

PART I. Multiple Choice (26 points, 2 points each). Circle all of the statements that are correct. Two points for each correct answer circled, **-1** for each incorrect answer circled, so do not guess wildly. There may be several correct answers to a question or none.

1. Lambda (λ) phage has two distinct life cycles in an infected bacterial cell.
 - a. repressor has higher affinity to O_L1 and lower affinity to O_R3 , following the order $O_R3 < O_R2 < O_L3 < O_L2 < O_R1 < O_L1$.
 - b. Only the Cro gene is expressed during the maintenance stage of the lysogenic cycle.
 - c. Lysogeny is terminated first by proteolysis of Cro protein.
 - d. The lytic pathway requires two protein factors. Q turns on the expression of immediate-early genes, whereas N affects the expression of delayed-early genes.
 - e. DNA damage induces the switch from the lysogenic to the lytic life cycle.

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 II 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. Centromeres are essential, whereas telomeres are dispensable, in designing a yeast artificial chromosome for cloning a large DNA fragment.
 - d. An essential feature of a cosmid is the lambda attachment sites which facilitate the propagation of a cosmid in bacteria.
 - e. Klenow enzyme can be used to convert a 3' overhang to a blunt end.

3. RNA processing is a posttranscriptional modification that is required to make RNA molecules fully functional.
 - a. The A residue in the branch point of the lariat intermediate during mRNA precursor splicing is joined to two nucleotides.
 - b. CstF is the catalytic enzyme that cleaves the precursor mRNA during 3' end processing.
 - c. Primase is the enzyme that adds a stretch of A residues at the 3' end of RNA.
 - d. U1 snRNP binds the 5' splice site to initiate the splicing of mRNA precursors.
 - e. A 7-methyl cytosine is often added to the 5' end of the mRNA precursor to protect the RNA molecules from being degraded by phosphatases and ribonucleases.

4. Transcription is a process that transmits the genetic information from DNA to RNA.
 - a. An A/T-rich sequence often found in bacterial promoters is recognized by the subunit of prokaryotic RNA polymerase.
 - b. Rho protein is the bacterial termination factor required to terminate all bacterial transcription.
 - c. TFIIA is the initiation factor that recognizes the A/T-rich region often found in the eukaryotic promoters.
 - d. α -amanitin can inhibit the function of both RNA polymerase II and III, but not RNA polymerase I.
 - e. In eukaryotes, RNA polymerase II is responsible for the synthesis of transfer RNA.

5. Prokaryotic transcription is controlled by many sequence-specific DNA-binding proteins.
 - a. The C protein encoded by the *araC* gene is a transcriptional repressor which can be converted into an activator after binding to L-Arabinose.
 - b. Binding of Lac repressor to the Lac operator inhibits the promoter recognition by RNA polymerase due to steric interference.
 - c. Inducible catabolic operons are globally regulated by CAP protein containing bound cyclic UMP.
 - d. Attenuation of the amino acid-synthesizing operons is mediated by the tight coupling of transcription and translation and is only found in prokaryotes.
 - e. A chimera protein containing an N-terminal α -helix from phage 434 and a C-terminal α -helix from phage P22 will bind to the phage 434 operator.

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. The snRNA genes are transcribed by RNA polymerase III and contain intragenic promoter elements.
 - d. TFIIF is the basal transcription factor that tightly associates with RNA polymerase II during the transcriptional process.
 - e. TFIIB mainly acts as an antirepressor to overcome the repressive functions of negative factors.

7. The structures of several DNA-binding domains have been solved.
 - a. The helix-loop-helix and ribbon are two commonly found motifs in prokaryotes.
 - b. TBP is a minor groove-binding protein which bends DNA after its binding.
 - c. Homeodomains are structurally similar to the helix-loop-helix DNA-binding domain.
 - d. The Cys₂-Cys₂ zinc-finger DNA-binding domains are very common in the nuclear hormone receptor superfamily.
 - e. The leucine zipper motif is sufficient for dimerization and DNA-binding.

8. The genetic code was deciphered more than 30 years ago through combined efforts in organic synthesis and in enzyme isolation.
 - a. The genetic code is non-degenerate and non-overlapping at the same reading frame.
 - b. UAA, UAG, and UGA are three commonly used termination codons.
 - c. AGG is the most commonly used initiation codon in prokaryotes and eukaryotes.
 - d. The genetic code is universal, so human cDNAs will always be recognized and expressed in bacteria.
 - e. An RNA-editing enzyme called cytidine deaminase can convert C to U.

PART II. Fill-in (19 points, 1 point each). Write in the blank the word or phrase that best completes the statement.

