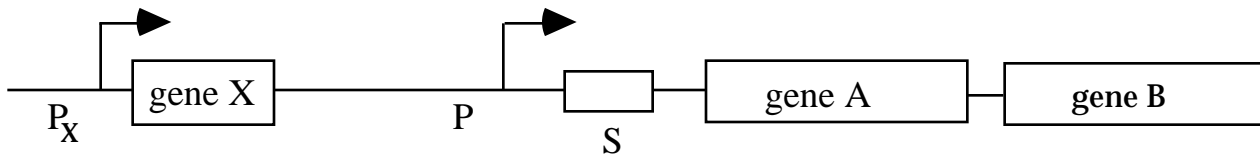


Question 1

In *E. coli*, the fictitious AB operon is induced by the presence of Compound W. A diagram of the operon, its regulatory proteins and regulatory sites is shown below:



- P_X promoter for the regulatory protein
- X gene for the regulatory protein of the AB operon
- P promoter for the AB genes
- S sequence shown to be important for regulation by W
- A structural gene for enzyme A
- B structural gene for enzyme B

The following table shows the genotypes of different *E. coli* strains with a wild-type AB operon and various mutant AB operons, and the number of molecules of protein A per cell in the absence or presence of Compound W (-W or +W, respectively).

a) In the table below, the symbol "+" indicates that the gene or control element is functional (wt) and "-" indicates that the gene or control element is non-functional. Assume the genes not listed are wild type.

Strain	X	P	S	A	Molecules of A		Expression
					-W	+W	
Wild type	+	+	+	+	0	200	inducible
M1	-	+	+	+	200	200	
M2	+	-	+	+	0	0	
M3	+	+	-	+	200	200	
M4	+	+	+	-	0	0	

i) For each strain on the table above, label the expression as either inducible, uninducible or constitutive.

ii) Based on the data shown above, does the regulatory protein X act as a repressor or an activator of the AB operon? Explain your reasoning.

Question 1, continued

b) You make partial diploids of various *E. coli* mutant strains using a single-copy plasmid introduced into the *E. coli* cell. In cells of the following genotypes, predict the number of molecules of enzyme A per cell (0, 200, 400) produced in the absence or presence of Compound W (-W or +W, respectively). Put the total number of molecules of enzyme A on the lines provided.

Genotype	# of molecules of enzymes A per cell	
	-W	+W
X ⁺ P ⁺ S ⁺ A ⁺	0	400
X ⁺ P ⁺ S ⁺ A ⁻		
X ⁺ P ⁺ S ⁺ A ⁺	0	200
X ⁺ P ⁺ S ⁺ A ⁻		
X ⁺ P ⁺ S ⁺ A ⁺		
X ⁻ P ⁺ S ⁺ A ⁺	_____	_____
X ⁺ P ⁺ S ⁻ A ⁺		
X ⁺ P ⁺ S ⁺ A ⁺	_____	_____
X ⁻ P ⁺ S ⁺ A ⁺		
X ⁺ P ⁺ S ⁺ A ⁻	_____	_____
X ⁺ P ⁺ S ⁻ A ⁺		
X ⁺ P ⁺ S ⁺ A ⁻	_____	_____
X ⁺ P ⁺ S ⁻ A ⁻		
X ⁺ P ⁺ S ⁺ A ⁺	_____	_____

Question 1, continued

You examine a different operon, the H operon, containing genes 1 and 2 that encode enzymes 1 and 2. In this operon compound H acts as an inducer. You look at the levels of DNA, mRNA and protein in a typical *E. coli* cell. The results you find are shown below:

