GENETICS 603

FALL 2005 Exam 1 Name_____

I. A small double stranded (ds) DNA virus called SP8 was used by Marmur and Greenspan (1963) to test a basic concept of genetics. A unique feature of SP8 is that one strand of the virus DNA is purine rich and the other is pyrimidine rich, meaning the 2 strands also differ in density.

A) Which bases are found in excess in the pyrimidine-rich strand? C and T

After isolating pure ds-virus DNA, key steps were 1) to convert double strands to single strands and 2) to collect the single strands in separate test tubes.
B) Suggest how these steps could be accomplished.

1) Heating will break the hydrogen bonds holding the 2 strands together

2) Density gradient centrifugation, as in the expt proving semi conservative replication

C) The next step was to isolate labeled mRNA which was done soon after SP8 was allowed to infect its host, *Bacillus subtilis*.

1) Suggest a method for labeling the mRNA. Grow in the presence of H3 or C14 radiolabelled Uracil for specific labeling of SP8 mRNA (just adding any label to the growing bacteria might work so was accepted)

D) When the labeled RNA was hybridized to the separated strands of DNA, hybrids formed only with the pyrimidine rich strand. What were Marmur and Greenspan able to conclude about transcription in SP8?

That only that one strand is a template for all messages made by SP8

II. How many "phosphates" does it take to make one peptide bond? Show or describe the steps involved. 4

2 from ATP for forming each aminoacyl-tRNA "high energy" bond
1 from GTP for getting each aa-tRNA onto the A site
1 from GTP for moving the mRNA etc after the peptide bond forms

III. Make list of differences in the synthesis of mRNA in eukaryotes and prokaryotes.

KEY ITEMS SOUGHT:

5' inverted GTP cap added only in making eukaryotic mRNAs
poly A tail at 3' end added only in making eukaryotic mRNAs
introns must be removed to make a functional mRNA only in eukaryotes
different sets of initiation factors required in each system
Other features that could be included
Different distance from TATA signal
Prokaryotic messages include Shine-Delgarno sequence for ribosome loading
Eukaryotic messages must leave nucleus before translation

IV. A) As DNA sequence data becomes available for many organisms, a trend is to find fewer 5'-CG-3' dinucleotides than one would expect due to "chance". How often would CG (or any dinucleotide) be expected to occur? (1/4) X (1/4) = 1 in 16 adjacent bases

Give a likely explanation as to why there are less. C next to G is often methylated and when 5MG is deaminated, this creates a T opposite a G. Since both strands have methylation at other sites, and only normal bases are present, repair may change the T:G base pair to T:A rather than C:G

What dinucleotide(s) would you predict to find in excess? Why?
TG since that is what you get when a 5MC next to a G gets changed to a T.

B) The C in a CG base pair in a mutant allele (kan^S) of an *E. coli* gene is methylated *in vivo* in strain B. Functional copies of the gene (kan^R) deactivate the antibiotic kanamycin; this occurs when the CG is mutated to TA.
In VSR^- strains, the rate of kan^S *to* kan^R mutation is 1.3×10^{-7} while in VSR^+ strains the rate of kan^S *to* kan^R mutation is 3×10^{-9}, the same as in strains where the C is not methylated. Propose a function for the VSR^+ gene.

VSR^+ appears to prevent 5MT from being deaminated or to repair T:G base pairs in favor of the G, that is back to C:G. It prevents the problem described above.
(VSR stands for "very short repair")

V. A mutation in the ICP gene of herpes simplex virus has been reported that prevents successful reproduction in host cells, even though antibody made against ICP27 can detect the presence of protein. Three revertants were found, one of which had the normal sequence with arg at position 440. A slow growing revertant had met at 440 and a rapid growing revertant had lys at this position.

Use the genetic code to give two possible mutation scenarios that will explain the sequence of events; also tell what type of mutation (DNA level) is involved in each step.

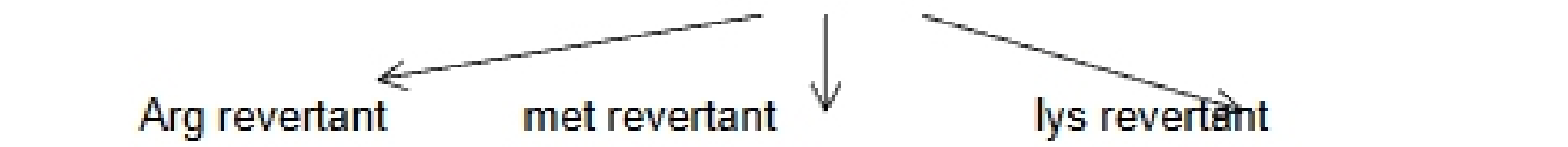
Explanation 1: (All must be single base substitutions to account for given information)
(For "silent" = samesense, start with AGA and CGA- others may work too!)

Original codon AGG mutant: codon ACG type: tranversion

Arg revertant	met revertant	lys revertant
Codon AGG	AUG	AAG
Type tranversion	transition	tranversion

Explanation 2:

Original codon AGA mutant: codon AUA type tranversion



Arg revertant	met revertant	lys revertant
Codon AGA	AUG	AAA
Type tranversion	transition	tranversion

VI. Which of the following 3 mutagens belongs to the classes listed, causes the effect(s) listed and would be expected to revert mutants of the given type: 1) EMS, 2) ethidium bromide, 3) 5BU

Type	Effect	Could Revert
Intercolating agent	transitions only	TA to AT
ethidium	5BU	EMS
Alkylating agent	+/- base pair	AT to GC
EMS	ethidium	5BU & EMS
Base analog	Transition&transversion	frameshift
5BU	EMS	ethidium