

Essay question #1 setup

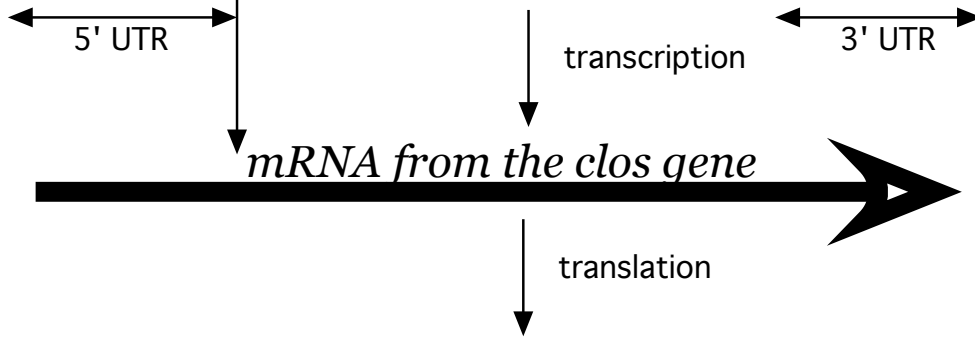
Your name: _____

The mutant form of the gene has single nucleotide change right here.

Activation domain

DNA binding domain

The clos gene



Wild type makes a transcription factor called CLOS.

The mutant makes a CLOS protein that is missing its activation domain but that still has its DNA binding domain.

Based on the translation lectures tell me what has happened to produce the mutant phenotype. Use just the space below for your answer.

The mutation has change the start codon to some other codon OR it has altered the Kozac's sequence so that the ribosome does not recognize it. Now the ribosome is scanning down the mRNA and finds another "AUG" that is useable. The second AUG probably has flanking sequences that resemble the Kozac's sequence. Translation starts here.

Essay question #2 setup

Your name: _____

I want you to describe **2** possible new HIV drugs (make them up). These drugs should NOT belong to the four categories of drugs that are currently in clinical use. Tell me how each drug works. Tell me how and why they interfere with the viral "life cycle". *Don't skip anything*. Use as much space as needed. **You are going to need to use information from all of HIV lectures to do a good job on this one.**

Below are provided just some of the possible answers. Some of my answers are truncated. I expected that you would provide details. You could not mention reverse transcriptase inhibitors (nucleoside or non-nucleoside), protease inhibitors or gp41 inhibitors (Enfuvirtide, T20) because they are in use.

1. Overexpress Tat

- lead to production of more tat
- shorten rev- part of the life cycle
- reduce time for nef to work
- increased CD4 on surface
- increase autolysis

Nef would not have sufficient time to work. Therefore, virus particles would begin to be assembled before the cellular antiviral defenses could be disarmed and before the cell could be prepped for the appearance of many viral proteins. Problems that would not be taken care of would include:

- removal of CD4 from the surface would be impeded
- more autolysis because CD4 would bind to gp120

Without Nef the virus cannot decrease MHC1 complexes on the cell surface

- >> (They do not need to state that this is specifically an inability to down-regulate HLA-A and HLA-B).

- >> cell would be better able to tell the rest of the immune system that it was infected. This results in increased recognition as infected by other immune cells.

The other cells would probably kill the infected cells.

NEF-mediated inhibition of apoptosis would not occur and so the infected cell would be more likely to undergo apoptosis.

No Nef induced release of cytokines which attract other immune cells. (NOT DISCUSSED IN 2008, DON'T TAKE AWAY POINTS IF THEY DON'T MENTION IT.)

Fewer uninfected immune cells would die because NEF could not act through the FAS-L system which can direct uninfected cells to undergo apoptosis. (FAS-L does not need to be mentioned by name since it was not discussed in Spring 2008.)

2. Bind or damage 99 bp flap

- Will prevent the importation into the nucleus of non-dividing cells. Dividing cells will not be protected.