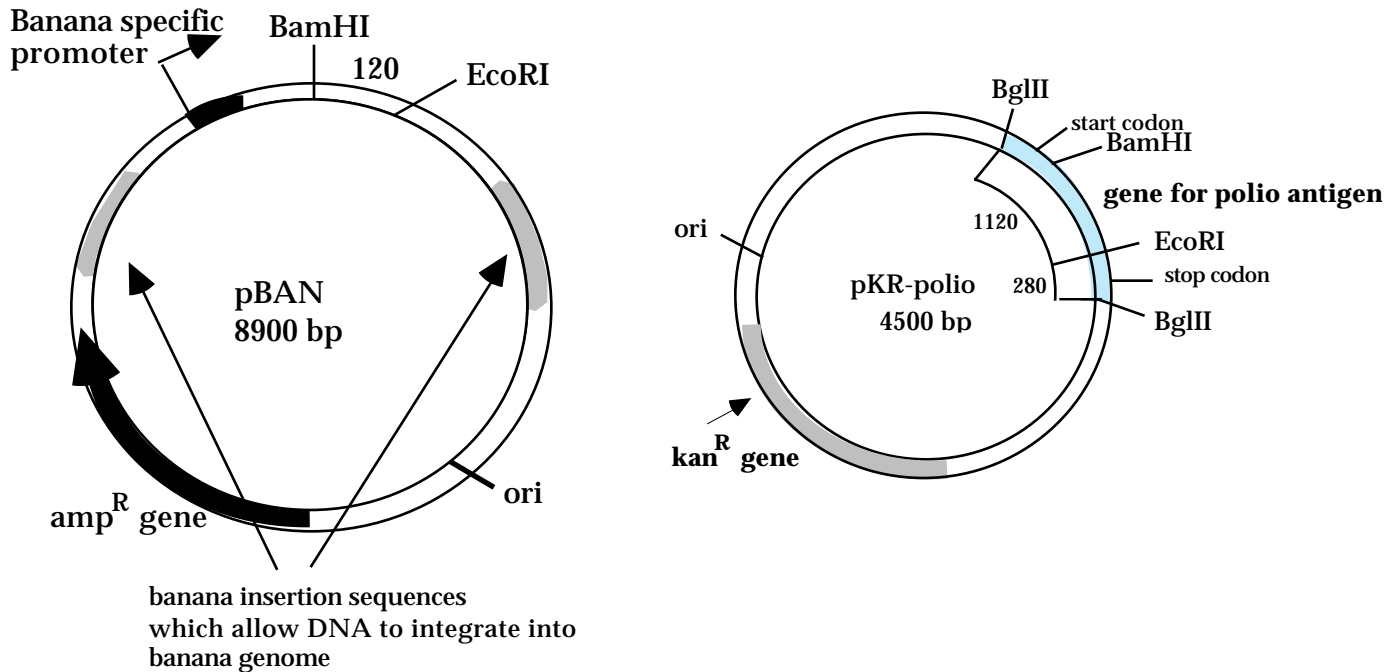
		-H	+H
DNA	# of copies of gene 1 per cell	1	1
mRNA	# of copies of gene 1 mRNA per cell	50	50
protein	# of molecules of enzyme 1 per cell	0	200

c) At which step of gene expression *E. coli* could this regulation occur? Explain your reasoning.

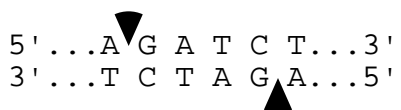
d) In the lac operon, which is regulated at the transcriptional level, repressor protein is present in *E. coli* at approximately 4 molecules per cell. In the H operon, the repressor protein is present at approximately 200 molecules per cell. Based on your answer to part c, why would you expect there to be more molecules of the repressor protein for the H operon per cell than molecules of the lac repressor per cell?

Question 2

Polio has been practically eliminated from the American population, however, in countries where people have little or no access to vaccinations, it is still prevalent. As a biologist with a global vision, you seek to create a transgenic banana that produces the protein used in the vaccine against polio. By consuming these bananas, individuals will develop immunity against the disease. The gene for this protein has already been cloned into a plasmid with a kanamycin-resistance gene (pKR-polio). You need to attach to the gene a banana-specific promoter and DNA sequences that will allow the gene to be incorporated into banana DNA. These sequences are contained in the pBAN plasmid, which carries a gene for ampicillin resistance. Maps of these two plasmids are shown below, including important restriction sites and distances (in base pairs) between the sites.



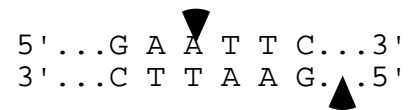
BglIII:



BamHI:



EcoRI:



a) An end generated by digestion with BamHI can be ligated to an end generated by digestion with BglII. Why is this possible?

