Indraprastha Institute of Information Technology Delhi (IIITD)
Department of Computational Biotechnology

BIO211 – Cell Biology and Biochemistry

End-Semester Exam (December 10, 2022)

Time duration: 2 hours (03:00-05:00 PM) **Total marks:** 80

These answers are only for reference, the answers for students may vary a bit.
So, please read the answers carefully and award marks accordingly.

Question 1. Neutral sphingomyelinase is an enzyme that converts sphingomyelin into ceramide and phosphocholine. A student studying the kinetics of this enzyme added 3 X 10^{-5} M of sphingomyelin to the reaction and observed an initial velocity of 6.0 µM/min. Calculate the K_M for this reaction, provided the V_{max} is 35 µM/min. **[3 marks]**

$V_0 = V_{max}$ [S] / (K_m + [S])

6 = (35 µM/min X 30 µM) / (K_M + 30 µM)

K_M = (1050 – 180)/6 = 145 µM

Question 2. Differentiate between **any 3** of the following: **[2 marks each]**

(i) Paracrine and autocrine cell signaling

In paracrine signaling, the signal molecules diffuse locally through the extracellular fluid, remaining in the neighborhood of the cell that secretes them. Thus, they act as local mediators on nearby cells. Eg. Many of the signal molecules that regulate inflammation at the site of an infection or that control cell proliferation in a healing wound.

It is a part of paracrine signalling where the cells can respond to the local mediators that they produce themselves. Eg. Cancer cells promote their own survival and proliferation by responding to the growth factors produce by them.

(ii) Transporters and Ion channels

Transporters: Undergo conformational transformation, Rate of transportation is less compared to channels, can couple the solute flow to an energy source to carry out active transportation

Channels: Ion passage depends on the diameter and shape of the ion channel and on the distribution of the charged amino acids that line it, Ion channels do not undergo conformational changes, Rate of transportation is much faster in comparison to transporters, does not allow activate transport of ions.

(iii) Cohesins and condensins

The sister chromatids are held together by protein complexes called cohesins, which assemble along the length of each chromatid as the DNA is replicated. These proteins are crucial for proper chromosome segregation, and it is broken completely only in late mitosis to allow the sisters to be pulled apart by the mitotic spindle.

Condensins help in chromosome condensation when the cell enters the M phase of cell division cycle. Condensins reduce mitotic chromosomes to compact bodies that can be more easily segregated within the crowded confines of the dividing cell.

(iv) DNA and RNA

- DNA is a double-stranded molecule, while RNA is a single-stranded molecule.
- DNA contains the sugar deoxyribose, while RNA contains the sugar ribose.
- DNA uses the bases- adenine, thymine, cytosine, and guanine; RNA uses adenine, uracil, cytosine, and guanine.

Question 3. The DNA isolated from two newly identified bacterial species (AB and XY) was found to have 14% and 38% adenine respectively.

(i) What relative proportional of adenine, guanine, thymine and cytosine are expected in these two DNA samples? What assumptions did you take into consideration for this answer?

AB: A= 14%, T=14%, G=36%, C=36% **[1 mark for each point]**
XY: A= 38%, T=38%, G=12%, C=12%
DNA is composed of only 4 nucleotides - A, T, G and C, where A always pairs with T and G always pairs with C. Therefore, [A] = [T] and [G] = [C].

(ii) Which of these two bacterial species you think was most likely isolated from a hot water spring (82^0C), and why? **[2 marks]**
Species AB with higher G+C content is most likely the thermophilic bacteria. Its DNA will have higher melting temperature and therefore will be more stable at the temperature of the hot spring.

Question 4. While working with cultured cells, the student released that the cells can uptake glucose only when the growth medium is supplemented with Na^+, else the cells die even after having surplus glucose concentration in the culture medium? Suggest a possible explanation for this situation. **[3 marks]**
The transporter that uptakes glucose is a symport that work along with Na^+.

