

PART I. Multiple Choice (30 points, 2 points each). Circle all of the statements that are correct. Two points for each correct answer circled, -1.5 for each incorrect answer circled, so do not guess wildly. There may be several correct answers or none within a general statement.

1. The genetic code was deciphered more than 30 years ago through combined efforts in organic synthesis and in enzyme isolation.
 - a. Polynucleotide phosphorylase uses ribonucleoside diphosphate as activated substrates and does not rely on a DNA template for RNA synthesis.
 - b. The genetic code is universal, so human cDNAs will always be recognized and expressed in bacteria.
 - c. The genetic code is non-degenerate and non-overlapping at the same reading frame.
 - d. AUG is the most commonly used initiation codon in prokaryotes and eukaryotes.
 - e. UAA, UAG, and UGG are three commonly used termination codons.

2. Recombinant DNA technology is facilitated by the discovery of enzymes that bind DNA and the development of cloning vectors.
 - a. Most commonly used enzymes for molecular cloning are type I restriction enzymes.
 - b. The DNA ends generated by Bam HI and Bgl II are compatible. Thus, Bam HI and Bgl II are defined as isoschizomers.
 - c. Yeast artificial chromosome can only accommodate DNA inserts less than 50 kb.
 - d. An essential feature of a cosmid is the lambda attachment sites which facilitate the propagation of a cosmid in bacteria.
 - e. T4 DNA polymerase can be used to convert a 3' overhang to a blunt end.

3. DNA replication is a highly coordinated event that requires many protein factors.
 - a. DNA gyrase introduces negative supercoils during prokaryotic DNA replication.
 - b. DNA polymerase I is responsible for lagging strand DNA synthesis, whereas DNA polymerase III holoenzyme is mainly needed for leading strand DNA synthesis.
 - c. Two subunits of the bacterial DNA polymerase III holoenzyme are required to form a DNA clamp on each DNA strand.
 - d. The subunit of DNA polymerase III holoenzyme has the proofreading activity.
 - e. Primase is required to remove the RNA primers made during DNA replication.

4. RNA processing is necessary to generate mature mRNAs in eukaryotes.
 - a. RNA triphosphatase and RNA guanylyltransferase are not sufficient to carry out the 5' capping reaction.
 - b. CF1 is a 3' end cleavage factor whose activity is inhibited by cordycepin.
 - c. Most introns begin at GU dinucleotides and end at AG dinucleotides.
 - d. The A residue in the branch point of the lariat intermediate formed during mRNA precursor splicing is joined to two nucleotides.
 - e. In mammalian cells, splicing begins with the recognition of the 3' splice site by U2 snRNP.

5. Many transcription factors are sequence-specific DNA-binding proteins.
 - a. The bacterial helix-turn-helix DNA-binding domain is structurally similar to that of the eukaryotic helix-loop-helix DNA-binding domain.
 - b. Binding of Lac repressor to the Lac operator inhibits the promoter recognition by RNA polymerase due to steric interference.
 - c. The C protein encoded by the *araC* gene is a transcriptional repressor which can be converted into an activator after binding to L-Arabinose.
 - d. A bacterial chimeric protein with a helix-turn-helix DNA-binding domain that has the N-terminal helix derived from phage 434 and a C-terminal helix derived from phage P22 will bind to the operator of P22.
 - e. Nuclear hormone receptors usually contains the Cys₂-Cys₂ zinc finger DNA-binding motif.

6. General transcription factors are accessory proteins required for accurate initiation by eukaryotic RNA polymerases.
 - a. UBF is a general transcription factor required for transcription by RNA polymerase I.
 - b. The TATA box is a core promoter element only found in the class II genes transcribed by RNA polymerase II.
 - c. TFIIF is the general transcription factor that tightly associates with RNA polymerase II during the transcriptional process.
 - d. RNA polymerase II holoenzyme is a large protein complex containing a subset of general transcription factors and proteins involved in chromatin remodeling or other biological processes in the cell.
 - e. The snRNA genes are transcribed by RNA polymerase III and contain intragenic promoter elements.

