

May 6, 2005

Name _____

ID number _____

CH462/562 Final Exam

There are **5** short answer questions worth **6** points each.
You must answer **4** of them.

There is **1** essay question worth **36** points.

There are **4** problems worth **24** points each.

If you are a CH462 student, you must answer at least **3** problems.

(If you do all 4, I will give you 2/3 credit on the problem on which you did poorest.)

If you are a CH562 student, you must answer all **4** problems.

The maximum amount of points is thus **132** (CH462) or **156** (CH562).

You are advised to look over the problems first before starting to work them. Time should not be a factor, if you are well prepared.

Short answer:		/24
Essay:		/36
Problems:	1	/24
	2	/24
	3	/24
	4	/24
Total		

No notes or books of any sort may be used during the exam.

I have neither given nor received aid on this exam.

(signature)

Given:

1) The genetic code

First letter of codon (5' end)

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

- 3) Show the reaction(s) catalyzed by aminoacyl-tRNA synthetases. Explain how they are able to discriminate between very similar amino acids.
- 4) Explain briefly why it is true to say that "the default transcriptional state of a eukaryotic gene is OFF." How is transcription activated in eukaryotes?

5) **Matching**

Match each enzyme process on the left with a polymerase activity on the right. If the process involves more than one activity, then indicate the *major* one. (Note: “dependent” in this context means “templated by”)

Enzyme or process	Activity
___ telomerase	(A) DNA-dependent DNA polymerase
___ replication of bacterial genome	
___ transcription	(B) DNA-dependent RNA polymerase
___ primase	
___ replication of eukaryotic chromosome	(C) RNA-dependent DNA polymerase
___ reverse transcriptase of HIV	
___ replication of influenza virus* transcription from influenza genome*	(D) RNA-dependent RNA polymerase
___ general recombination (to repair a double-stranded break)	

*Influenza is an RNA virus that does not go through a DNA intermediate.

Essay question (36 points)

Explain the Central Dogma of molecular biology.

- a) Draw out the traditional depiction of the Central Dogma showing the flow of information between the 3 macromolecules (DNA, RNA, polypeptide). Explain what each of the 3 arrows means: what process does it describe and which proteins/enzymes are involved?

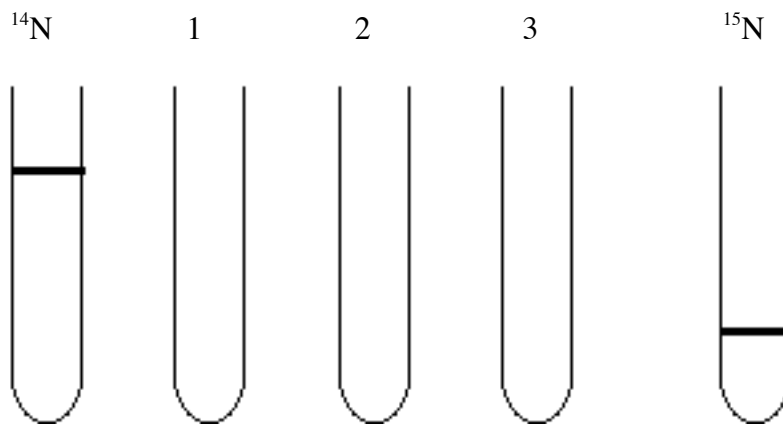
- b) Write out one paragraph as if you were explaining the Central Dogma to someone without a scientific background.

- c) Using the *lac* operon as a paradigm, discuss how a genetic regulatory network can be set up using proteins and the specific arrangement of DNA sequences.

Problems (24 points each)

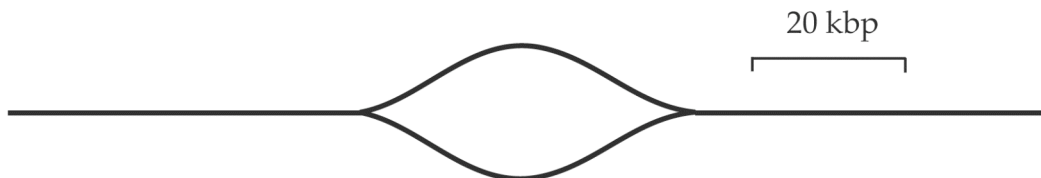
Problem 1. DNA replication in a prokaryote