Question 2, continued

b) You want to insert the gene encoding the polio antigen into pBAN. Devise a strategy to accomplish this. Identify the enzyme(s) you would use to cut pBAN, the enzyme(s) you would use to cut pKR-polio, and the steps necessary to generate the intact plasmid.

c) You next transform *E. coli* with the plasmids you have made. You grow the transformed cells on media containing (circle one):

ampicillin

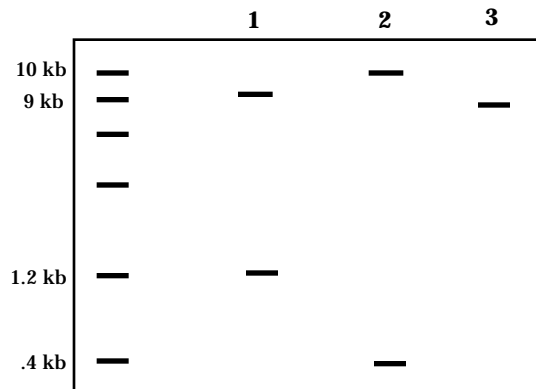
kanamycin

both ampicillin and kanamycin

neither ampicillin nor kanamycin

Why?

d) You isolate plasmid DNA from three colonies that pass your antibiotic resistance test. You digest the DNA with the restriction enzyme EcoRI. You size separate the resulting fragments from each plasmid on an agarose gel. You find the following results. DNA fragment sizes are indicated to the left.



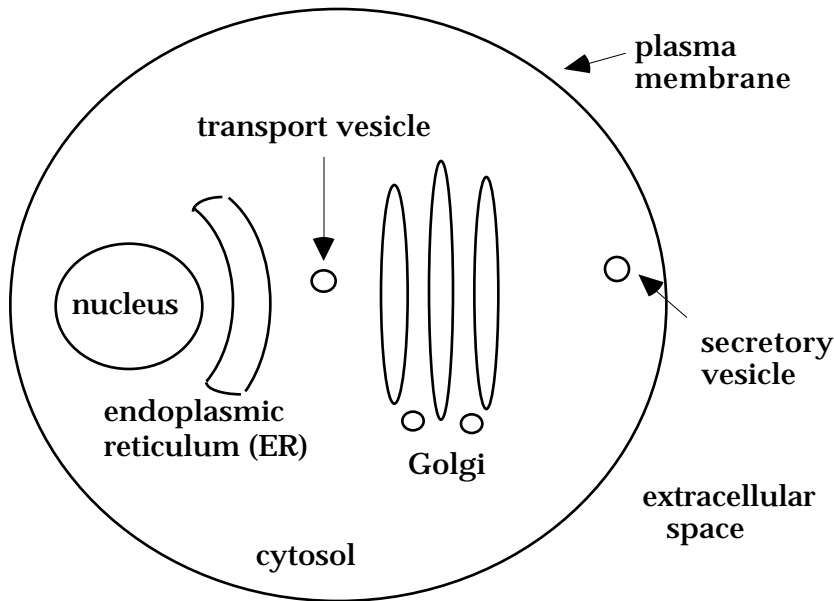
Question 2, continued

- d) i) Using the data on the previous page, draw plasmids associated with the colonies 1, 2, and 3. Indicate all relevant features such as the promoter, the origin of replication, the genes for ampicillin resistance and the polio antigen,

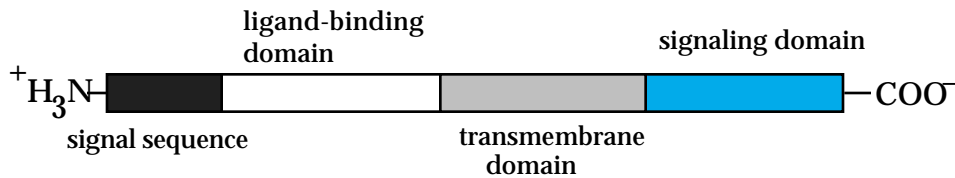
ii) Which of the three plasmids would allow synthesis of the protein in a banana? Explain your reasoning.

Question 3

Use this diagram of a cell as a guide when answering the following questions.



a) Consider the following receptor protein:



i) Where would this receptor protein be localized if it's the cell was lacking SRP? Would it be functional?

ii) Where would this receptor protein be localized if its signaling domain were deleted? Would it be functional?

iii) What common characteristic do the side chains of the amino acids found in a transmembrane domain share, and why?

iv) Which domain (**ligand binding, transmembrane, or signaling**) of the wild-type receptor protein is inserted into the ER lumen during translation? Why must the receptor protein be inserted in this fashion to become functional?