1. **Southern** blotting is a technique used to identify the complementary DNA sequence that hybridizes with the DNA probe.
2. **Polynucleotide phosphorylase** is an unusual RNA polymerase that uses ribonucleoside diphosphates as active substrates and does not require DNA template for RNA synthesis.
3. The helical winding of the DNA strands around each other is defined as **twist**.
4. **Aphidicolin** is used to inhibit DNA synthesis and is also used to synchronize eukaryotic cells at the G1 phase.
5. The β subunit of DNA polymerase III holoenzyme is the sliding clamp that enhances the processivity of prokaryotic DNA polymerase, and **PCNA** is a protein that enhances the processivity of eukaryotic DNA polymerase .
6. **Telomerase** is the enzyme replicating the ends of chromosomes and has been implicated in the aging process.
7. **Guide** RNAs contain 3' oligo(U) tails which are used to provide U residues during RNA editing.
8. The group I self-splicing intron requires a **guanosine** cofactor to initiate the transesterification reaction.
9. **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.
10. 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**.
11. There are three core promoter elements identified in eukaryotic protein-encoding genes: the TATA box, the initiator element, and the **downstream promoter element**.
12. **TFIID** is a TBP-containing complex required for transcription by RNA polymerase II.

13. An enhancer can activate transcription in a position-independent and an orientation-independent manner.
14. TAF_{II}s, USA, and Mediator are the three types of transcriptional coactivators required for most activator function in eukaryotes.
15. Micrococcal nuclease is an enzyme that releases individual nucleosomes after complete digestion.

PART III. Short Answers (credit as specified).

1. (8 points) In the arabinose operon, araO₂ and araI are 211 bp apart. Inserting 5 extra base pairs anywhere between araO₂ and araI causes the level of repression of the araBAD operon to be greatly diminished. However, insertion of 11 base pairs in this same region has no effect. Explain why.

Insertion of 5 bp in the region between araO₂ and araI adds a half turn to the DNA helix. This causes araO₂ to be on the opposite face of the DNA relative to araI and therefore makes it harder for the DNA to loop around and be repressed by araC.

Insertion of 11 bp places in an entire turn to the DNA enabling araO₂ and araI to be again on the same face when the DNA is looped around.

2. (14 points) A lot of proteins required for *E. coli* DNA replication are well characterized. List seven bacterial proteins involved in the initiation of prokaryotic DNA replication, and describe in a few words the role of each enzyme in bacterial DNA replication.

<u>Protein</u>	<u>Role</u>
DNA gyrase	introduces negative supercoils
Helicase	unwinds the double helix
SSB	stabilizes single-stranded regions
Primase	synthesizes RNA primers
DNA polymerase III holoenzyme	synthesizes DNA
DNA polymerase I	erases primer and fills gaps
DNA ligase	joins the ends of DNA

Part IV True / false section: circle appropriate letter (15 points; 1 point each)

1. T / F Asn-linked GlcNAc is a sugar found on some cytosolic proteins, and is thought to function analogously to phosphorylation.
2. T / F The tRNA synthetase which charges Met-tRNA^{MET} recognizes only N-formyl-methionine.
3. T / F A primary function of the large ribosomal subunit is to catalyze peptide bond formation, whereas the small ribosomal subunit binds mRNA and tRNA.
4. T / F Endoplasmic reticulum bound ribosomes have a subunit not found in soluble ribosomes.
5. T / F Translation of eucaryotic mRNAs is initiated after recognition of the shine dalgarno sequence by the ribosome.
6. T / F Transport of a lysosomal enzyme from the endoplasmic reticulum to the lysosome occurs via a process of transmembrane transport.
7. T / F Proteolytic activities within the proteasome are maximal due to maintenance of a low internal pH.
8. T / F Proteins imported into mitochondria require a signal sequence which is an amphipathic alpha helix.
9. T / F Multiple sclerosis results in deficiency in action potential propagation as a result of autoimmunity (antibodies recognize and destroy parts of the nervous system) against the Na⁺ channels required for the action potential rising phase.
10. T / F Mucins contain carbohydrates which can confer structural as well as specific recognition properties.
11. T / F Isoprene units are the precursors of the myristoyl groups attached to proteins.
12. T / F Zymogens are important for blood clotting, digestive enzymes, and apoptosis.
13. T / F The catalytic mechanism of aspartyl proteases (e.g. pepsin) involves an acyl enzyme intermediate.
14. T / F The proteasome is involved in the proteolytic activation of caspases.
15. T / F Lysosomal function requires ATP.