- a) You extract DNA from *E. coli* cells that have been growing in normal medium (containing ^{14}N nitrogen source) and run it on a CsCl gradient, where DNA will migrate and form a band according to its density. You obtain the result shown below (" ^{14}N "). You then shift the cells into medium containing $^{15}\text{NH}_4\text{Cl}$ as their only source of nitrogen. After 1 generation time (the time in which the cells double once), you extract DNA again. You do the same after the second and third generation time. After they have doubled many times in ^{15}N , you get the last result shown (" ^{15}N "). **Show what you would expect to see in the first 3 generations after the shift to ^{15}N .**



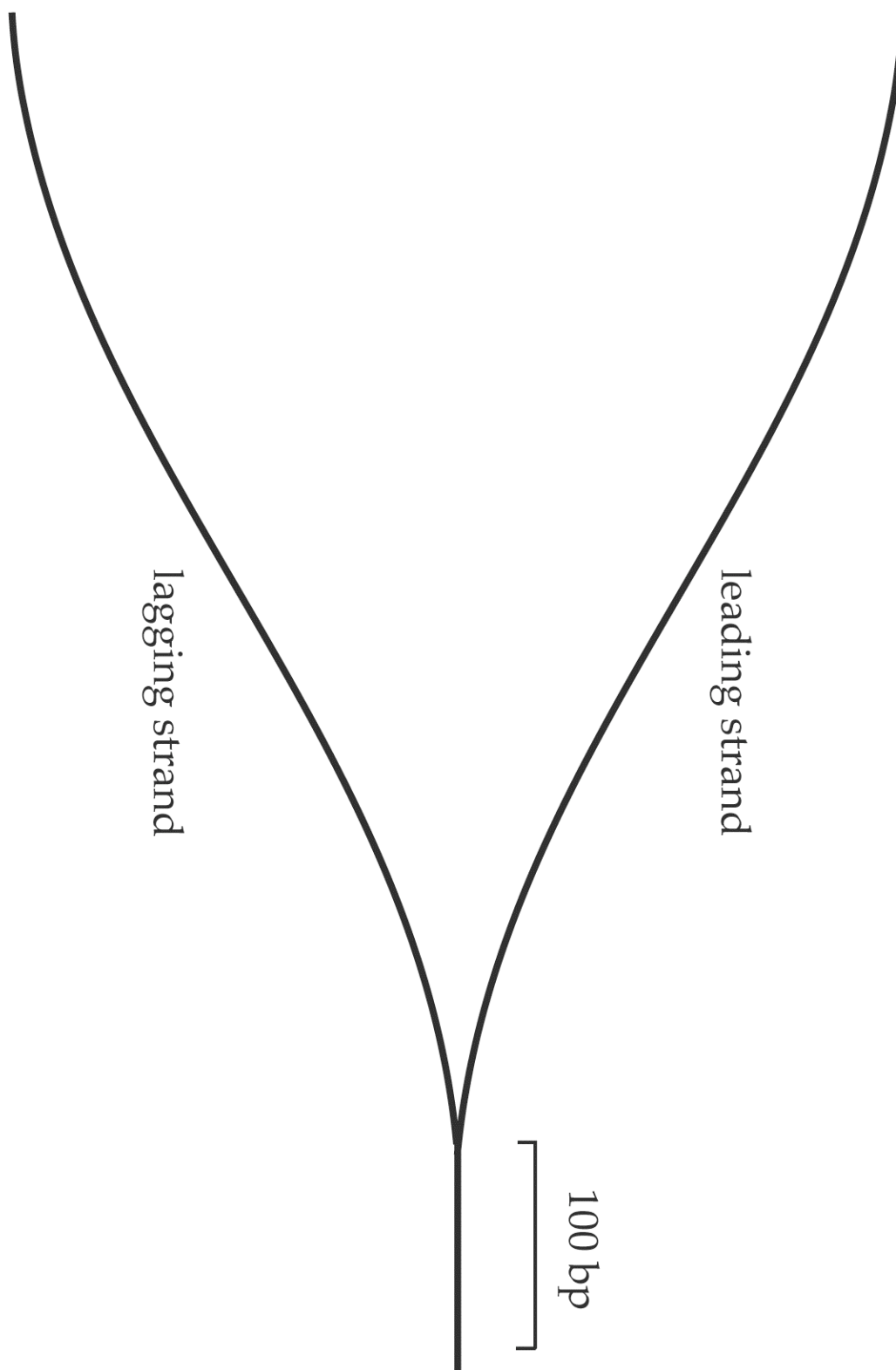
- b) You add [^{14}C]-thymine to *E. coli* cells, which take it up, incorporate it into dTTP, and then use it for DNA synthesis. After a few minutes, you gently extract DNA and spread it onto an electron microscope grid and perform autoradiography. **Show where you would expect to see labeling in the picture below.**

Question: Why is isotopically labeled *thymine* the best choice over all other nucleobases?