Question 3, continued

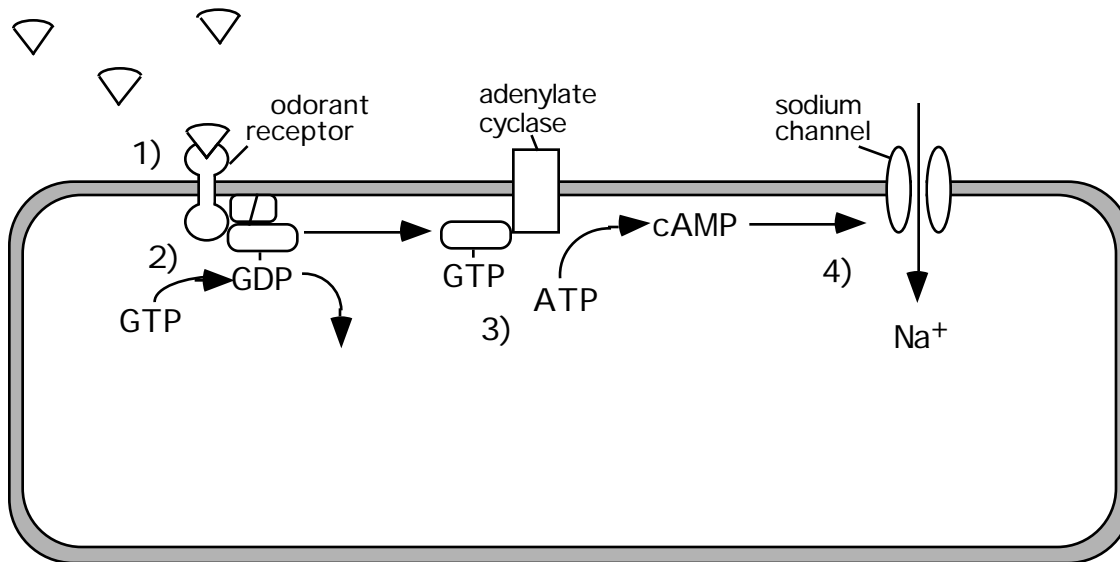
b) The mature mRNA encoding a protein destined for secretion specifies a polypeptide chain of 497 amino acids; yet the protein that is actually secreted by the cell is 475 amino acids long.

i) How can you account for this size difference?

ii) Would a transmembrane protein also show this disparity? Briefly explain your answer.

Question 4

Consider the following signal transduction pathway involved in smelling pumpkin pie.



1) A pie odorant molecule (), one of the many aromatic compounds present in pumpkin pie, binds to a specific G protein-coupled olfactory receptor in an olfactory cell in your nose.

2) The receptor-odorant complex activates a G protein, which displaces GDP and then binds to a molecule of GTP.

3) The subunit of the G protein dissociates and activates adenylate cyclase, which catalyzes the production of cAMP from ATP.

4) cAMP binds to a sodium channel, opening it and allowing Na⁺ to enter the cell. This creates a nerve stimulus, which travels to the brain. Your brain integrates the odorant signal with signals from other olfactory receptors in your nose. The brain interprets the signal as the odor of pumpkin pie. You think, "Mmm...pie."

Question 4, continued

a) How does binding of the odorant molecule stimulate the receptor protein and how does this activate the G protein?