7. Chromatin is a higher order structure commonly found in eukaryotes.
 - a. H1 is the most conserved histone, whereas H4 is more divergent through evolution.
 - b. The length of the linker DNA remains constant among various species and different cell types.
 - c. The histone fold motifs in the core histones are important for both histone-histone and histone-DNA interactions.
 - d. SWI/SNF has both chromatin assembly and chromatin remodeling activities.
 - e. Histone acetyltransferase and histone deacetylase both regulate the acetylation state of histones which is important in modulating gene activity.

PART II. Fill-in (15 points, 1 point each). Write in the blank the word or phrase that best completes the statement.

1. The helical winding of the DNA strands around each other is defined as twist.
2. Northern blotting is a technique used to identify the complementary RNA sequence that hybridizes with the DNA probe.
3. Actinomycin D is a cyclic peptide-containing antibiotic that can inhibit transcription via integration into DNA between neighboring base pairs.
4. Spliceosome is a large protein complex that carries out the splicing of mRNA precursor, whereas cleavage and polyadenylation specificity factor (CPSF) is the protein that recognizes the AAUAAA polyadenylation signal in eukaryotes.
5. Guide RNAs contain 3' oligo(U) tails which are used to provide U residues during RNA editing.
6. Most bacterial operons specifying the expression of enzymes involved in the metabolism of lactose, arabinose, and galactose were not induced in the presence of glucose. This phenomenon is known as catabolite repression.
7. There are three core promoter elements identified in eukaryotic protein-encoding genes: the TATA box, the initiator element, and the downstream promoter element.
8. An enhancer can activate transcription in a position-independent and an orientation-independent manner.
9. TAF_{II}s, USA, and Mediator are the three types of transcriptional coactivators required for most activator function in eukaryotes.
10. The JAK-STAT pathway is activated by interferons and through phosphorylation and translocation, thereby regulating specific gene expression.
11. Micrococcal nuclease is the enzyme used to cleave chromatin and releases the nucleosomes formed by the histone octamer and its associated DNA.
12. A splooning model explains how RNA polymerase can transcribe through a chromatin template without dissociating the histone octamer from the DNA template.

PART III. Explanation and Long Answers (credit as specified).

1. (8 points) Many genes that are involved in the same biochemical pathway are clustered together on the prokaryotic chromosome and are called operons. These operons usually have one promoter directly upstream which controls transcription of the operon. Name two advantages of having this kind of system.
 - 1) **Transcription can be turned on or off by a single regulatory element and the corresponding binding factor.**
 - 2) **The ratio of the amount of each component of a pathway is maintained constant without having each one separately regulated.**

2. (14 points) Yeast two-hybrid system has become an important tool to define protein-protein interactions. Assume your favorite human protein “CMC” has no transcriptional activity. How will you design a yeast two-hybrid assay to screen for the CMC-interacting human proteins? Outline the procedure in steps.

Step 1, I will construct a yeast expression plasmid producing a fusion protein containing the CMC protein linked to the Gal4 DNA-binding domain (DBD).

Step 2, I will construct (or request) a human cDNA library with individual DNA inserts linked to an activation domain-coding sequence (for instance, the Gal4 activation domain) in a yeast expression vector.

Step 3, I will make (or request) a yeast strain harboring a LacZ reporter gene driven by a yeast core promoter linked to (e.g., 5 copies) Gal4-binding sites.

Step 4, I will introduce the CMC-Gal4 DBD fusion expression plasmid into a yeast strain harboring the reporter gene obtained from step 3.

Step 5, I will introduce the human cDNA library obtained from step 2 into a yeast strain containing both a reporter gene and the CMC-Gal4 DBD fusion expression plasmid (see step 4), and plate the library onto X-gal-containing plates.

Step 6, I will pick up the blue colonies obtained from step 5 and isolate the DNA for sequence analysis.

Now, I have probably obtained the cDNA clones expressing CMC-interacting human proteins!