Explain briefly how the replication is initiated at the *oriC* origin of replication in *E. coli*. (Use the back of the page, if necessary.)

- c) Here is what a close-up of a replication fork might look like. **Label where you would expect to see the following proteins:** DNA pol I, DNA pol III (you do not need to indicate dimeric state), helicase, topoisomerase, primase, ligase, SSB. **For each one, write a brief description (~1 phrase) of its role.**



Problem 2. Transcription and translation in a eukaryote

Consider the following DNA sequence, which is part of a gene encoding a cytochrome P450 enzyme:

AAATCACGCGCGTTTTTAACCAACCAATCGAAATCGGCAAATCC

AAATCAAATGCGCTTACCGAGATAGGGTTGAGTCGCGCGCAGT

CAAGAGTCCCAATTTAAAGAACGTGGACTCCAACGTCAAAGGGC

CCGTCTATATGCGCTTAGATGGCCCACTACTTAGATAACACCCT

TTTTTGGGGTCGAGGTGCCGTAAAGCACCAATCGGAACCCTAAA

TATAAATTTAGAGCTTGACGGGGAAAGCCGGCTCACACTGGCGAG

GAAAGCATGAGTAAAGTATACGATTGGTTTGAAGAACGTTTAGAAA

TAGCAATTGCTGATGATATTACAAGTAAATATGTTCCACCACACGT

It is known that transcription of this gene requires several transcription factors:

Factor	Enhancer sequence bound
CTF1	CCAAT
SP1	CGCGCG
Fite4	ATGCGCTTA
STF-2	TTANNTAA (N = any base)

The consensus for the start site of transcription (Inr) is:

YYAN(A/T)YY (Y = pyrimidine, R = purine, N = any)

It is usually found ~30 nucleotides downstream of the TATA box.

a) Label the sequence above with the following:

- 1) Inr (initiator)
- 2) TATA box
- 3) Any of the enhancers above

- b) Now write out the first 50 bases of the RNA transcript**
(the sequence above is the coding strand, not the template strand):

Then draw out the chemical structure of the first 3 bases, including the 7-meG cap:

- c) Go back to your RNA sequence and find the translation start site and label it.**

Translate the first 10 amino acids and write out their sequence below (use full name, 3-letter code, or 1-letter code):

Now draw out the chemical structure of the first 6 residues of the polypeptide:

d) When the polypeptide is fully translated into cytochrome P-450, it will be inserted into the endoplasmic reticulum (ER) membrane by the same machinery that translocates proteins destined for secretion.

1. Briefly describe how this is carried out.

2. Also describe very briefly how the protein is kept in the ER.

3. Finally, describe briefly the function of cytochrome P-450.

Problem 3. Translational cycle in a prokaryote

At each stage, indicate the steps involved in prokaryotic translation.
Please draw a picture.

- a) **Initiation** (Go through steps to build up an initiation complex):
Start with: mRNA, 30S ribosomal subunit, 50S ribosomal subunit, mRNA, fMet-tRNA^{fMet}, IF-1, IF-2, IF-3, GTP

b) Elongation

Show 1 round of elongation.

Be sure to indicate where proofreading takes place.

Start with: 70S initiation complex, amino acyl-tRNAs,
EF-Tu, EF-Ts, EF-G, GTP

c) Termination

Show how termination is achieved upon entry of a stop codon into the A site.

