

A. Choose the correct one: (30%)

1. In Alzheimer's disease, which protein is considered to be alternatively miss-folded:
  - a. hsp70
  - b. amyloid
  - c. GroEL
  - d. prion
2. For Ras, a small GTP binding protein, which protein is the GEF factor:
  - a. Grb
  - b. SOS
  - c. NF1
  - d. Raf
3. What is correct for acetylcholine receptor?
  - a. Six subunits each contains M2 alpha helix.
  - b.  $Ca^{2+}$  ion channel.
  - c. Neuron attenuates the signal by endocytosis of acetylcholine.
  - d. Abundant in neuromuscular junctions
4. Which of the following histone proteins is not found in nucleosome cores?
  - a. H1
  - b. H2a
  - c. H2b
  - d. H3
  - e. H4
5. Which of the following DNA polymerases is responsible for most of the nuclear DNA replication in eukaryotic cells?
  - a. DNA polymerase I
  - b. DNA polymerase II
  - c. DNA Polymerase  $\alpha$
  - d. DNA Polymerase  $\delta$
6. Which of the following proteins directly binds and recognizes the NLS for transport of a protein into the nucleus?
  - a. Importin beta
  - b. Importin gamma
  - c. Importin alpha
  - d. Ran-GTP
  - e. Ran GDP
7. Which protein is required for pinching off Clathrin coated vesicles?
  - a. Clathrin
  - b. Snap
  - c. v-Snare
  - d. Dynamin

8. What calcium dependent plasma membrane protein is responsible for cell-cell adhesion and is connected to cytoplasmic cytoskeletal filaments?
  - a. Cadherin
  - b. Integrin
  - c. Selectin
  - d. ICAM
9. In TGF-beta signaling pathway:
  - a. TGF-beta directly binds to dimmer form of receptor
  - b. The TGF-beta receptor owns tyrosin kinase activity
  - c. STAT binds to the phosphorylated receptor
  - d. Smad 4 binds to phosphorylated R-smad.
10. For the osteopetrosis disease, which is most prevalent among postmenopausal women. Which signaling molecule may contribute to the calcium resorption regulation?
  - a. AKT
  - b. G protein
  - c. OPG
  - d. PLC
11. In determination of intracellular  $Ca^{2+}$  levels, which dye can be used?
  - a. EtBr
  - b. Fura-2
  - c. SNARF-1
  - d. Cy3
12. Which repair system uses methylation as a cue to repair only the newly made strand of DNA shortly after replication.
  - a. Excision and mismatch repair
  - b. Recombinational repair.
  - c. SOS repair
  - d. Error-prone replication.
13. "Apolipoprotein A" is the major component of
  - a. chylomicron
  - b. LDL
  - c. VLDL
  - d. HDL
14. Which molecule is related to degradation of cyclin B in metaphase.
  - a. Cdc20
  - b. Cdh1
  - c. securin
  - d. Sic1
15. What is the major factor release from mitochondra that leads to apoptosis?
  - a. caspase 3
  - b. caspase 9
  - c. cytochrome c
  - d. p450

B. Answer the following questions (70%)

1. Describe the structure of basal lamina, including the major components and the interaction. (10%)
2. Please illustrate major classes of membrane G proteins, and indicate the mechanism of how they are activated and transduce the signal, and how the signal is attenuated. (10%)
3. Describe the mechanism that how DNA damage induce p53 mediated G1 arrest. (10%)
4. PKA (cAPK, protein kinase A), PKB (protein kinase B), and PKC (protein kinase C) are important Ser/Thr protein kinases in cell. What are the major pathways to activate them (Please address the ligand/receptor, and how the kinase is activated?) (10%)
5. What is "loss of heterozygosity" in cancer cells? Explain the possible mechanisms? (10%)
6. Reading the abstract and answer questions (20%)

Title: Identification of pathways regulating cell size and cell-cycle progression by RNAi