Part IV True / false section: circle appropriate letter (25 points; 1 point each)

1. T / **F** The energy driving amino acylation of a tRNA molecule comes from hydrolysis of GTP.
2. T / **F** Puromycin cause polypeptide chain termination because it resembles Release Factor 3.
3. **T** / F Each amino-acyl-tRNA linkage is activated for addition of the next amino acid to the growing peptide chain rather than for its own addition.
4. T / **F** Wobble base pairing occurs between the first position in the codon and the third position in the anticodon.
5. T / **F** The primary function of the small ribosomal subunit is to catalyze peptide bond formation, whereas the large ribosomal subunit binds mRNA and tRNAs.
6. T / **F** Inserting a delay between the binding of a charged tRNA to the ribosome and its utilization in synthesis increases fidelity and speed of protein synthesis by giving improperly base-paired tRNAs an opportunity to diffuse off the ribosome.
7. T / **F** Asn-linked GlcNAc (NAG) is a sugar found on some cytosolic proteins, and is thought to function analogously to phosphorylation.
8. **T** / F Some O-linked sugars are linear unbranched polymers of repeating disaccharides.
9. T / **F** Some N-linked sugars are linear unbranched polymer of repeating disaccharides.
10. T / **F** Nuclear import occurs by a process of transmembrane transport.
11. **T** / F Fusion of a virus with a host cells endocytic vesicle membrane involves a pH change which drives a conformational change of the viral fusion protein.
12. **T** / F An IRES sequence in mRNA allows eucaryotic protein synthesis to begin with initial recognition of the mRNA by the ribosome occurring in the middle of the mRNA.
13. T / **F** Proteolytic activities within the proteosome are maximal due to maintenance of a low internal pH.
14. **T** / F Isopeptide bonds are found in bacterial cell walls and in ubiquitinated proteins.
15. T / **F** GTP is used to help unfold proteins prior to insertion into the proteosome for degradation.

16. T / **F** Unlike protein and nucleic acid synthesis, sugar chains on proteins are heterogeneous because sugar encoding genes are recombined somatically.
17. **T** / F Proteoglycans have their sugar chains attached in Serine-Glycine (Ser-Gly; S-G) repeat sequences which are flanked by acidic residues.
18. T / **F** Apoptosis involves the transcriptional activation of pro-apoptotic genes necessary to carry out the cell death program.
19. T / **F** Transport of a lysosomal enzyme from the endoplasmic reticulum to the golgi occurs via a process of transmembrane transport.
20. T / **F** Isoprene units are the precursors of the myristoyl groups attached to proteins.
21. T / **F** Nitric oxide is a gaseous second messenger which is derived from citrulline.
22. T / **F** Clathrin coated vesicles remain coated from donor to acceptor membrane.
23. **T** / F SNARE proteins involved in membrane fusion events are the targets of botulinus toxin produced by bacteria.
24. T / **F** The GTP bound form of Ran has a high affinity for importin .
25. **T** / F Proteins imported into mitochondria require a signal sequence which is an amphipathic alpha helix.

Part V Fill in the blank section (17 points; 1 point for each blank)

1. The common theme seen in translocation of proteins across the ER membrane, across the mitochondrial membrane, across the chloroplast membrane, and into the proteosome is that proteins must be **unfolded** to pass.
2. A nucleotide triplet on a tRNA that forms a stable, hydrogen-bonded complex with a complementary triplet in mRNA is called a(n) **anticodon** ; its complementary triplet on the mRNA is called a(n) **codon** .
3. The **EF-Ts** is the GEF (guanine nucleotide exchange factor) for EF-Tu?
4. The three generally recognized stages of protein synthesis in both prokaryotes and eukaryotes are **initiation** , **elongation** , and **termination** .
5. Stop codons are recognized by proteins called **release (termination)** factors.

6. _____ **cytochrome C** and _____ **ATP** _____ are the two low molecular weight cofactors required for Apaf (Ced4) protein activation (involved in apoptosis pathway).