3. Overexpress rev+

shorten rev- part of the life cycle

Nef would not have sufficient time to work. Therefore, virus particles would begin to be assembled before the cellular antiviral defenses could be disarmed and before the cell could be prepped for the appearance of many viral proteins. Problems that would not be taken care of would include:

- removal of CD4 from the surface would be impeded
- more autolysis because CD4 would bind to gp120

Without Nef the virus cannot decrease MHC I complexes on the cell surface

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Fewer uninfected immune cells would die because NEF could not act through the FAS-L system which can direct uninfected cells to undergo apoptosis. (FAS-L does not need to be mentioned by name since it was not discussed in Spring 2008.)

4A. Delete/inhibit/suppress/block/RNAi against CCR5

viruses could not enter macrophages because the CD4 CCR5 combination is used for entry into these cells. This would be effective very, very early in the infection. In fact, once infected it would probably be too late. Probably, this would work prophylactically.

4B. Delete/inhibit/suppress/block/RNAi against CXCR4

Same as 4A except that it would affect T-cells and would not just work prophylactically.

5. Drug that mimics TAR

Tat binds the wrong target and then is not available to activate transcription from the provirus. The processivity of RNAPII from HIV LTR would never improve.

NOT DISCUSSED IN LECTURE BUT NOT INCORRECT---> Also TAT enhances the initiation rate by directly recruiting TBP. This would not happen.

Both events would reduce the number of transcripts produced from the HIV provirus.

6A. Drug that mimics RRE (RRE decoy)

Blocks REV activity.

Prevent transition to REV+. Only 2000 n class of mRNAs get to the cytoplasm and so can only make Tat, Rev & Nef. Cannot make viruses.

6B. NOT DISCUSSED IN 2008. RevM10 is a mutant in REV. Engineer this into a lymphocyte stem cells then replace the stem cell population with the modified variants. RevM10 does

not support Rev enucleation. Will block the action of Rev. Same effects as Drug that mimics RRE.

7. Drug that enhances REV+ activity. Speeds transition to REV+ stage. Not enough time for Nef to work. All of the problems caused by a Nef inhibitor happen albeit less severely.
8. Drug blocks the ribosomal frameshift that is required for translation to pol. No reverse transcriptase, integrase or protease could be made.
9. Nef inhibitor
removal of CD4 from the surface would be impeded
more autolysis

Without Nef the virus cannot decrease MHC I complexes on the cell surface

- >> (They do not need to state that this is specifically an inability to down-regulate HLA-A and HLA-B).
- >> cell would be better able to tell the rest of the immune system that it was infected. This results in increased recognition as infected by other immune cells.

The other cells would probably kill the infected cells.

NEF-mediated inhibition of apoptosis would not occur and so the infected cell would be more likely to undergo apoptosis.

No Nef induced release of cytokines which attract other immune cells. (NOT DISCUSSED IN 2008, DON'T TAKE AWAY POINTS IF THEY DON'T MENTION IT.)

Fewer uninfected immune cells would die because NEF could not act through the FAS-L system which can direct uninfected cells to undergo apoptosis. (FAS-L does not need to be mentioned by name since it was not discussed in Spring 2008.)

10. Block the enzyme that adds the fatty acid to NEF. This would probably reduce Nef activity. All of the problems caused by a Nef inhibitor happen albeit less severely.
11. Drug which blocks the interaction between Gp120 and Gp41.
12. Stimulate CD4 expression so that Nef cannot down-regulate it.
13. Block base-pairing required to form TAR. Would have the same effects as drugs that inhibit TAR. See above.
14. Block RNase H activity.
15. Ribozymes
16. Toxic polyprotein. Engineer this into a lymphocyte stem cells then replace the stem cell population with the modified variants. This might be a component in a suicide cell. Once HIV protease is introduced (cell is infected) the cell kills itself.
17. Create mutant chemokine receptors. CCR5 for macrophages, CXCR4 for T cells. This would prevent the virus from infecting the cells. Engineer this into a lymphocyte stem cells then replace the stem cell population with the modified variants.
18. Stimulate expression of CEM15. Anti retroviral mechanism.
20. Whole virus vaccines. You had to note something about variability etc to get more than 1.8 for this one.
21. Inhibit Vif. This will help CEM15 work better.
22. Injection of something that has some of the binding properties of RANTES. Binds CCR5