Many high-throughput loss-of-function analyses of the eukaryotic cell cycle have relied on the unicellular yeast species *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. In multicellular organisms, however, additional control mechanisms regulate the cell cycle to specify the size of the organism and its constituent organs. To identify such genes, here we analysed the effect of the loss of function of 70% of *Drosophila* genes (including 90% of genes conserved in human) on cell-cycle progression of S2 cells using flow cytometry. To address redundancy, we also targeted genes involved in protein phosphorylation simultaneously with their homologues. We identify genes that control cell size, cytokinesis, cell death and/or apoptosis, and the G1 and G2/M phases of the cell cycle. Classification of the genes into pathways by unsupervised hierarchical clustering on the basis of these phenotypes shows that, in addition to classical regulatory mechanisms such as Myc/Max, Cyclin/Cdk and E2F, cell-cycle progression in S2 cells is controlled by vesicular and nuclear transport proteins, COP9 signalosome activity and four extracellular-signal-regulated pathways (Wnt, p38<sup>β</sup>MAPK, FRAP/TOR and JAK/STAT). In addition, by simultaneously analysing several phenotypes, we identify a translational regulator, eIF-3p66, that specifically affects the Cyclin/Cdk pathway activity.

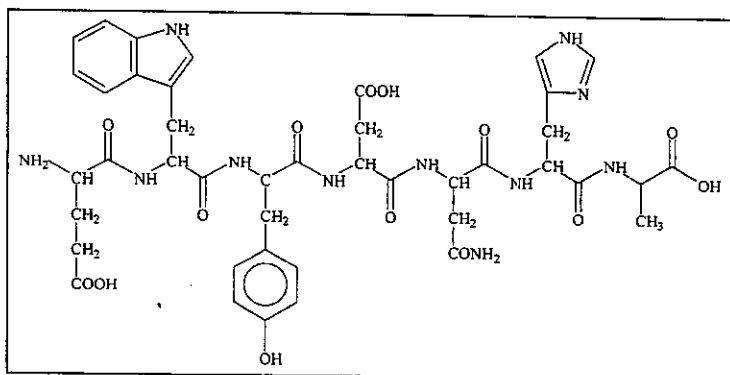
Questions:

- a. S2 cells are used in this study, what is the animal source of the cell line: (5%)
- b. Explain how flow cytometry is used for cell cycle analysis.(5%)
- c. Please describe the principle of RNAi technique, and describe how you can use RNAi technique to search for the genes involved in cell cycle control.(5%)
- d. Please describe the roles of Myc/Max, Cyclin/Cdk, and E2F in cell cycle control. (5%)

Choice the correct **ONE** to each question (2 points each)

- (1) **The number of hydrogen bonds between the two strands of the duplex oligonucleotide illustrated below is**  
 AGCTC  
 TCGAG  
 (a) 5 (b) 13 (c) 26 (d) 15
- (2) **Metals found as natural constituents of proteins include all of the following EXCEPT**  
 (a) Cobalt (b) Mercury (c) Iron (d) Copper
- (3) **In the transamination of amino acids all of the following statements are true EXCEPT**  
 (a) Pyridoxal phosphate is a coenzyme (b) The catalytic mechanism is Ping-Pong mechanism  
 (c) The amino acid acceptor is typically an  $\beta$ -keto acid (d) Ammonium is neither consumed nor produced
- (4) **During collagen synthesis, intracellular events that take place include**  
 (a) Hydroxylysine is incorporated into collagen during protein synthesis (b) Vitamine C is a cofactor for the biosynthesis of collagen (c) Formation of globular structure (d) The secondary structure is  $\beta$ -sheet
- (5) **The following sequence could appear during the following situations EXCEPT**  
 5'-GTAAC-3'  
 3'-CAUUG-5'  
 (a) Transcription (b) Alternative splicing (c) Hybridization (d) Reverse transcription
- (6) **The binding of the following substances can interact with its receptor and finally enter into cell nucleic to regulate the gene expression EXCEPT**  
 (a) Estrogen (b) Androgen (c) Vitamin D (d) Epinephrine
- (7) **Even when a gene is available and its sequence of nucleotides is known, chemical studies of the protein are still required to determine:**  
 (a) the location of disulfide bonds (b) the number of amino acids in the protein (c) the amino-terminal amino acid (d) molecular weight of the protein.
- (8) **Two enzymatic activities that catalyze the bulk of the nitrogen flow from amino acids to ammonia are**  
 (a) transaminases and glutaminases (b) Transaminases and glutamate dehydrogenase (c) Transaminases and Aspartases (d) Transaminases and amino acid oxidases
- (9) **All of the following statements regarding the zymogen are correct EXCEPT**  
 (a) Pepsinogen is activated by autocatalysis (b) Trypsinogen is activated by chymotrypsin (c) Blood clotting is associated with activation of zymogen (d) Zymogen is activated by proteolytic cleavage
- (10) **Which of the following is not normally found in proteins?**  
 (a) 4-hydroxyproline (b)  $\gamma$ -carboxyglutamic acid. (c) ornithin (d) glutamine

