MCB110
FINAL

Dec 13, 2005

_____.
Your name and student ID

QUESTION	POINTS
1 (25 points)	
2 (10 points)	
3 (15 points)	
4 (10 points)	
5 (20 points)	
6 (20 points)	
7 (15 points)	
8 (20 points)	
9 (25 points)	
10 (40 points)	
11 (30 points)	
12 (20 points)	
13 (14 points)	
14 (21 points)	
15 (9 points)	
16 (6 points)	

TOTAL (300 points)

WARNING: Your exam will be taken apart and each question graded separately. Therefore, if you do not put your name and ID# on every page or if you write an answer for one question on the backside of a page for a different question you are in danger of irreversibly LOSING POINTS!

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Constants that you may need in the exam: R = 0.025 kcal/(mol . degree centigrade); N_A = 6.02 10^{23} mol^{-1}; F = 23.06 kcal/mol.V

1. – Indicate true of false for the following statements (25 points, 5 each):

(a) Lipids rafts are regions in biological membrane where the fluidity of lipids is reduced
(b) Lipids rafts are enriched in sphingolipids
(c) Lipids rafts are enriched in cholesterol
(d) Lipids rafts are enriched in GPI-anchored proteins
(e) Lipids rafts diffuse on the lipid bilayer

All true

2. - Which are the two main protein folds for the transmembrane region of integral membrane proteins? (5 points) Which one of them can be predicted from hydropathy plots and why? (5 points)

The two main transmembrane domain structures are all alpha helical, or beta barrels. In the first case the fold requires stretches of 20 or more hydrophobic residues that can be detected in hydropathy plots. In the second hydrophobic and hydrophilic residues alternate, a pattern that will not be detected by hydrophathy plots, in which the average hydrophobic character around a given residue is calculated.

3. – You are studying a protein that resides in the smooth ER and have created a GFP fusion to visualize it by fluorescence microscopy in living cells. What technique will you use to determine if the protein is able to diffuse in the membrane of the ER? (5 points) What results would you expect if the protein were fully mobile or unable to diffuse at all? (10 points).

I would use FRAP (fluorescence recovery after photo-bleaching). If the protein were totally mobile I would expect close to total recovery of the bleach area. It totally unable to diffuse the bleached area will remain dark (no recovery).

(And if a student chooses SPT as the technique, will you take that as a valid answer? NO, the whole point is that they created a GFP!)

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4. – The concentration of Cl^- ions in and out of a cell is 10 and 100 mM, respectively (maintained by an ATPase pump). In which direction will Cl^- ions move when Cl^- channels open? Show how you came to that conclusion (15 points).

The ΔG for movement of a solute into the cell is given by

$$\Delta G = 2.3RT \log_{10} \frac{[C_i]}{[C_o]} + zF\Delta E$$

with ΔE=-0.07 V

$$\Delta G = (1.4\,kcal/mol) \log \frac{10}{100} + (-1)(23.06\,kcal/mol.V)(-0.07\,V)$$

$$= -1.4 + 1.7 = 0.3\,kcal/mol$$

It moves out of the cell

5. – Indicated YES or NO in the grid below (16 points). You can make small clarifying comments if you think they are necessary.

Property	**Channel**	**Facilitative Transporter**	**Active ATPase Transporter**	**Cotransporter**
Movement down a concentration gradient	Yes	Yes	No	Yes, for one of the substances
Conformational change accompanying transport	No	Yes	Yes	Yes
Requires ATP	No	No	Yes	No
Requires a preexisting gradient	Yes	Yes	No	Yes

Why do you think that transport through a channel is faster than through a facilitative transporter? (4 points)

Because it does not involve a protein conformational change

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6.- Define the role of SRP in cotranslational translocation. In your answer refer to what it recognizes, how it affects translation, and what role GTP has in targeting (20 points).

The SRP binds signal sequences in nascent polypeptide chains at the exit channel in the ribosome and stall translation by blocking the biding site of elongation factors and tRNAs. Binding to the ribosome results in the exchange of GDP for GTP in the SRP. This GTP-bound particle now is able to bind to its GTP-bound receptor in the cytosolic surface of the rough ER. This in turn brings the stalled ribosome in close proximity to the translocon. Interaction of the signal sequence open the translocon and results in the hydrolysis of GTP in both the SRP and its receptor, which detach from the each other and the ribosome. Translation then resumes simultaneously with the translocation of the polypeptide chain into the ER through the translocon.

(Could you assign points to each portions of the answer to facilitate the grading process? I rather not, as they may use different sentences in different order and me giving points for each of my sentences may biased graders against a more flexible answer. Please use your discretion…)

7. – Deduce the cytosolic or extra cellular location of the N- and C-terminus for the following integral membrane proteins (draw it schematically) (15 points) :

Example: A protein with a signal sequence and a single transmembrane segment?
N-terminus – Extra cellular
C-terminus – Cytosolic

a) A protein with a signal sequence and two transmembrane segments?

b) A protein with an internal signal-anchor sequence (far from the N-terminus) (SAII)?

c) A protein with an SAII and one internal stop-transfer anchor sequence (STA)?