- chemokine receptor and would interfere with proper gp120 binding to CD4/CCR5 combination. Would block viral entry into macrophages.
- 22b. Injection of something that has some of the binding properties of SDF-1. Binds CXCR4 chemokine receptor and would interfere with proper gp120 binding to CD4/CXCR4 combination. Would block viral entry into T-helper lymphocytes.
 23. Reverse transcriptase enhancer. This would reduce diversity.
 24. Vif resistant cytidine deaminase (CEM15 substitute)
 25. Cholesterol decreasing genes. Affects capacity of CD4 to bind GP120.
 26. Some sort of LTR endonuclease. Damage the provirus itself. Can't transcribe.
 27. Vpu inhibitor
Prevents the removal of CD4 from the ER. Get increased autolysis. Loose some Gp120 in ER. Cell will not live as long and should produce fewer virions during this time.
 28. Vpr Inhibitor
Prevents virus from entering the nuclei of non-dividing cells.
 29. There are more possibilities. Some of which students presented.

Essay question #3 setup

Your name: _____

Describe how the original PAM250 matrix was made. Include the "philosophy" behind it and include any shortcomings in this "philosophy".

Philosophies in its designs

1) chemical properties and other logical approaches concerning knowledge about amino acids did give one a scoring system that had good predictive value

2) a problem is that amino acids seem to be able to substitute for one another with varying degrees of success.

3) The overarching philosophy is that one can get "evolution" to tell us what is an acceptable change in a protein sequence.

How was it done?

Identify examples of the same protein in different species (or two copies of the same in a single organism). You might have 2 examples of protein A, 3 or protein B, 2 or protein C, etc. The protein A's are aligned to each other, the protein B's are aligned to each other and the protein C's are aligned.

Because these proteins perform the same function they any changes in their amino acid sequence are obviously acceptable to evolution. We know this because the organism is alive.

Now determine the frequency of differences in individual amino acids between each of the proteins. A change that is not acceptable to evolution will be selected against and will be less common. Changes are acceptable will not be selected against or will be weakly selected against and will be more common.

The frequency of changes are tabulated and written down in a table. Then this frequency is expressed (normalized) as a function of 10 million years of evolution over a 100 amino acid window. This is a PAM 1 table and is only useful for very short epochs of evolutionary time.

Usually this is expressed as a logarithm

The frequency of change is also normalized to the frequency of the appearance of the amino acid
 $\log_{10}(\text{freq of change} / \text{freq of amino acid}) * 10$

The frequency that the amino acid stays the same is also measured.

Then the matrix of these values is multiplied by itself over and over to produce the PAM250 table.

-----**RNAi**-----

- 1. The trigger for an RNAi response must be**
 - A) double stranded RNA *
 - B) RNA that base-pairs to the promoter region of a gene
 - C) RNA that can base-pair to a virus genome
- 2. In the RNAi response**
 - A) DICER produce siRNAs from the trigger molecule. These become part of a RISC complex. The RISC complex contains Argonaute and a siRNA. It is this complex that cuts up the mRNA.*
 - B) DICER produce triggers from the siRNA molecule. These become part of a RISC complex. The RISC complex contains RdRp and a siRNA. It is this complex that cuts up the mRNA.
 - C) RISC produces siRNAs from the trigger molecule. These become part of a DICER complex. The DICER complex contains Argonaute and a siRNA. It is this complex that cuts up the mRNA.
 - D) RISC produces siRNAs from the trigger molecule. These become part of a DICER complex. The DICER complex contains R2D2 and a siRNA. It is this complex that cuts up the mRNA.

-----**RNA Editing**-----

Please use the multiple choice to fill in the blanks with the correct answers.