7. The _____ **bcl2** _____ family of proteins regulates the activation of Apaf (Ced4) which is required for apoptosis.

8. _____ **cyclin dependent kinase (cdc2/cdk/p34)** and _____ **Cyclin (mitotic cyclin)** _____ are the two protein components of maturation promoting factor (MPF, also called Mitosis promoting factor). _____ **Thr kinase** _____, and _____ **Tyr Kinase** _____, and _____ **phosphatase (Tyr phosphatase)** are three enzymatic activities which are required for MPF activation, while _____ **proteolysis (destruction by ubiquitin/proteosome)** is the enzymatic activity responsible for the termination of MPF function.

Part VI short answer: use only the space provided, single words or phrases are sufficient (40 points total, points for each question indicated in ())

1. (2) What enzymatic feature of some aminoacyl tRNA synthetases increases the fidelity of protein synthesis, and what reaction is performed (independent of the ribosome)?

hydrolytic site of synthetase rejects (hydrolyzes) incorrectly charged amino acids

2. (2) What 2 features of procaryotic mRNA direct the start site of polypeptide synthesis?

Shine dalgarno sequence, AUG codon

3. (2) An aminoacyl tRNA linkage is labile (subject to hydrolysis by water). Why isn't it hydrolyzed in the aqueous environment of the cytoplasm?

complexed with (sequestered by) EF-Tu

4. (2) The genetic code is degenerate. What are the two sources of degeneracy?

**multiple codons (and corresponding tRNAs) encode a single amino acid;
a single tRNA can recognize multiple codons (wobble pairing)**

5. (Points as indicated) Nitroglycerin is an effective antianginal drug.
5a) (1) What product is liberated from nitroglycerin in the human body which makes this chemical an effective antianginal drug (i.e. it reverses blood vessel constriction which causes heart muscle pain)?

nitric oxide (NO)

- 5b) (3) What is the mechanism of action of this drug (what is the target of this product, what is the effect of this product on this target, and what is the next step in this pathway)?

NO activates guanylate cyclase inducing cGMP production, which activates a cGMP dependent protein kinase and ultimately smooth muscle relaxation and vessel dilation.

6. (2) What is the major difference between *ex vivo* gene therapy and *in vivo* gene therapy?

ex vivo cells are transformed outside the body and then returned, in vivo the target is transformed in the intact organism

7. (2) How is negative feedback established in the TGF β signal transduction pathway?

**a gene activated by this pathway is an inhibitor of the pathway,
or
inhibitory smad genes are expressed / turned on**

8. (4) The protein *inaD* is involved in visual signal transduction in *Drosophila* photoreceptor cells. This protein is composed of 5 PDZ domains. What do these domains do (in a general sense), and what is the advantage of this arrangement for visual signal transduction?

bind multiple molecules involved in the signal transduction cascade (act as a scaffold)

physical proximity maximizes speed and fidelity (minimizing potential cross-talk)

9. (3) Sarin is an effective nerve gas which causes death by inactivating acetylcholinesterase. What is the biochemical mechanism of inactivation (a simple statement is appropriate), and what mechanistically similar reaction is inactivated by the sarin-like molecule diisopropylphosphorofluoridate (DIPF)?

covalent modification of active site residues (reacts with active site serine)
serine proteases are inhibited by DIPF using a similar mechanism

10. (3) What three processes contribute to termination of synaptic transmission at the neuromuscular junction?

diffusion, reuptake, degradation of neurotransmitter (ACh)

11. (3) During the rising phase of the action potential Na^+ channels allow sodium to flow into the cell causing a depolarization which in turn causes more Na^+ channels to open. What type of regulation does this exemplify, and what two events reverse the process of depolarization (allowing the falling phase of the action potential)?

positive feedback

Na^+ channel inactivation (closing), and delayed K^+ channel opening

12. (2) Why is the Na⁺/K⁺ ATPase “electrogenic” (how / why does this pump causes a change in the charge distribution across the plasma membrane)?

it pumps 3 Na⁺ ions out for each 2 K⁺ ions in

13. (points as indicated) Cadherins and integrins are families of proteins which can mediate cell-cell interaction (binding).

13a) (2) Cadherins are capable of mediating homophilic interactions between cells. What does this mean, and what additional requirement (cofactor) is required for cadherin mediated homophilic binding?

two cells which express the same cadherin can bind each other, Ca²⁺ is required.