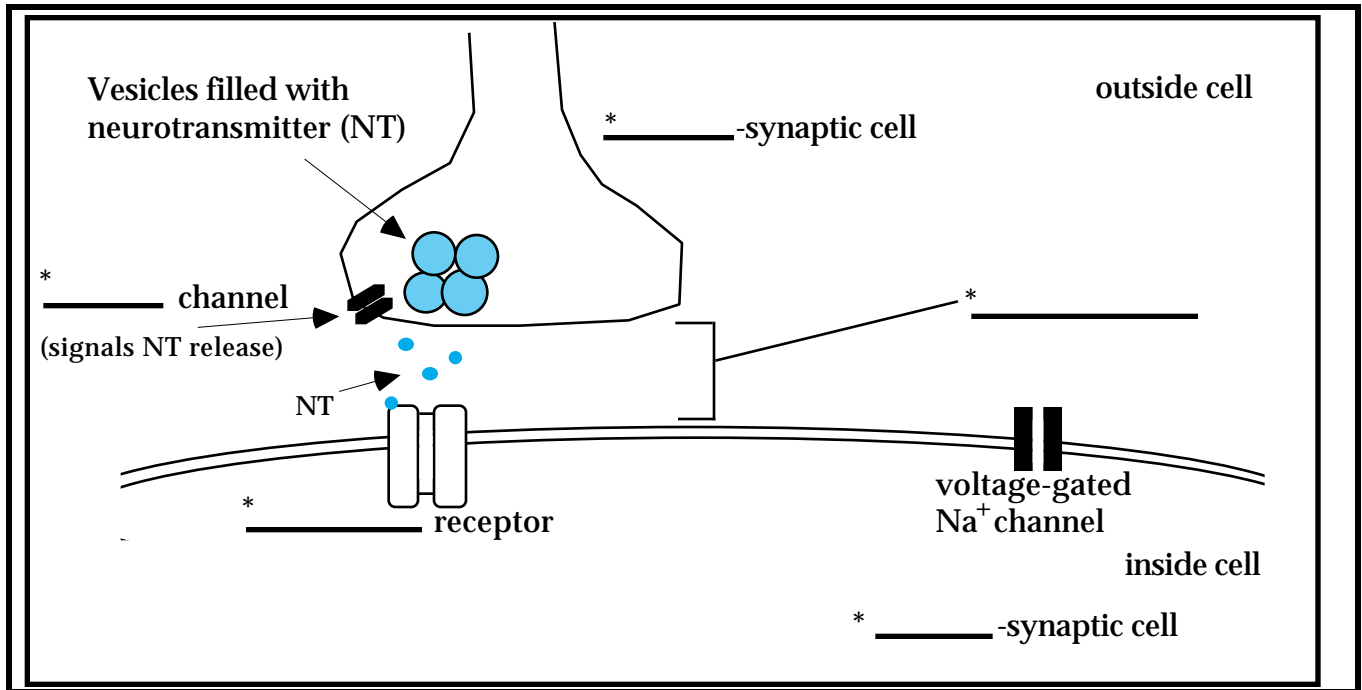
b) Although it might be nice to have the sensation of smelling pie continuously, once the odorants are gone, signaling stops. How is the signaling pathway turned off? Discuss why once the G protein is activated, it does not continuously activate adenylate cyclase. What chemical reaction is involved in this process?

c) List two advantages to having multiple steps in a signal transduction pathway.

d) A turkey odorant molecule comes in contact with the odorant receptor. Would this have an effect on the nerve stimulus being sent from this olfactory cell? Explain your answer.

Question 5

a) Fill in the blanks (*____) on the schematic of the **neuromuscular junction** below.



b) Briefly describe the events that trigger the release of neurotransmitter at this synapse.

c) Once neurotransmitter is released, briefly describe the events that result in a depolarization large enough to cause a muscle twitch.

SOLUTIONS

Question 1

a)

i)

Strain	X	P	S	A	-W	+W	Expression
Wild type	+	+	+	+	0	200	inducible
M1	-	+	+	+	200	200	constitutive
M2	+	-	+	+	0	0	uninducible
M3	+	+	-	+	200	200	constitutive
M4	+	+	+	-	0	0	uninducible

ii) Protein X acts as a repressor. In mutants 1 and 3 that lack either the repressor or the repressor binding site, expression is constitutive (high with or without W).

b) Put the total number of molecules of enzyme A on the lines provided.

Genotype	<u># of molecules of enzymes A per cell</u>	-W	+W
X ⁺ P ⁺ S ⁺ A ⁺		0	400
X ⁺ P ⁺ S ⁺ A ⁺			
X ⁺ P ⁺ S ⁺ A ⁺		0	200
X ⁺ P ⁺ S ⁺ A ⁻			
X ⁺ P ⁺ S ⁺ A ⁺		0	400
X ⁻ P ⁺ S ⁺ A ⁺			
X ⁺ P ⁺ S ⁻ A ⁺		200	400
X ⁺ P ⁺ S ⁺ A ⁺			
X ⁻ P ⁺ S ⁺ A ⁺		0	200
X ⁺ P ⁺ S ⁺ A ⁻			
X ⁺ P ⁺ S ⁻ A ⁺		200	200
X ⁺ P ⁺ S ⁺ A ⁻			
X ⁺ P ⁺ S ⁻ A ⁻		0	200
X ⁺ P ⁺ S ⁺ A ⁺			

Question 1, continued

c) **At which step of gene expression could this regulation occur? Explain your reasoning.**