- 3. Choose the best answers for the blanks. A _____ binds intronic sequence and helps to position a _____ so that it can act on a nucleotide in an exon.**
Two blanks two answers per choice
The choices for the blanks are:
 - A) deaminase and guide RNA
 - B) guide RNA and deaminase *
 - C) guide RNA and RNAase H nuclease
 - D) U1 SNRP and deaminase
 - E) U1 SNRP and reductase
- 4. The enzyme will convert _____ it to _____, this latter nucleotide behaves like guanine with respect to base pairing.**
 - A) adenosine, hypoxanthine
 - B) adenosine, inosine *
 - C) cytidine, uracil
 - D) guanine, adenosine,
 - E) thymine, alanine
- 5. The enzyme that produces the change is called _____.** The nucleotide change produced by the enzyme can cause a change in a codon that can cause an amino acid change in the protein. For instance, editing can cause a ACG->GCG codon change and this causes a threonine to alanine change in the protein.
 - A) adenosine deaminase acting on RNA (ADAR) *
 - B) adenosine reductase (AR)
 - C) cytidine reductase (CR)
 - D) RNAase H activity
 - E) uracil deaminase acting on RNA (UDAR)

Translation

6. The Shine-Dalgarno sequence is

- A) AUG
- B) a sequence in a eukaryotic rRNA that interacts with the eIF4 and the 5'-CAP of the mRNA. It is essential for the recognition of mRNAs
- C) a sequence in a prokaryotic rRNA that interacts with the eIF4 and the 5'-CAP of the mRNA. It is essential for the recognition of mRNAs
- D) a sequence in a prokaryotic mRNA preceding the start codon that base pairs with the 3' end of the 16S rRNA *
- E) a sequence in a eukaryotic mRNA preceding the start codon that base pairs with the 3' end of the 16S rRNA

7. A reticulocyte is starved for heme.

- A) HCR is stimulated to phosphorylate eIF2alpha. This makes it impossible for eIF2alpha to release eIF2B which prevents the reuse of eIF2. This represses translation ELONGATION in the reticulocyte.
- B) HCR is stimulated to phosphorylate tRNAiMet. This represses translation ELONGATION in the reticulocyte.
- C) HCR is stimulated to phosphorylate tRNAiMetinitiator. This represses translation INITIATION in the reticulocyte.
- D) HCR is stimulated to phosphorylate eIF2alpha. This makes it impossible for eIF2alpha to release eIF2B which prevents the reuse of eIF2. This represses translation INITIATION in the reticulocyte.

Bioinformatics

8. Fickett's testcode makes use of the fact that

- A) different organisms have distinct codon biases, meaning that protein exons will have this bias but that non-coding regions will not show this bias.
- B) stop codons appear in regions that do not encode proteins while stop codons can be found other DNA.
- C) a protein coding exon should be represented by an entry in a cDNA database
- D) protein encoding exons show a bias of nucleotides at position 1, 2, and 3 while DNA not encoding a protein does not have the same bias of nucleotides at these positions.

This is a dynamic programming matrix. You are comparing two amino acid sequences (X1XXXX and Y1YYYY). X1 refers to the first amino acid of the X protein. Y1 refers to the first amino acid of the Y protein. The # refers to numbers that you write in while solving the matrix. Final matrix would have lots of arrows in it. I HAVE ONLY SHOWN TWO OF THESE ARROWS.

	X1	X2	X3	X4	X5	
	0	-2	-3	-4	-5	-6
Y1	-2	#	#	#	#	#
Y2	-3	#	#	#	#	#
Y3	-4	#	#	#	#	#
Y4	-5	#	#	#	#	#

9. What do these two arrows mean?

- A) These arrows mean that you are inserting spaces in front of the X1 amino acid.
- B) These arrows would mean that you are inserting spaces in front of the "Y1" amino acid. *
- C) These arrows mean that you have decided to align X1 and Y1, and then X2 and Y2.
- D) These arrows meant that you have decided to align X3 with Y1.

10. Which is the correct trace-back for this sequence?

		X1	X2	X3	X4	X5	
	0	-2	-3	-4	-5	-6	A) X1 X2 X3 X4 X5 - Y1 Y2 Y3 Y4
Y1	-2	#	#	#	#	-2	B) X1 X2 X3 X4 X5 Y1 - Y2 Y3 Y4
Y2	-3	#	#	#	#	3	C) X1 X2 X3 X4 X5 - Y1 Y2 - Y3
Y3	-4	#	#	#	#	4	D) X1 X2 X3 X4 X5 - Y1 Y2 Y3 - *
Y4	-5	-4	2	3	3	1	

11. Please calculate the value for cell marked by the star.

30	I	D	I	O	M	A
L	32	30				
E	30	☆				
N						
G						
U						

- A) 3 B) 28 C) 32
D) 35 * E) 92

12. In the matrix above, which way should the arrow go?

- A) left to right horizontal
B) right to left horizontal
C) top to bottom vertical
D) bottom to top vertical
E) diagonal *

13. You are trying to find the protein encoding exons within a large piece of DNA. Which is the best program or algorithm for this endeavor?