13b) (1) Integrins can mediate intercellular interactions but in contrast to cadherins this interaction is fundamentally different. What is this difference?

heterophilic (the integrin binds a different molecule on the second cell)

14. (4) Binding of the G alpha subunit of a heterotrimeric G protein to GTP is partially mediated by switch regions I, II, III of the G alpha protein. What protein-protein interactions are also mediated (at least in part) by these regions of the G alpha subunit, and in which nucleotide bound states do these interactions occur?

**binding to the beta-gamma subunit occurs when GDP is bound
binding to effector proteins (e.g. adenylate cyclase or phospholipase C) occurs when GTP is bound**

15. (2) You knock out (inactivate) the glucosidase present in the ER which removes the inner most glucose residues from the core structure of N-linked glycoproteins. What do you predict happens to glycoproteins with N-linked sugars and why.

retained in ER (and or degraded in ER). Calnexin will not allow export of monoglucosylated glycoproteins from ER.

Part VII short essay: use only the space provided. (51 points total, points for each question indicated in ())

1. (4) List two means of generating an oncogene from a proto-oncogene (not the genes affected but what happened in the chromosome to change the genes). Give the general mechanism and the outcome relative to the protooncogene (e.g. is the change qualitative or quantitative in nature and how so)?

point mutation activating an oncogene

insertional mutagenesis altering the function or expression of a proto-oncogene

chromosomal rearrangements resulting in the altered function or expression

amplification of a chromosomal locus

virus encodes an altered or misregulated protooncogene

2. (5) List five features which characterize a cell undergoing apoptotic cell death.

surface blebs, surface signals for phagocytosis, cell shrinkage, release contacts with neighbors, chromatin condensation, chromatin cleavage, fragmentation of cell into membrane bound vesicles

3. (points as indicated) You identify a virus which is present in 100% of ear-lobe cancers, and show that the virus is the causative agent underlying this type of cancer. When you sequence the genome of the virus you realize that it is a retrovirus; it has the genes common to all retroviruses as well as one additional open reading frame similar to a heterotrimeric G-protein alpha subunit.

3a) (7) what are the genes you identify which are common to all retroviruses, and what is the function of each of these genes?

GAG: capsid protein, surrounds genome in core of virus

POL: encodes reverse transcriptase and integrase

ENV: viral surface (envelope) glycoprotein, ligand for host cell receptors

3b) (3) What types of alterations (mutations) do you think you might find in the additional open reading frame relative to the cellular homologue of the viral open reading frame (i.e. what function of the G α is likely to be altered, will it be enhanced or inhibited, what is the functional outcome of the mutation in terms of signaling: think about the enzymatic activity of a G α subunit)?

GTPase activity is likely decreased or absent so that signaling is constitutive

3c) (4) Independent virus isolates from different persons with the same disease each have different additional open reading frames (i.e. they all have the genes common to retroviruses but each has a different, single, additional gene). These additional open reading frames are homologous to proteins which influence heterotrimeric G-protein alpha subunit function, but are not themselves heterotrimeric G-protein alpha subunit homologues. What two classes of proteins are these likely to encode, and what mutations in these genes are likely to be found (what function of these proteins will be altered and in which way will this function be altered)?

GAP with activity lost

GPCR constitutively activated or ligand independent GEF activity

4. (6) What are the three methods of compartmentalizing proteolysis (give the name of the structures and the general mode of compartmentalization)?

stomach (intestine) is an organ compartmentalized proteolytic system
lysosome is an organellar compartmentalized proteolytic system.
proteasome is a proteinaceous compartmentalized proteolytic system.

5. (4) Give the major differences between the life cycle of a lytic virus an episomal virus.

lytic viruses replicate and cause cell lysis which releases virions from the ruptured host, while episomal viruses are maintained in the host as a plasmid and divide with the host chromosomes to allow passage of the viral genome to daughter cells

6. (points as indicated) Activation of the PDGF receptor results in autophosphorylation of tyrosine residues.