The regulation occurs at translation. In both the absence and presence of Compound H, the numbers of DNA and mRNA copies are the same. However, the number of molecules of enzyme 1 increase in the presence of inducer. Since only protein levels vary in the presence or absence of inducer, the regulation must be occurring at protein synthesis.

d) **Based on your answer to part c, why would you expect there to be more molecules of the repressor protein for the H operon per cell than molecules of the lac repressor per cell?**

In this case, the repressor protein binds to a site on the mRNA and blocks translation. Since the mRNA is present at approximately 50 copies per cell, an excess of repressor is necessary to regulate it. In the lac system, the lac repressor binds to the operator on the DNA. Each haploid cell contains only one copy of the lac operon and only has one site to which lac repressor can bind. Because of this, the lac repressor is only required at very low levels.

Question 2

a) **An end generated by digestion with BamHI can be ligated to an end generated by digestion with BglII. Why is this possible?**

Digestion with BglII or BamHI produce the same overhangs, or sticky ends. Thus the ends produced by cutting with BglII are complementary to the ends produced with BamHI, base pairing and ligation can occur.

b) **You want to insert the gene encoding the polio antigen into pBAN. Devise a strategy to accomplish this. Identify the enzyme(s) you would use to cut pBAN, the enzyme(s) you would use to cut pKR-polio, and the steps necessary to generate the intact plasmid.**

Cut pBAN with BamHI to linearize.

Cut pKR-polio with BglII, this gives a 1400 bp fragment containing the gene for the polio antigen and a 3100 bp fragment.

Size select the DNA from the pKR-polio plasmid to obtain the 1400 bp fragment.

Ligate the 1400 bp fragment together with the cut pBAN plasmid.

c) **You next transform *E. coli* with the plasmids you have made. You grow the transformed cells on media containing (circle one):**