Part V Fill in the blank section (25 points; 1 point for each blank)

1. prenylation or fatty acylation can direct proteins to associate with the cytosolic face of the plasma membrane, while GPI addition can direct proteins to associate with the extracellular face of plasma membranes (classes of post-translational modification).
2. tRNA charging and fatty acid activation both use ATP as an energy source, and PPi hydrolysis by pyrophosphatase drives these reactions forward (makes the reactions irreversible)?
3. Cadherins mediate homo philic, while integrins mediate hetero philic interactions.
4. The common theme seen in translocation of proteins across the ER membrane, across the mitochondrial membrane, across the chloroplast membrane, and into the proteasome is that proteins must be unfolded to pass.
5. During translation, mRNA is read in the 5' to 3' direction.
6. Proteins are synthesized from amino to carboxyl termini.
7. EF-Ts is the GEF (also referred to as GNRP, GNRF, nucleotide exchange factor) for EF-Tu.
8. GPC-R is the GEF for heterotrimeric G-proteins.
9. monoglucosylation or terminal glucosylation or glucosylation is a sugar modification which can keep a protein from leaving the ER:
10. Mannose-6-PO₄ is the sugar modification which can direct a protein to the lysosome:
11. Proteolytic activities within the lysosome are maximal due to H⁺ ATPase which generates a low internal pH.

12. Proteoglycans contain a high content of _____ **carboxylated** and **sulfated** sugars which gives them a strongly negative charge (sugar modifications).
13. Binding of activated tRNAs by _____ **EF-Tu** _____ protects the amino acyl linkage from hydrolysis by water.
14. _____ **Inosine** _____ is frequently found in the _____ **5'** _____ position of the anti codon of a tRNA because it maximizes the number of codons which can be recognized.
15. The tertiary structure of a tRNA molecule requires tertiary base pairing interactions between the _____ **“T ψ C loop”** _____ and _____ **“DHU loop”** _____ loops.

Part VI short answer: use only the space provided, single words or phrases are sufficient (57 points total, points for each question indicated in ())

1.(2) Unlike proteins and nucleic acids, the exact sequence and branching of carbohydrates present on proteins is heterogeneous. Why does this heterogeneity exist?

sugar chain addition to proteins has no genetic template but nucleic acids and proteins do

2.(2) cAMP acts on a regulatory subunit of protein kinase A (PKA) to change it's conformation. In the absence of cAMP, what is the basis of the inhibition of PKA catalytic subunits by the regulatory subunits?

regulatory subunit pseudosubstrate binds in active site of catalytic subunit

3.(2) If a single cadherin mediated interaction is insufficient to mediate cell-cell binding, how can cadherins mediate strong cell-cell binding without a change in binding affinity, and what is this called?

multiple cadherins (zipper-like) leads to increased avidity

4.(3) Many signal transduction pathways involve cascades of enzymatic activities. What properties does this arrangement confer on the pathways (list three)?

amplification, sites for integration, sites for regulation, sites for divergence and convergence

5.(1) Some cells can respond to adrenaline (epinephrine) by increasing cAMP while other cells respond by decreasing cAMP. How can the same ligand elicit different responses?

different receptors which both bind same ligand

6.(6) What three processes contribute to termination of synaptic transmission at the neuromuscular junction, which one of these is inhibited by nerve gas (sarin), and what is the mechanism of this inhibition?

diffusion, reuptake, degradation of neurotransmitter (ACh)

degradation, covalent modification of acetylcholinesterase active site serine

7.(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 (giving rise to the falling phase of the action potential)?

positive feedback; Na^+ channel inactivation (closing OK), and delayed K^+ channel opening

8.(4) What feature of ras differs from the heterotrimeric G alpha subunit and underlies the resistance of ras to activation by AIF? Would you expect EF-Tu to be inhibited by AIF?

**much weaker GTPase so that AIF⁻ can not stabilize transition state
EF-Tu NO**

9.(2) TGFbeta signal transduction involves activation of “excitatory” smad proteins to alter gene expression. However, one of the genes which is turned on encodes a class of inhibitory smad proteins (which inhibit excitatory smads). What type of regulatory mechanism does this exemplify?

autoregulatory negative feedback

10.(2) In a hypothetical receptor system it is found that two different transmembrane kinases (A and B) are required for signal transduction. You find that mutational activation of receptor A makes the signaling independent of ligand, while mutational inactivation of receptor A makes signaling nonfunctional. What do you conclude about the flow of information through this receptor system (think growth hormone)?

**ligand activates B which in turn must activate A which in turn causes the signal
(A is downstream of B)**

11. Start kinase and MPF have a common subunit and a divergent subunit. These complexes control separate checkpoints in the cell cycle.

a)(3) What is the common component, what are the different components, and what does this suggest about the mechanism of substrate recognition by these complexes?

Cdc2 (Cdk, p34, kinase) is common; the associated cyclin is different. The cyclin must play a role in determining substrate recognition.

b)(3) Which of these components undergoes cyclic changes in amount, and what is the basis of this cycle?

cyclin, ubiquitin mediated degradation

12. You identify a gene product which is involved in curtailing progression of cells through the cell cycle (inhibits cell cycle progression).

a)(2) What criteria would have to be met for mutations in this gene to give rise to cancer (e.g. would the mutations enhance or inhibit function and how many copies must be affected)?