- (11) The following statement is a posttranslational modification EXCEPT  
 (a) Glycosylation (b) Sumoylation (c) Acetylation (d) Guanidination
- (12) The phase transition temperature of membrane is affected by EXCEPT  
 (a) Flipase (b) Phospholipids (c) Membrane proteins (d) Length of fatty acid chain
- (13) The following compounds are derived from cholesterol EXCEPT  
 (a) Progesterone (b) Testosterone (c) Bile acid (d) Lanosterol
- (14) What is the sequence of the following peptide  
 (a) Glu-Trp-Phe-Asp-Asn-His-Ala. (b) Glu-Trp-Tyr-Asp-Asn-His-Ala (c)  
 Glu-Trp-Tyr-Asp-Asn-His-Leu (d) Glu-Tyr-Trp-Asp-Asn-His-Ala.



- (15) Correct statements regarding arachidonic acid include all of the following EXCEPT  
 (a) It is a precursor of thromboxane A<sub>2</sub> (b) It can be derived from linoleic acid (c) It is an activator of phospholipase A<sub>2</sub> (d) It activates lipoxygenase
- (16) Allosteric modulators influence enzyme activities by  
 (a) competing for the catalytic site with the substrate (b) binding to the site on the enzyme molecule distinct from the catalytic site (c) changing the nature of the product formed (d) Changing the substrate-specificity
- (17) Which one of the following sugars is NOT commonly found in cell surface glycoproteins  
 (a) Galactose (b) Sialic Acid (c) Ribose (d) Mannose
- (18) Double-stranded regions of RNA:  
 (a) are less stable than double-stranded regions of DNA (b) can be observed in the laboratory, but probably have no biological relevance (c) can form between two self-complementary regions of the same single strand of RNA. (d) have the two strands arranged in parallel (unlike those of DNA, which are antiparallel).
- (19) Identify the INCORRECT statement  
 (a) The quaternary structure of a protein describes the interaction of two or more polypeptide chains (b) The quaternary structure of a protein could be elucidated by X-ray crystallography (c) The tertiary structure of a protein could be identified by

circular dichroism measurement (d) The secondary structure of protein could be assessed by bioinformatics methods

(20) The activity of aspartate transcarbamoylase is affected by ATP acting as a  
(a) homotropic inhibitor (b) heterotropic inhibitor (c) homotropic activator (d) heterotropic activator

(21) Which one of the following is a glycosaminoglycan

(a) Keratin (b) Desmosine (c) Heparin (d) Dextrin

(22) The type of motion least common in biological membranes is:

(a) flip-flop diffusion of phospholipid from one monolayer to the other (b) lateral diffusion of individual lipid molecules within the plane of each monolayer. (c) lateral diffusion of membrane proteins in the bilayer. (d) random motion of the fatty acyl side chains in the interior of the phospholipid bilayer.

(23) Of the following lipid components found in the human erythrocyte plasma membrane, which one is likely to be present only in the outer monolayer of the lipid bilayer forming the plasma membrane

(a) cholesterol (b) lecithin (c) phosphatidylethanolamine (d) sphingomyelin

(24) Which one of the following statements is INCORRECT?