ampicillin

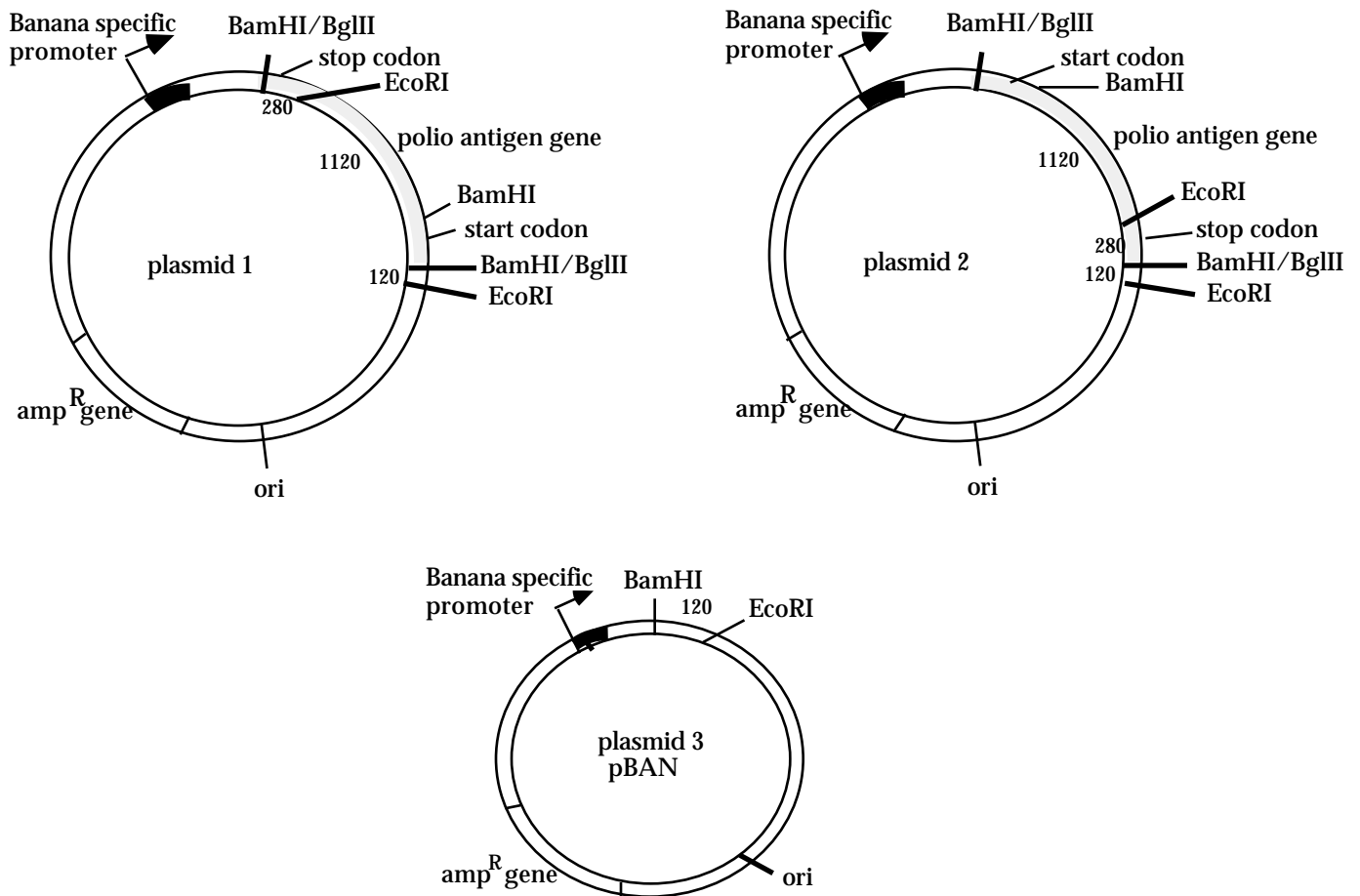
kanamycin

both ampicillin and kanamycin

neither ampicillin nor kanamycin

Why? *The media should contain ampicillin only, because the plasmid with the promoter and banana insertion sequences has a gene for ampicillin resistance.*

- d) i) Draw plasmids 1, 2, and 3, indicating all relevant features such as the promoter, the origin of replication, the genes for ampicillin resistance and the polio antigen, restriction sites and distances (in base pairs) between the sites.



- ii) Which of the three plasmids would allow synthesis of the protein in a banana? Explain your reasoning.
Only plasmid two would allow synthesis of functional protein. The polio antigen gene must be correctly oriented with respect to the promoter.

Question 3

- a) i) Where would this receptor protein be localized if the cell was lacking SRP?
The receptor protein would be found in the cytosol. It would be non-functional.
- ii) Where would this receptor protein be localized if its signaling domain were deleted?
It would be inserted into the plasma membrane, but would not be functional without a signaling domain.
- iii) What common characteristic do the side chains of the amino acids found in a transmembrane domain share, and why?
The transmembrane domain, is composed of amino acids with hydrophobic side chains because the interior of the lipid bilayer is hydrophobic.

Question 3, continued

iv) Which domain (**ligand-binding, transmembrane, or signaling**) of the wild- type receptor protein is inserted into the ER lumen during translation? Why must the receptor protein be inserted in this fashion to become functional?

The ligand-binding domain is inserted into the ER lumen. Upon secretory vesicle fusion with the plasma membrane, the interior of the ER becomes the outside of the cell where the ligand-binding domain must be in order to bind ligand.

b) i) How can you account for this size difference?

A signal sequence of 22 amino acids is cleaved during translation into the ER.

ii) Would a transmembrane protein also show this disparity? Briefly explain.

Yes. A transmembrane protein also has a signal sequence, which is also cleaved during translation into the ER.

Question 4

a) How does binding of the odorant molecule stimulate the receptor protein and how does this activate the G protein?

The binding of a odorant molecule to the extracellular domain of the receptor changes the conformation of the protein. This allows the intracellular domain of the receptor to bind the G protein heterotrimer. This binding induces the subunit to exchange GDP for GTP, activating the subunit and releasing it from the subunit.

b) Although it might be nice to have the sensation of smelling pie continuously, once the odorants are gone, signaling stops. How is the signaling pathway turned off? Discuss why once the G protein is activated, it does not continuously activate adenylate cyclase. What chemical reaction is involved in this process?

The subunit of the G protein has an intrinsic GTPase activity. This activity converts GTP bound to the subunit to GDP. Since the GTP is required for the subunit to be active, the conversion of GTP to GDP deactivates the subunit of the G protein. The inactive subunit then can rejoin the subunit to await another signal from the receptor.

c) List two advantages to having multiple steps in a signal transduction pathway.

Multiple steps in the pathway can provide a large amplification of the signal. It also allows multiple points of regulation. In addition, if several different receptors work through the same transduction pathway, integration of different inputs is possible.