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8.- Describe the function of GEFs and GAPs in G-protein activity (10 points). Can you think of the GEF for the α subunit of trimeric G proteins? And for the small G protein Ras? (10 points)

GEFs (guanosine exchange factors) change the conformation of the GDP-bound G protein resulting in a loss of affinity for GDP. GTP, more abundant in the cell, readily binds to the empty site resulting in G protein activation.
GAPs (GTPase activating proteins) catalyze hydrolysis of GTP by their corresponding G protein, resulting in their inactivation.
G protein-couple receptors (GPCR) acts as GEFs for their corresponding α G protein.
Sos is the GEF for Ras

9.- What signaling events are needed for the activation of protein kinase C? (15 points)

Receptors, likely RTKs, will activate kinases specific for PI (phosphatidyl inositol) and create PIP2. Activation of PLC (phospholipase C) by RTKs or GPCRs will result in the breakdown of PIP2 into DAG and IP3. IP3 will diffuse in the cytosol and bind to the IP3 receptor (or IP3-gated Ca++ channel), opening it. This in turn results in the influx of Ca++ into the cytosol from the ER. Ca++ will then bind to inactive PKC (protein kinase C) and bringing it to the plasma membrane, where interaction with DAG activates its kinase activity.

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10. – Indicate whether or not the following mutations will result in an overproliferative phenotype in the cell. Explain your answer (40 points):

a) A mutation in growth hormone RTK that results in constitutive dimerization and kinase activity

Yes, this is equivalent to maintaining the pathway constantly activated, even in the absence of growth hormones

b) A mutation that inhibits nucleotide exchange in Ras

No, this would actually shut down the pathway inhibiting proliferation

c) A mutation that inhibits GTP hydrolysis in Ras

Yes, this is a classical oncogenic mutation that will result in a constitutively activated pathway

d) A mutation that inhibits the interaction of Ras with Raf

No, this is an inhibitory mutation for the activation of the MAP kinase pathway

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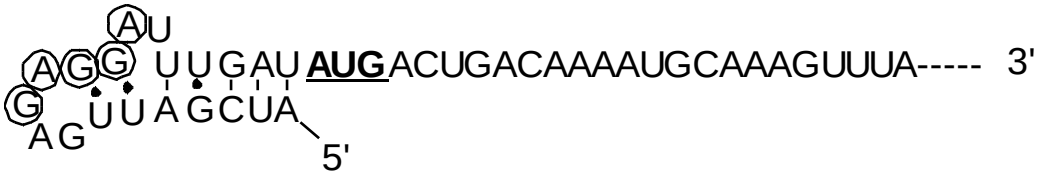
Question 11 (30 pts)

(a) Explain how an increase in the level of σ32 proteins in E. coli cells can lead to the induction of heat shock-responsive genes (14 pts).

σ32 interacts with the RNA polymerase core enzyme (α2ββ') to form a special holo-enzyme that can recognize specific promoter elements present in the promoter region of many heat shock-responsive genes. An increased level of σ32 will result in an increase in transcription of these genes.

(b) Recent data suggest that the σ32 mRNA contains a built-in thermosensor at its 5' end. The predicted secondary structure of the 5' end of the σ32 mRNA is shown below with the initiation codon underlined and the Shine-Delgarno sequence as letters in small circles. Explain how the σ32 thermosensor may function to increase the σ32 protein level in response to heat shock (16 pts).



The S/D sequence base pairs with the 3' end of 16S rRNA in the 30S ribosomal subunit, allowing the proper positioning and entry of the ribosome at the correct initiation codon. Because of the secondary structure within the 5' end of the σ32 mRNA, the S/D sequence is not fully exposed for the interaction with the ribosome, leading to a low level of translation of the mRNA. When E. coli cells are subjected to heat shock, partial melting of mRNA secondary structure at high temperature exposes the S/D sequence and enhances ribosome entry and translational initiation without involvement of other cellular components. Thus, intrinsic mRNA stability serves as a built-in thermosensor that controls synthesis of a transcriptional regulator.