(a) Supercoiling can occur only in DNA that is topologically constrained  
(b) Naturally occurring DNA within cells is generally negatively supercoiled  
(c) Positive supercoiling could occur in DNA behind of a replication fork  
(d) DNA gyrase can introduce negative supercoiling into DNA

(25) Agarose gel electrophoresis typically separates DNA fragments on the basis of

(a) Size (b) Single base differences (c) Charges per unit length (d) The binding with ethidium bromide

(26) For a pure enzyme

(a)  $K_m$  is maximum (b)  $K_m$  is minimum (c) The specific activity is a minimum (d) The specific activity is maximum

(27) Double reciprocal plots of kinetic data for an enzyme in the presence or absence of a reversible inhibitor were straight lines. The following information was obtained from these graphs

No inhibitor present  $1/V_{max} = 50 \text{ sM}^{-1}$   $1/[S] = -4 \times 10^2 \text{ M}^{-1}$

Inhibitor present  $1/V_{max} = 80 \text{ sM}^{-1}$   $1/[S] = -4 \times 10^2 \text{ M}^{-1}$

What is the value for the  $K_{cat}(\text{inhibitor})/K_{cat}(\text{no inhibitor})$  ratio?

(a) 1.6 (b) 1 (c) 0.625 (d) 0.8

(28) 5-Bromouracil can be incorporated into DNA because it is an analogue of

(a) Uridine (b) Thymine (c) Cytosine (d) Deoxyuridine 5'-triphosphate

(29) Which of the following compounds would have the most direct adverse on RNA synthesis in cells

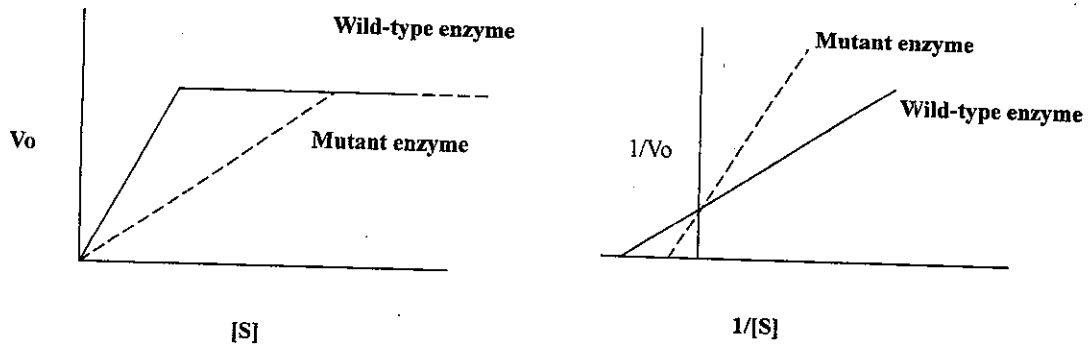
- (a) Chloramphenicol (b) Cycloheximide (c) Actinomycin D (d) Tetracycline
- (30) Which of the following statements comparing A and B form of DNA is TRUE?**
- (a) Both are left handed helices (b) The major groove and minor grooves are identical in both forms of DNA (c) The sugar puckers are different in both forms of DNA (d) The DNA-RNA hybrid structure is similar to B-DNA structure
- (31) The p53 gene product is**
- (a) a G protein (b) a DNA-binding protein (c) A tyrosine kinase (d) a serine/threonine kinase
- (32) A mutation of a particular growth factor receptor protein results in tyrosine kinase activity even in the absence of the appropriate growth factor. If this mutation led to tumor formation, it would be called**
- (a) tumor suppressor gene (b) oncogene (c) protooncogene (d) anti-oncogene
- (33) Eukaryotic gene transcription is associated with**
- (a) constitutive heterochromatin (b) facultative heterochromatin (c) euchromatin (d) Barr bodies
- (34) Given the following strand of mRNA (CUCAAGUGCUUC), identify the strand of DNA from which it was made**
- (a) CUCAAGUGCUUC (b) GAGTTCACGAAG (c) AGACCTGTAGGA (d) GTTGCACCTTGTG