**both alleles
inhibit function**

b)(1) What is the common term for this type of protein?

tumor suppressor

13.(3) Based upon your knowledge of apoptotic pathways, would injection of cytoplasm from cells undergoing apoptosis drive a recipient cell into apoptosis in the absence of external signals or damage to the recipient cell? Why or why not?

Yes, the caspase cascade is activated and will activate the cascade in the recipient.

14.(2) several examples of a mechanism of enhancing biochemical interactions were talked about in class including: bFGF binding to a high abundance low affinity receptor prior to interaction with signalling transmembrane receptor, alpha and gamma subunits of heterotrimeric G-proteins both having attached lipid chains, and growth hormone binding to sequential receptors. What is the principle illustrated by all of these examples.

reducing the dimensionality of the reaction from 3 dimensions to 2.

15.(3) Why does removal of a Tyrosine residue in the carboxyl terminus of src result in constitutive activation of the src tyrosine kinase activity (i.e. what does this tyrosine normally do and how)?

It is phosphorylated and binds to an SH2 domain of src (intramolecular) which inhibits kinase activity.

16.(3) What glycosylation reaction (and which step in the pathway) is blocked by tunicamycin and why?

N-linked glycosylation, transfer of UDP GlcNAc to dolichol-phosphate structural analog of UDP- GlcNAc

17.(6) Name the three species of RNA involved in translation and briefly (1-5 words) give their function

mRNA: informational

tRNA: adapter / charged intermediate

rRNA: structural / catalytic

18.(4) What are four types of genetic alterations which can give rise to oncogenic transformation (not the genes affected but what happened in the chromosome to change the genes)?

translocations, insertions, amplifications, point mutations

Part VII short essay: use only the space provided. (36 points total, points for each question indicated in ())

1. You identify a virus which is present in 100 % of cuticle cancers. When you sequence the genome of the virus you realize that it is a retrovirus. In addition to the genes common to all retroviruses, you find an open reading frame similar to a heterotrimeric G-protein alpha subunit.

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

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

b) (2) independent isolates from different persons with the same disease have different additional open reading frames. These 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 class 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

or

GPCR constitutively activated or ligand independent GEF activity

2. Gene X1 is mutated (and not expressed at all) in a progressive nasal disease. Its function is required in nasal epithelia where it is normally expressed at high levels. It is one member of a family of proteins (X1, X2, and X3). X2 and X3 are expressed at lower levels in the same tissues as gene X1. The following describe a retrovirus based method of gene therapy which could be tried to alleviate the disease caused by the X1 mutation.

a) (1) What gene would be in the vector (i.e. delivered to the nasal epithelial cells to try to correct the defect) in order to minimize the likely hood of an adverse immune response?

X2 or X3

b) (2) What potential drawback is there to using this gene?

it may not have identical functions as X1

c) (2) What other elements must be present in the vector (i.e. cis acting elements)?

psi (packaging signal), LTRs

d) (5) What is the function of a packaging cell line, and what must be expressed in the packaging cell line (i.e. supplied in trans)?

generate viral particles which contain gene of interest but lack other viral genes; GAG, POL, ENV

3. (6) SH2, SH3, PDZ, and PTB domains are all modular protein-protein interaction domains. Pick two of these and give the motif which they bind, and the type of interaction between the motif and the domain (biochemical basis of the interaction).

SH2-> binds PY-> 2 pronged plug, deep insertion, charge neutralization of PY and deep insertion and hydrophobic pocket for +3 residue

SH3->polyproline-> shallow groove hydrophobic interactions not hydrogen bonds

PDZ-> c-terminal E(S/T)DV-> added beta strand to a beta sheet

PTB-> binds PY-> additional strand to anti-parallel beta sheet, hydrogen bonding interactions, PY after beta turn recognized at edge of sheet

4. a) (12) Sketch the flow of information (protein-protein interaction cascade) in the ras-MAP kinase pathway from the activated cell surface receptor (e.g. transmembrane Tyr kinase growth factor receptor) to an activated nuclear transcription factor (assume no branching or crosstalk with other pathways). Briefly identify the function of the proteins listed.

Receptor->PY(binding site)->

Grb (adaptor protein)->SOS (ras GEF)->Ras (GTP binding protein)->Raf (kinase)->MAPKK(kinase)->MAPK(kinase)

->transcription factor activated

b) (3) What two events are triggered on the receptor (transmembrane Tyr kinase growth factor receptor) upon ligand binding to initiate this cascade?

dimerization, followed by trans-autophosphorylation giving PY receptor