- A) A program that uses the PAM250 table
B) Dynamic programming
C) Fickett's testcode *
D) Smith-Waterman local alignment algorithm

14. You are trying to find the protein encoding exons within a large piece of DNA. You have the DNA sequence in your computer. You translate it to look for possible protein coding regions.

- A) There is one possible computer-generated (conceptual) translation.
B) There are two possible computer-generated (conceptual) translations—one forward and one backward translation.
C) There are 3 possible computer-generated (conceptual) translations.
D) There are 6 possible computer-generated (conceptual) translations. *

15. **What is codon bias and what is it used for?**

- A) refers to the "dialect" of codons used by different organisms *
- B) refers to the fact that codons can only sensibly read in one direction
- C) refers to the fact that the G and C nucleotides are more common in codons
- D) refers to the position parameter part of the Fickett's testcode algorithm

16. **In Fickett's Testcode which is the A position parameter and which is the A content parameter?**

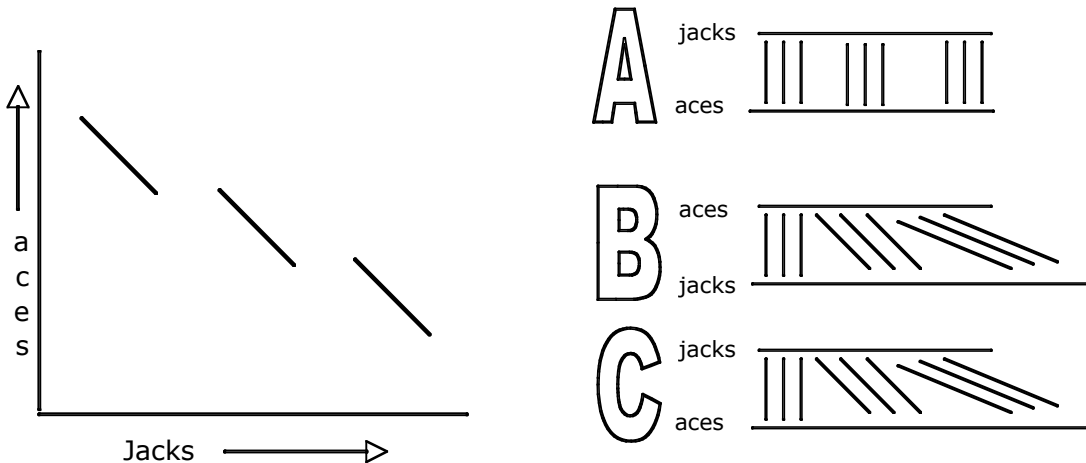
- 1) $\frac{\max(\#Apos1, \#Apos2, \#Apos3)^*}{\min(\#Apos1, \#Apos2, \#Apos3)+1}$
- 2) $\frac{\min(\#Apos1, \#Apos2, \#Apos3)}{\max(\#Apos1, \#Apos2, \#Apos3)+1}$
- 3) % of A's in the sequence
- 4) % of A's in position 3

- A) # 1 is the A position parameter and #4 is the A content parameter
- B) #1 is the A position parameter and #3 is the A content parameter *
- C) #2 is the A position parameter and #4 is the A content parameter
- D) #3 is the A position parameter and #1 is the A content parameter
- E) #4 is the A position parameter and #2 is the A content parameter

17. **The Fickett's testcode algorithm Which is the BEST answer?**

- a. can identify exons in genomic DNA
- b. can identify exons transcribed by RNA polymerase II in forward reading frames but not if the sequence is presented in the backward reading frame
- c. can identify protein-encoding exons regardless of their reading frame
- d. can identify transcribed regions of genomic DNA