**For the following statement, select all correct answers, there may be more than one (2 points each)**

- (35) If the  $\Phi$  and  $\Psi$  angles of each peptide unit in a protein are known, the following will also be determined:**
- (a) Complete secondary structure (b) Complete primary structure (c) Functional site of the protein (d) Complete tertiary structure (e) Complete quaternary structure
- (36) Select the CORRECT ones for the phospholipid bilayer**
- (a) Asymmetric distribution (b) is entirely hydrophobic (c) contains cholesterol (d) contains lipid raft (e) the fluidity is not affected by temperature
- (37) Which statement is not true of membrane carbohydrates?**
- (a) Most are bound to proteins (b) They increase membrane fluidity (c) Proteoglycan is not membrane carbohydrate (d) They all are added to proteins in the Golgi complex (e) Glycoproteins could be classified as N-type and O-type
- (38) Starting from double stranded DNA with a G content of 35% answer the following question**
- (a) The A content is 65% (b) After translation, the content of A in the resulting mRNA is 15% (c) After translation, the content of T in the resulting mRNA is 15% (d) After translation, the content of G in the resulting mRNA is 35% (e) After duplication, the

content of G in the resulting double stranded DNA is 70%

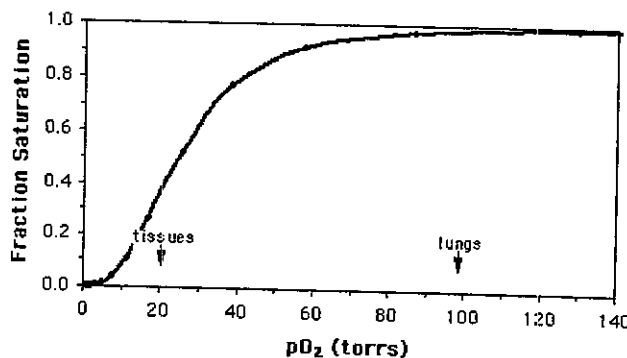
(39) A mutant enzyme has an increased  $K_m$  and unchanged  $V_{max}$  as compared to the wild-type enzyme. Based on this information, which of the following are true?



- (a) The mutation has increased the enzyme's ability to bind substrate
  - (b) The mutation has increased the rate at which the enzyme-substrate complex dissociates to enzyme and free product
  - (c) The mutation has decreased the rate at which the free substrate and enzyme are converted to the substrate-enzyme complex
  - (d) The mutated site is involved in the change of  $K_{cat}$  value
  - (e) The enzymatic activity follows Michaelis-Menten equation
- (40) Which of the amino acid sequences bellows is most likely to be the  $\alpha$ -helical structure
- (a) LSFAAMMLAG (b) GVHLDIEVDA (c) KEKAKGKSEE (d) INEGFDLLRSG
  - (e) AGPGPADEKEG

Short answer (each 5 points)

(1) The graph shows the  $O_2$  saturation curve for human hemoglobin (Hb A). Draw a



curve on the graph for the  $O_2$  saturation expected for fetal hemoglobin (Hb F). Briefly explain why your Hb F curve corresponds to an advantage for the developing fetus.

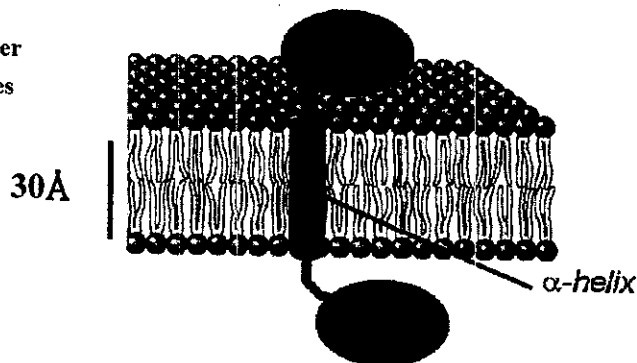


(2) A biochemist is attempting to separate a DNA-binding protein (protein X) from other proteins in a solution. Only three other proteins (A, B, and C) are present. The proteins have the following properties:

	pI (isoelectric point)	size Mr	bind to DNA
protein A	7.4	82,000	yes
protein B	3.8	21,500	yes
protein C	7.9	23,000	no
protein X	7.8	22,000	yes

What type of protein separation techniques might she use to separate: (a) protein X from protein A? (b) protein X from protein B? (c) protein X from protein C?

(3) A protein that span the lipid bilayer contains  $\alpha$ -helix. How many residues would be required to span the membrane?



(4) Compare the gel mobility shift and DNase footprinting methods of assaying specific DNA-Protein interactions. What information does DNase footprinting provide that gel mobility shift does not?