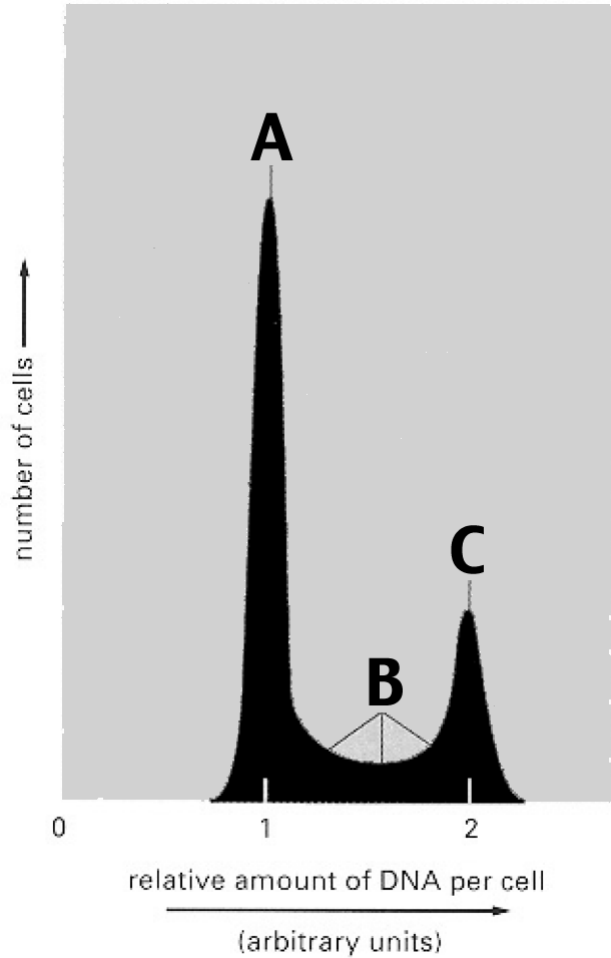
6a) (2) One of the resulting tyrosine phosphate residues has a high affinity for the src (a soluble tyrosine kinase) SH2 domain. What is the significance of this interaction?

PDGF PY interaction competes for src SH2-PY interaction relieving autoinhibition of the kinase and thus activation.

6b) (2) How is this mode of regulation of src kinase activity circumvented in the rous sarcoma virus version of the src kinase (the oncogenic version)?

the src tyrosine residue is deleted (which is normally subject to phosphorylated) such that autoinhibition does not occur and the kinase is thus constitutively active.

7. (4) Refer to the flow cytometry histogram shown. The fluorescence of a DNA marker is plotted on the X axis, while the number of cells within each bin of fluorescence is plotted on the Y axis (i.e. this is a histogram of DNA content). State the phase of the cell cycle which describes the cells indicated by the letters A, B and C (at what phase of the cell cycle are the cells with the fluorescence value at A, B, and C).



A: G1

B: S

C: G2 and M

8. (6) lymphocyte extravasation (white blood cells going from the lumen of the blood vessel to the surrounding tissue by passing between the blood vessel endothelial cells) occurs by a multi-step process. Briefly, describe the three major steps involved in this process, and the protein-protein interaction responsible for each of these steps.

rolling mediated by selectin-mucin interactions

chemokine-GPCR mediated integrin activation (inside out signaling)

integrin-ICAM mediated strong adhesion and migration

9. (points as indicated) You have defined a novel transmembrane tyrosine kinase which contains several cytoplasmic tyrosine residues which are subject to autophosphorylation upon receptor activation.

8a) (2) How would you ascertain whether any of the individual tyrosine residues (which are subject to autophosphorylation) are functionally involved in signal transduction mediated by the activated receptor (a simple and direct test)?

mutant the individual residues to phenylalanine, and assess functionality.

8b) (2) Assuming that the above mentioned assessment reveals a functional role for a given tyrosine residue, what is the likely function of this residue in the phosphorylated state?

it recruits / binds a downstream protein (adaptor, or scaffold, or anchor, or SH2 domain protein, or PTD domain protein)