∴ missense mutation in the codon for the third amino acid, where a U was inserted in the first base instead of a C (therefore the mutation changed from proline → serine); therefore serine is made instead of proline

Mutant 2: This is a nonsense +1 mutation which leads to premature mRNA termination (the first codon in the 4 amino acid was changed to a U instead of a C)

Mutant 3: This is a frameshift +1 mutation, due to the addition of one nucleotide. This shifts the whole reading frame.

Mutant 4: This a deletion +2 mutation (therefore a frameshift mutation), that lead to the reading frame getting changed.

Mutant 5: This is an addition +3 mutation, where three nucleotides were added together (therefore 2 leucines are present). This leads to increased expression of leucine

Name: [redacted] 12

11. A miRNA was recently identified that promotes differentiation of mouse bone cell precursors (osteoblasts) into mature bone cells (osteocytes) by regulating post-transcriptional expression of a histone deacetylase. (12 points)

a) How do histone deacetylases (HDACs) regulate gene expression? 3

histone deacetylases, deacetylates DNA so ~~than gene expression is possible~~ ~~that gene the DNA goes back to a compact structure it increases~~ gene expression is ~~increased~~ decreased.

it turns DNA back to a compact structure therefore decreasing gene expression

b) How would expression of the miRNA affect expression of the HDAC?

~~the~~ Expression of the miRNA would decrease the expression of HDAC because miRNA would degrade the mRNA for histone deacetylase 3

c) How would expression of the miRNA affect the expression of the genes normally regulated by the HDAC?

Expression of the miRNA would decrease the expression of HDAC, therefore it would increase the expression of the genes regulated by HDAC. 3

d) If the gene encoding the miRNA were silenced how would mouse bone formation be affected?

If the gene for miRNA was silenced, this would lead to the decrease in the formation of mature mouse bone cells (osteocytes).

3

Name: [redacted] 6

12. Scientists at a textile company are interested in developing a flame-retardant chemical that can be used to treat clothing and bedding in the hopes of reducing deaths from fire. In the 1970's several widely used flame-retardants were removed from the market after it was found that they were potential mutagens and carcinogens. What test do you suggest the scientists perform to determine if the new flame-retardant chemical is safe to use? Describe the procedure that the scientists should use. (6 points)

el would use the Ames test because this helps to gauge the carcinogenity of a chemical. The scientist should have a control which is just his⁻ bacteria w/ an enzyme & then have a tube w/ a known conc. of mutagen & the enzymes → place the filters on the ~~mutagen~~ experimental plate & add the bacteria & see how many bacteria (on both plates) revert to his⁺ form. The more the reversions, (in the experimental tube relative to the control) the more likely that the chemical is carcinogenic

13. A cell-free system for protein synthesis requires addition of GTP. What step(s) of protein synthesis would be blocked if GTP were omitted? (3 points)

el GTP were omitted, the DNA would not open/be available for transcription; ~~the mRNA would not be able~~ therefore an mRNA wouldn't form & translation wouldn't take place & protein synthesis would not occur

elongation factors

Name: _____

First Position (5′ end)	Second Position: U		Second Position: C		Second Position: A		Second Position: G		Third Position (3′ end)
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	*Stop*	UGA	*Stop*	A
	UUG		UCG		UAG	*Stop*	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G