Question 5. Arrange the following in increasing rate of simple diffusion across the lipid bilayer membrane: Glucose, CO_2, Na^+, Water
Na+ < Glucose < Water < CO_2 **[0.5 mark for each in the correct order]**

Question 6. Explain the molecular mechanism that gets activated at the G1-to-S transition to prevent cells from replicating the damaged DNA. **[4 marks]**
DNA damage in G1 causes an increase in both the concentration and activity of a protein called p53, which is a transcription regulator that activates the transcription of a gene encoding a Cdk inhibitor protein called p21. The p21 protein binds to G1/S-Cdk and S-Cdk, preventing them from driving the cell into S phase. The arrest of the cell cycle in G1 gives the cell time to repair the damaged DNA before replicating it. If the DNA damage is too severe to be repaired, p53 can induce the cell to kill itself by undergoing a form of programmed cell death called apoptosis. If p53 is missing or defective, the unrestrained replication of damaged DNA leads to a high rate of mutation and the production of cells that tend to become cancerous.

Question 7. Show the rearrangement of Michaelis-Menten equation for Lineweaver-Burk representation. Draw a schematic of Lineweaver-Burk plot and mark the various parameters related to enzyme kinetics that can be estimated from the plot. **[4 marks]**

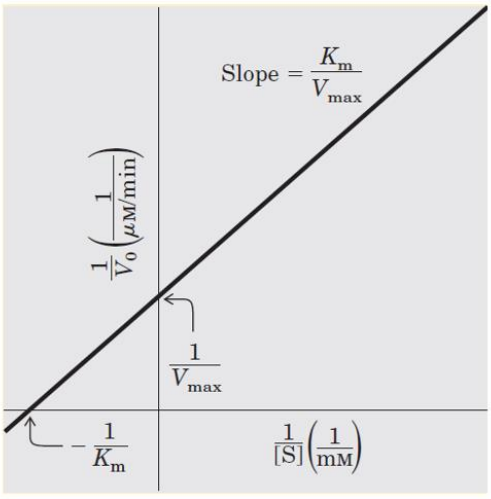
Rewritten in the following manner, the equation gives a straight line, which is useful to determinate the parameters K_m and V_{max} (Lineweaver-Burk representation):

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{max}[S]}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

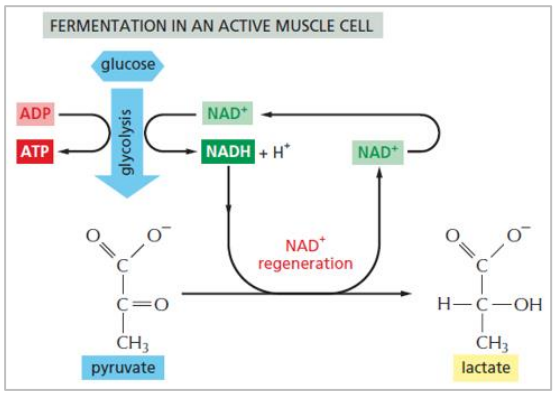


Question 8. Briefly discuss the three catabolic routes through which pyruvate formed by glycolysis is further metabolized. **[2 marks for each of the route]**
Pyruvate formed by glycolysis is further metabolized via one of three catabolic routes:

1. In aerobic organisms or tissues, under aerobic conditions, glycolysis is only the first stage in the complete degradation of glucose. Pyruvate is oxidized, with loss of its carboxyl group as CO_2, to yield the acetyl

group of acetyl-coenzyme A; the acetyl group is then oxidized completely to CO_2 by the citric acid cycle. The electrons from these oxidations are passed to O_2 through a chain of carriers in the mitochondrion, to form H_2O. The energy from the electron-transfer reactions drives the synthesis of ATP in the mitochondrion.

2.