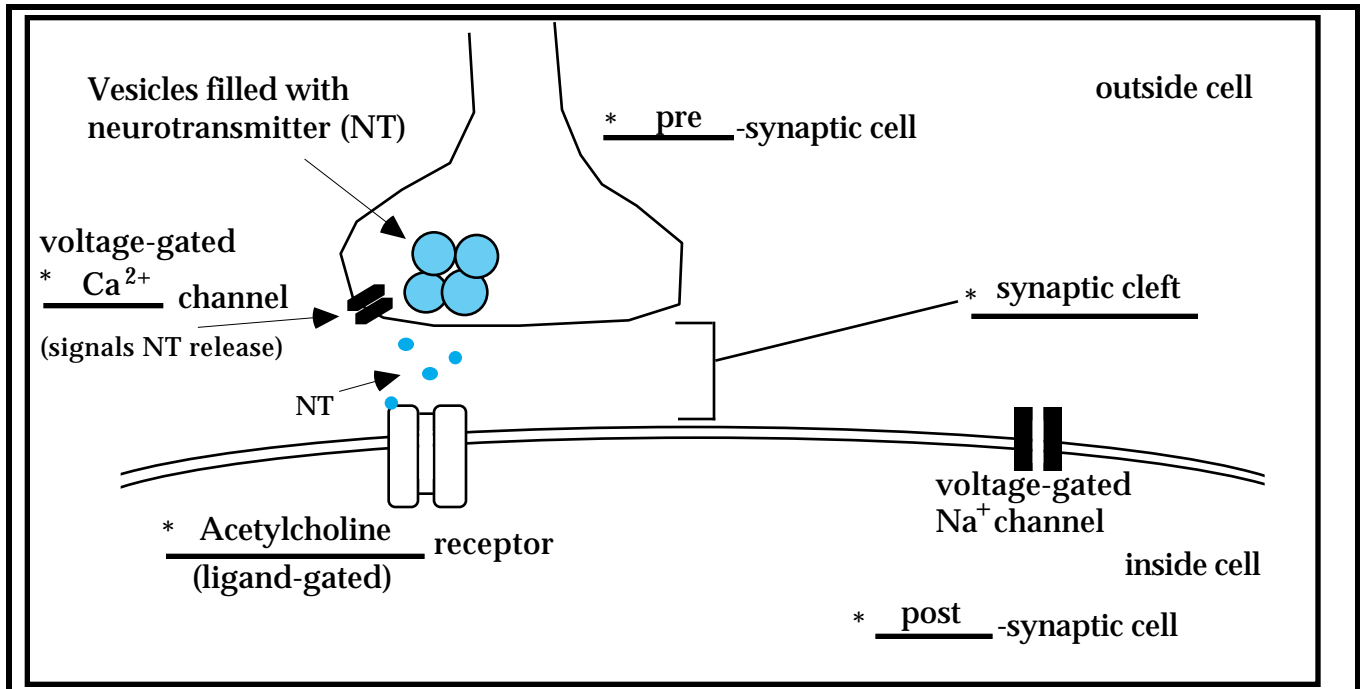
d) A turkey odorant molecule comes in contact with the odorant receptor. Would this have an effect on the nerve stimulus being sent from this olfactory cell?

Explain your answer.

No, a turkey odorant molecule would have no effect on the stimulus from this cell. The odorant receptor specifically recognizes the odorant. However, the turkey odorant molecule would affect olfactory cells specialized for turkey.

Question 5

a) Fill in the blanks (*____) on the schematic of the **neuromuscular junction** below.



b) Briefly describe the events that trigger the release of neurotransmitter at this synapse?

An action potential invades the terminus of the pre-synaptic cell, depolarizing it.

Voltage-gated Ca^{2+} channels open, allowing Ca^{2+} into the cell.

Ca^{2+} triggers the fusion of the vesicles containing acetylcholine with the plasma membrane, releasing NT.

c) Once neurotransmitter is released, briefly describe the events that result in a depolarization large enough to cause a muscle twitch?

Acetylcholine (ACh) binds to the receptor causing a conformation change that allows the receptor to function as a Na^+ channel. Na^+ move into the muscle cell and depolarizes it. If the depolarization is large enough, voltage-gated Na^+ channels open on the muscle cell resulting in a large depolarization of the muscle.