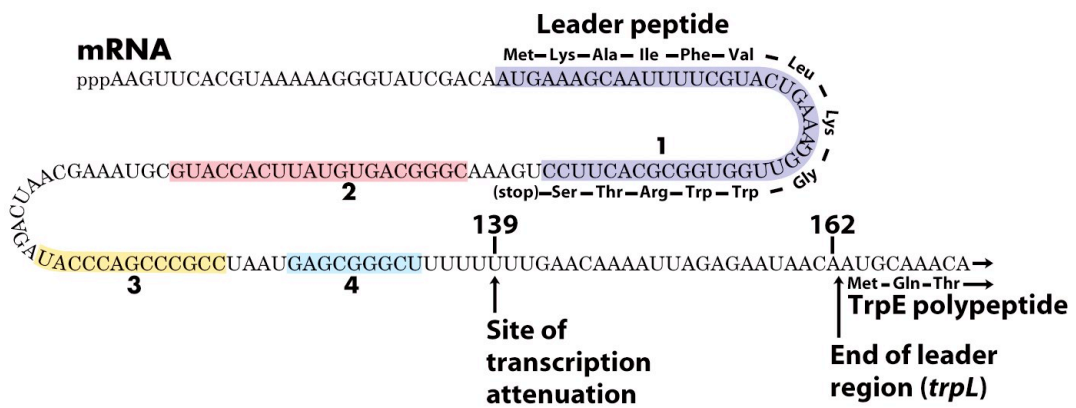
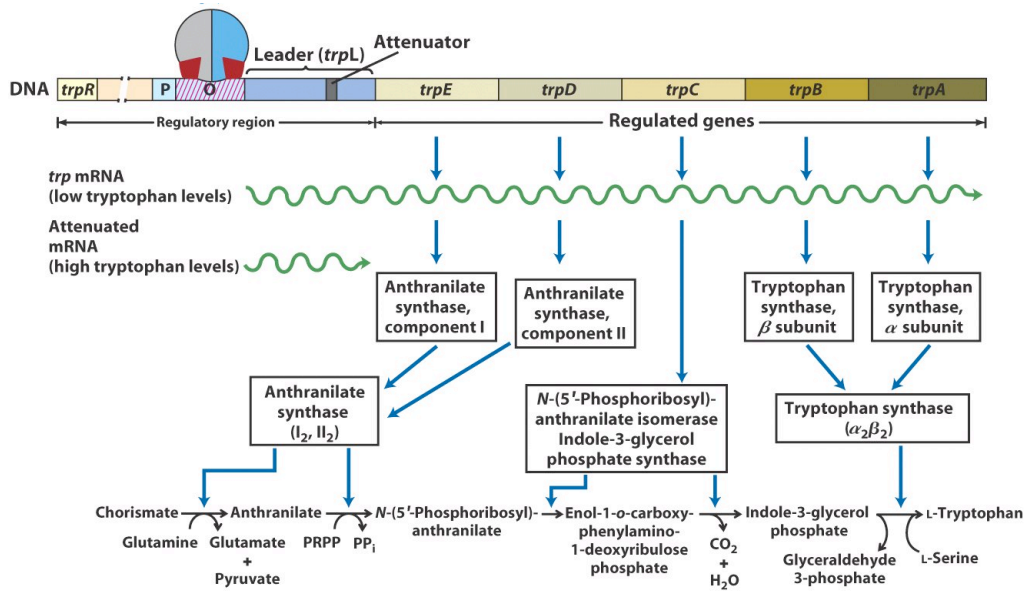
Factors: RF-1, RF-2, RF-3

Problem 4. Regulation of expression in prokaryotes and eukaryotes

- a) Using what you know about how the *lac* operon is regulated, predict the effect of the following perturbations upon production of β -galactosidase, which is encoded by *lacZ* (one of the structural genes of the *lac* operon). Use the following symbols:
 - (no transcription), +/- (weak transcription), + (strong transcription)

Presence of glucose	+	+	-	-
Presence of lactose	-	+	-	+
normal				
In a <i>lacI</i> mutant				
In a <i>lacI^s</i> mutant that makes a lac repressor that cannot bind allolactose				
A <i>lacO^c</i> mutant that cannot bind repressor				
In a mutant with a defective CRP/CAP protein				
A CRP mutant with a much higher affinity for cAMP				
In a mutant with a defective adenylyl cyclase				
In a mutant with a defective cyclic nucleotide phosphodiesterase (catalyzes hydrolysis of cAMP)				
In a mutant having both the <i>lacO^c</i> and the <i>lacZ</i> mutations on the chromosome and possessing a plasmid with a normal <i>lac</i> operon on it. Genotype = <i>lacI lacO^c lacZ</i> [<i>lacO lacZ</i>] (Brackets usually indicate genes not on the chromosome.)				
In a mutant having both the <i>lacI^s</i> and the <i>lacZ</i> mutations on the chromosome and possessing a plasmid with a normal <i>lac</i> operon on it. Genotype = <i>lacI^s lacO lacZ</i> [<i>lacO lacZ</i>]				