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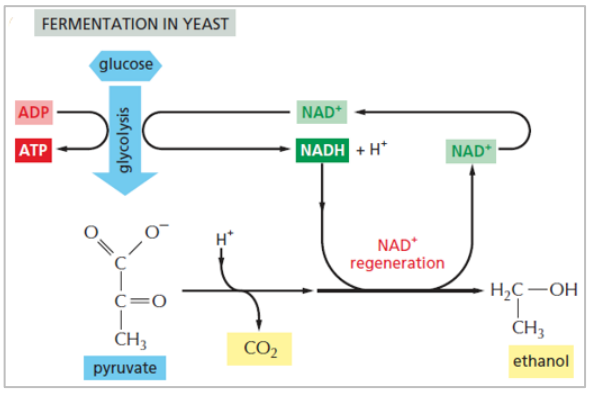


OR

Answer the following:

(i) Why phosphorylation of glycolytic intermediates considered important?

To retain phosphorylated intermediates in the cell. **[1.5 mark]**

(ii) Give the net equation of the overall glycolytic process.

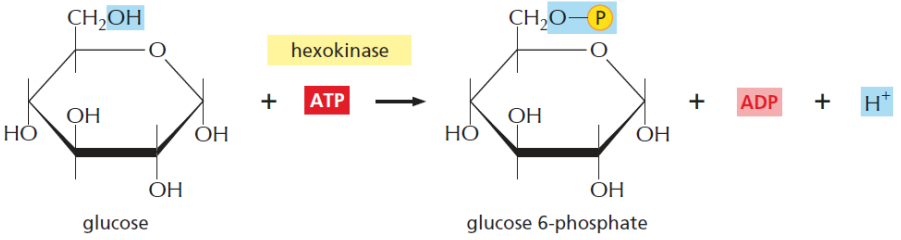
Glucose → 2 Pyruvate + 2 ATP + 2 NADH **[1.5 marks]**

(iii) Give the three steps in glycolysis that strongly favor the direction of glucose breakdown making the process effectively irreversible.

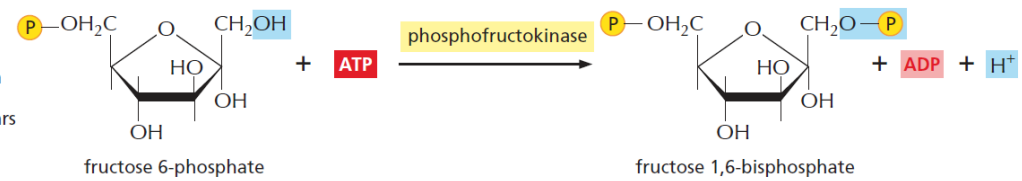
Structures are not required. If reaction has been explained with correct reactants, products and enzyme involved, give marks. **[1 mark for each step]**

(Step 1, 3 and 10) → this is wrong answer

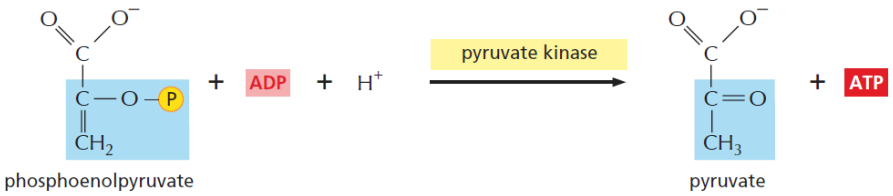
Step 1 Glucose is phosphorylated by ATP to form a sugar phosphate. The negative charge of the phosphate prevents passage of the sugar phosphate through the plasma membrane, trapping glucose inside the cell.



Step 3 The new hydroxyl group on carbon 1 is phosphorylated by ATP, in preparation for the formation of two three-carbon sugar phosphates. The entry of sugars into glycolysis is controlled at this step, through regulation of the enzyme *phosphofructokinase*.



Step 10 The transfer to ADP of the high-energy phosphate group that was generated in step 9 forms ATP, completing glycolysis.



Question 9. Answer the following: **[1 mark for each correctly filled blank]**

(i) Kinetochores attach the chromosomes to the mitotic spindle.
(ii) Dihedral angles/torsion angles account for the four body atomic interactions.
(iii) Microtubules and actin are the two cytoskeletal structures that mediate the M phase in animal cell cycle.
(iv) Peroxisomes contain hydrogen peroxide to inactive toxic molecules.
(v) Presence of phosphate makes a nucleotide negatively charged.
(vi) Histone proteins help in the condensation of DNA molecules.
(vii) The ends of eukaryotic chromosomes are replicated with the help of telomerase enzyme.
(viii) Coding regions within a gene are known as exons.
(ix) The initiator tRNA carries formylated methionine amino acid in bacteria.