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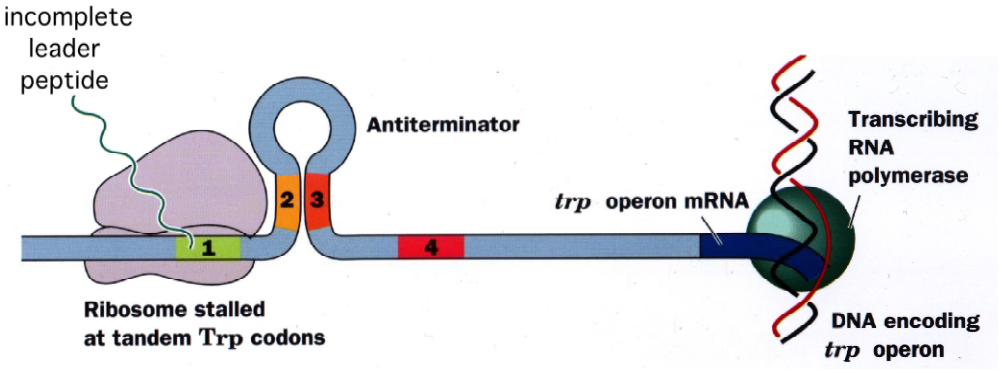
Question 12 (20 pts)

When cellular tryptophan levels are low, the leader mRNA of the trp operon, which contains 4 segments, adopts a conformation shown below.



a) How does the availability of tryptophan affect the attenuation process of the trp operon? (10 points)

When the cellular level of tryptophan is low, ribosome translating the leader mRNA is stalled at tryptophan codons located in segment 1, which results in base-pairing of segment 2 with 3. As a result of this, segments 3 and 4 cannot pair to form the stem and loop termination signal and transcription continues. When tryptophan is abundant, segment 1 is fully translated. Segment 2 enters the ribosome, which enables segments 3 and 4 to base-pair to form the transcription termination signal.

b) Part of the RNA sequence in segment 4 reads: 5'-CGGGC-3'. Based on what you have known about the regulation of the trp operon, please predict a 5-nucleotide sequence that can be found in segments 2 and 3. (10 points)

Segment 2: 5'-CGGGC-3'.

Segment 3: 5'-GCCCG-3'.

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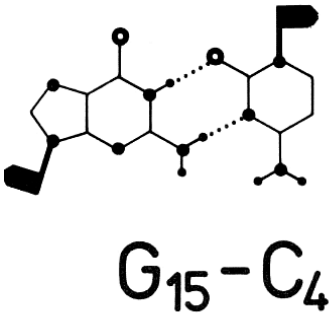
13A. (4 pts.) The figure below shows a G-C base pair observed in the crystal structure of yeast Phe tRNA. The flags represent the backbone orientations. List two ways that this base pair differs from a standard "Watson-Crick" base pair.



A. Different H-bonds and
B. backbone orientations.
C. One base is flipped compared to a standard W-C bp.

13B. (4 pts.) What is meant by the term "coaxial stacking" to describe a common feature of RNA 3-dimensional structure.

Separate stems directly contact each other end on with the axis of each stem pointed in roughly the same direction.

13C. (6 pts.) Not counting coaxial stacking or simple base pairing in stems, list three common RNA structural motifs that introduce or increase stacking interactions.

AA platform
Tetraloop
Cross-strand purine stacks
Tetraloop receptor
4-helix junction

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14A. (12 pts.) List three different enzymes that create RNA:DNA hybrids. For each enzyme, indicate whether the reaction creates the DNA or the RNA polymer.

Primase: RNA
Telomerase: DNA
Reverse transcriptase: DNA
Any others?

14B. (9 pts.) Briefly discuss what special features of RNA:DNA hybrids are exploited for each of these three functions In other words, why are these functions not carried out by double-stranded DNA or double-stranded RNA?

A. RNA marks the start of the Okazaki fragment with an A-form duplex. This allows low-fidelity segments to be preferentially removed and coordinates the placement of sliding clamps at the site where DNA pol III begins elongation.

B. The RNA in telomerase forms a conserved structure that is recognized by the Tert RT protein.

C. Reverse transcriptase converts RNAs into DNA copies to allow stable incorporation into the host chromosomes or readout. The genomes are RNAs, so an enzyme that uses RNA substrates is crucial.

15. (9 pts.) Briefly outline an experimental method you would use to find all the homologs of Your Favorite Gene expressed in the human brain?

A. PCR a cDNA library using primers corresponding to conserved regions of the gene.

B. Make cDNA from fresh brian tissue and probe a microarray containing sequences representing all homologs.

Partial credit

C. (4) Use BLAST to search fo homologs in an EST database of human brain transcripts.

D. (3) Purify proteins from human brain extract and identify all proteins with the activity of YFG.

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16. (6 pts.) Over a third of the human genome is made up of LINEs and SINEs. What is the difference between a LINE and a SINE?

LINE=Long interspersed element. This transposon contains terminal repeats and open reading frames encoding an RNA binding protein and a bifunctional reverse transcriptase/DNA endonuclease. The LINE contains all activities needed for transposition.

SINE=Short interspersed element. This shorter element contains the terminal regions that are substrates for the LINE enzymes, but the ORFs have suffered deletions that block autonomous transposition.