18. This plot on the right is a dotplot of two sequences. One sequence is called aces, the other is called Jacks. In A, B & C. the horizontal lines represent these sequences. The lines between the horizontals shows us which areas are similar between the two. Which of these three could have produced the dotplot? **Correct Answer is B.**



19. **In order to detect functionally conserved protein domains between distantly related proteins, it would be best to use a**

- A) Fickett's testcode algorithm
- B) global alignment algorithm
- C) local alignment algorithm

HIV

20. **Select the true statement concerning HIV.**
- A. HIV is a haploid retrovirus
 - B. Reverse transcription uses the host's DNA polymerase
 - C. *Reverse transcription occurs immediately after the virus first enters the cell
 - D. Reverse transcription occurs in the nucleus
21. **During the process of reverse transcription**
- A) an RNAi response against host cell mRNAs weakens the cell's defenses.
 - B) genetic recombination between viral genomes occur *
 - C) new viral genomes are made that are encapsulated into infectious virus particles
22. **Which statement is INCORRECT concerning HIV?**
- A) Reverse transcription uses partially degraded HIV RNA genome as a primer during DNA synthesis.
 - B) Reverse transcription uses a random DNA primer to begin cDNA synthesis. *
 - C) Reverse transcription uses a tRNA as a primer to begin cDNA synthesis.
 - D) Complete reverse transcription requires RNase H activity.
23. **Which statement is INCORRECT concerning CCR5**
- A. found on macrophages
 - B. helps to transport the virus DNA into the nucleus *
 - C. one mutant allele of the CCR5 gene is an aids resistance gene (ARG)
 - D. with CD4 interacts with gp120
24. **What year was HIV first isolated?**
- A) 1981 B) 1983 * C) 1993
25. **In 2003 how many people are estimated to have HIV in the world (rounded).**
- A) 900,000 B) 38 million * C) 100 million
26. **In Dr. Atkinson's opinion, what is the most convincing evidence that HIV causes AIDS?**
- A) Drugs designed to specifically interfere with the viral life cycle suppress AIDS symptoms and extend human life. *
 - B) Recipients of contaminated blood subsequently develop AIDS.
 - C) Because of experiments performed in the laboratory of Kary Mullis.
27. **The recombination that is important for generating HIV diversity occurs**
- A) between different proviruses in the nucleus.
 - B) between RNA molecules in the virus particle.
 - C) during reverse transcription of the viral genome. It occurs in the cytoplasm.
28. **One proteins binds CD4 and then changes shape and binds a chemokine receptor. Then the other protein causes a fusion between the outer membrane around HIV and the cell membrane.** What TWO proteins am I describing.
- A) env and pol B) rev and tat C) gp120 and gp41 * D) vif and vpu
29. **What are AIDS resistance genes (ARGs)?**
- A) HIV genes whose products defend the against defenses of the host cell.
 - B) Host cell genes whose job is to defend against viral attack.
 - C) These are genes belonging to the cell. The encoded protein participates in some normal process in the healthy cell. Natural variants in the gene interferes with viral life cycle. *

30. **An HIV virus variant appears that has fantastically high levels of REV expression. Which is more likely:**
- A) The virus is quickly cleared by the infected host. *
 - B) The virus replicates more rapidly and kills the host more quickly.
31. **Nef**
- A. can block the action of Rev
 - B. can block the action of Tat
 - C. can cause CD4 to persist on the cell surface
 - D. can remove CD4 from the cell surface. *
 - E. is only made late in the infection of the cell
32. **How does HIV kill cells?**
- A) Autofusion, syncytial cell formation and apoptosis *
 - B) necrosis, autofusion and a programmed lysis event
 - C) programmed cell lysis
 - D) It does not. Infected cells are always killed by killer macrophages.
33. **HIV recombination**
- A. may have originated as a side-effect of a mechanism that allows the virus to reverse transcribe nicked or damaged genomes. *
 - B. occurs at a low frequency
 - C. usually occurs between viruses that have the same sequence
34. **Which enzyme activities are involved in HIV recombination?**
- A. env + nef
 - B. nef + vpr
 - C. rev + tar
 - D) reverse transcriptase + RNAase H activity *
 - E) vif + vpr
35. **What does TAT bind?**
- A) An RNA stem loop called TAR *
 - B) An DNA sequence called TAR
 - C) A sequence called REV
 - D) CD4
36. **Without this protein the RNA polymerase II that transcribes HIV provirus tends to terminate transcription prematurely. With this protein the RNA polymerase tends to complete transcription of the provirus.**
- A) NEF
 - B) REV
 - C) TAT*
 - D) VIF
 - E) VIP
37. **During reverse transcription a mutation breaks the rev gene making it non-functional. No active rev is produced.**
- A) This virus only makes tat, nef and non-functional rev *
 - B) This virus only makes tat, nef, and env
 - C) This virus only makes tat and pol
38. **Maturation of pol polyprotein produces**
- A) HIV reverse transcriptase, RNAase H activity, protease and integrase *
 - B) HIV gp120 and gp41
 - C) HIV p7, p17 and P24