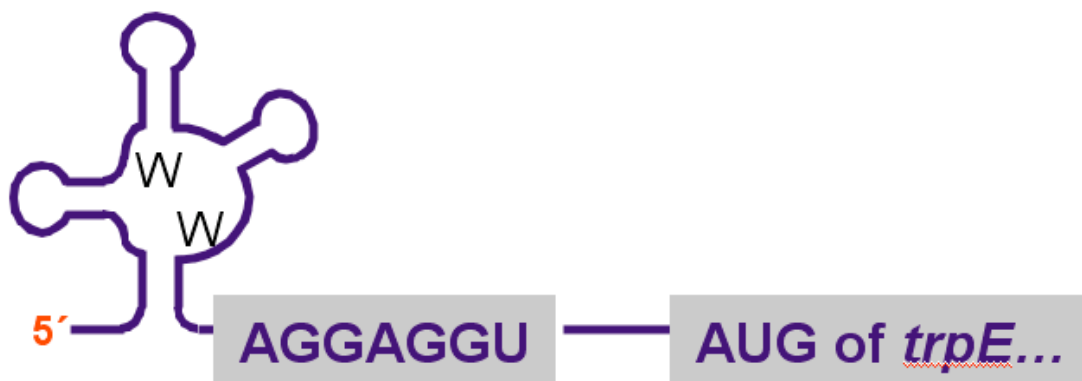
b)



A schematic model of the 5' end of the *trp* operon is shown on the preceding page. Based on your understanding of how the *trp* operon is regulated, predict the effects of the following perturbations. In each case, you will measure the level of TrpE protein (the first polypeptide of the operon) while the cells are being grown under either high or low external tryptophan. The normal situation is already filled in for you. Use the following symbols:
 - (no transcription), +/- (weak transcription), + (strong transcription)

Condition	Expression of <i>trpE</i>	
	High Trp	Low Trp
Normal	-	+
Mutation of Trp repressor that deletes its helix-turn-helix motif		
Mutation of tRNA ^{Trp} -Trp synthetase that raises its K_M for tRNA ^{Trp}		
Introduction of a second tRNA ^{Trp} that is modified in the acceptor stem such it is recognized and charged by the tRNA ^{Gly} -Gly synthetase		
Deletion of the tandem Trp codons are deleted from leader sequence 1		
Replacement of leader sequence 2 with 20 bases of random sequence		
poly-T tract following sequence 4 in the leader is converted to poly-G		

- c) In another species of bacterium, *S. witchii*, the regulation of the *trp* operon is achieved by a different mechanism. Expression of the operon is regulated by the presence of tryptophan at the level of translation. In the 5' UTR (untranslated region) of the mRNA are several sequences that can form RNA hairpins that interact to form a complicated 3-dimensional structure (see picture below). This structure is right next to the Shine-Dalgarno sequence and is thought to occlude it, blocking translation. The structure, which is called a *riboswitch*, is stabilized by binding to at least two tryptophans (“W” in the picture). In the absence of tryptophan, the structure falls apart, and translation is allowed.



Given all this, predict the effects of the following perturbations. In each case, you will measure the level of TrpE protein (the first polypeptide of the operon) while the cells are being grown under either high or low external tryptophan. The normal situation is already filled in for you. Use the following symbols:

- (no transcription), +/- (weak transcription), + (strong transcription)

Condition	Expression of <i>trpE</i>	
	High Trp	Low Trp
Normal	-	+
Deletion of the riboswitch, leaving the Shine-Dalgarno sequence intact		
Deletion of the Shine-Dalgarno sequence		
Mutation of the riboswitch at the bases that contribute to the Trp-binding site to lower their affinity for Trp		
Insertion of 100 bases between the riboswitch and the Shine-Dalgarno sequence		

d) Using what you know about how the *GAL* genes are regulated in yeast, predict the effect of the following perturbations upon transcription of the *GAL* genes.

Use the following symbols: - (no transcription), + (strong transcription), +/- (weak transcription)

Presence of galactose	-	+
Normal situation	-	+
Mutation that deletes the <i>GAL4</i> gene (<i>gal4Δ</i>)		
Mutation that deletes the <i>GAL80</i> gene (<i>gal80Δ</i>)		
Mutation that deletes the <i>GAL3</i> gene (<i>gal3Δ</i>)		
Mutation of the acidic sequence in Gal4 to convert many of the Asp/Glu residues to Asn/Gln		
Double mutant: <i>gal4Δ gal80Δ</i>		
Double mutant: <i>gal3Δ gal80Δ</i>		