39. **The so-called Rev minus part of the HIV life-cycle provides time for what to occur?**
A) for NFkB to stimulate transcription from the HIV promoter
B) for the tat protein to activate CD4 expression
C) Nef to disarm the cell *
D) synthesis of viral coat proteins that are needed for the production of complete viruses.
40. **How many transcriptional promoters does HIV have?**
A) one* B) four C) nine D) twelve
E) none-it uses a promoter from one of the host's genes.
41. **Inhibition of HIV protease prevents**
A) the processing of the vif and vpr proteins
B) the processing of the tat, nef and rev proteins
C) the processing of the pol, env and gag polyproteins. *

Poster questions

42. **This sequence, YSPTSPS, is the repetitive amino acid sequence found in the CTD of RNA polymerase II. What happens if you mix this peptide into an in vitro splicing reaction?**
A. Splicing switches from exon definition to intron definition.
B. Splicing occurs at a much higher rate.
C. RNA polIII mRNA can no longer be transcribed.
D. Splicing is inhibited. *
43. **This question has to do with the Weaver et al 2007 DNA methylation paper. These investigators described two sites that were important for interaction of the GR promoter with NGFI-A. These sites are called the 5' site and a 3' site. What was important about the 3' site?**
A. Regardless of the methylation status, that particular base had to be a C or NGFI-A binding was abolished. *
B. When the 5' site was also methylated mother rats would engage in licking and grooming (LG) behavior.
C. It was the first time that methylation of DNA had been observed in scientific literature.
D. When the authors tried an experiment, that piece of DNA could not pull down proteins when attached to a bead unless it was methylated.

Mutation Data Matrix (250 PAMs)

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	2																			
R	-2	6																		
N	0	0	2																	
D	0	-1	2	4																
C	-2	-4	-4	-5	12															
Q	0	1	1	2	-5	4														
E	0	-1	1	3	-5	2	4													
G	1	-3	0	1	-3	-1	0	5												
H	-1	2	2	1	-3	3	1	-2	6											
I	-1	-2	-2	-2	-2	-2	-2	-3	-2	5										
L	-2	-3	-3	-4	-6	-2	-3	-4	-2	2	6									
K	-1	3	1	0	-5	1	0	-2	0	-2	-3	5								
M	-1	0	-2	-3	-5	-1	-2	-3	-2	2	4	0	6							
F	-4	-4	-4	-6	-4	-5	-5	-5	-2	1	2	-5	0	9						
P	1	0	-1	-1	-3	0	-1	-1	0	-2	-3	-1	-2	-5	6					
S	1	0	1	0	0	-1	0	1	-1	-1	-3	0	-2	-3	1	2				
T	1	-1	0	0	-2	-1	0	0	-1	0	-2	0	-1	-3	0	1	3			
W	-6	2	-4	-7	-8	-5	-7	-7	-3	-5	-2	-3	-4	0	-6	-2	-5	17		
Y	-3	-4	-2	-4	0	-4	-4	-5	0	-1	-1	-4	-2	7	-5	-3	-3	0	10	
V	0	-2	-2	-2	-2	-2	-2	-1	-2	4	2	-2	2	-1	-1	-1	0	-6	-2	4