Question 10. Arrange the following events in order of their occurrence after a presynaptic neuron releases acetylcholine into the synaptic cleft.

(i) Vesicles carrying the neurotransmitter fuse with the cell membrane.
(ii) Ligand-gated Na^+ channels open, causing an influx of Na^+ ions.
(iii) Voltage-gated Na^+ channels open in the axon.
(iv) Membrane depolarization triggers voltage-gated Ca^{2+} channels to open.
(v) Local membrane depolarization in the axon triggers an efflux of K^+.

ii, iii, v, iv, i **[2 marks for the correct order]**

Question 11. For each of the following categories, give one example of biological process that can be defined using that timescale. **[3 marks]**

(i) Procedural time − Polymerization of nucleotides by RNA/DNA polymerase
(Replication, transcription or translation are wring answers)
(ii) Relative time − Cell division cycle
(iii) Manipulative time − Enzyme catalyzed reactions in a living cell

Question 12. Define **any 5** of the following terms. **[2 marks each]**

(i) Electrochemical gradient.

The net force driving a charged solute across a cell membrane is a composite of two forces: (i) the concentration gradient and (ii) the membrane potential. This net driving force, called the solute's electrochemical gradient, therefore determines the direction that each solute will flow across the membrane by passive transport.

(ii) Standard free-energy change ($\Delta G'^{o}$)

Under standard conditions (298K = 25^{o}C), when reactants and products are initially present at 1 M concentrations or, for gases, at partial pressures of 101.3 kilopascals (kPa), or 1 atm, the force driving the system toward equilibrium is defined as the standard free-energy change, $\Delta G'^{o}$.

(iii) Nucleophiles

A nucleophile is electron rich species and donates electron pairs to electron deficient species. Examples include carbanions, halogen anions (I^-, Cl^-, Br^-), the hydroxide ion (OH^-).

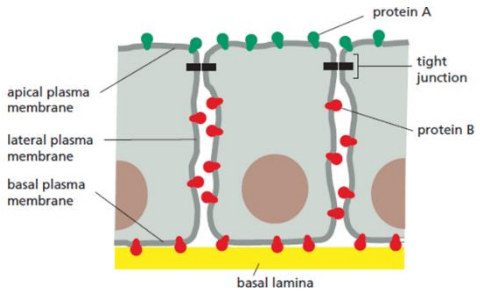
(iv) Substrate level phosphorylation

Substrate-level phosphorylation is a metabolic reaction that results in the production of ATP or GTP by the transfer of a phosphate group from a substrate directly to ADP or GDP.

(v) Cell cortex

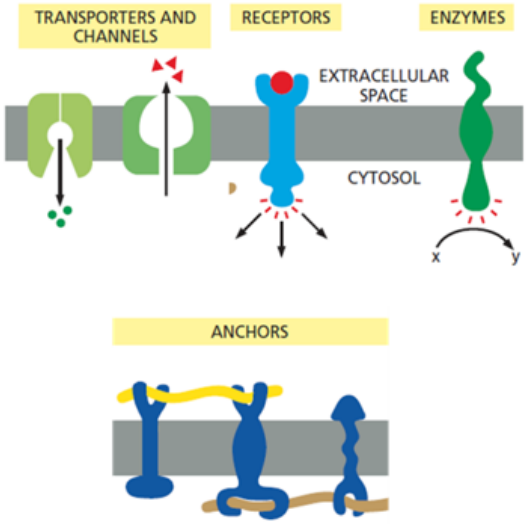
The cell cortex is typically defined as a thin layer of actin meshwork that uniformly underlies the plasma membrane of the entire cell.

(vi) Tight junctions



These are diffusion barriers can restrict particular membrane components to one membrane domain.

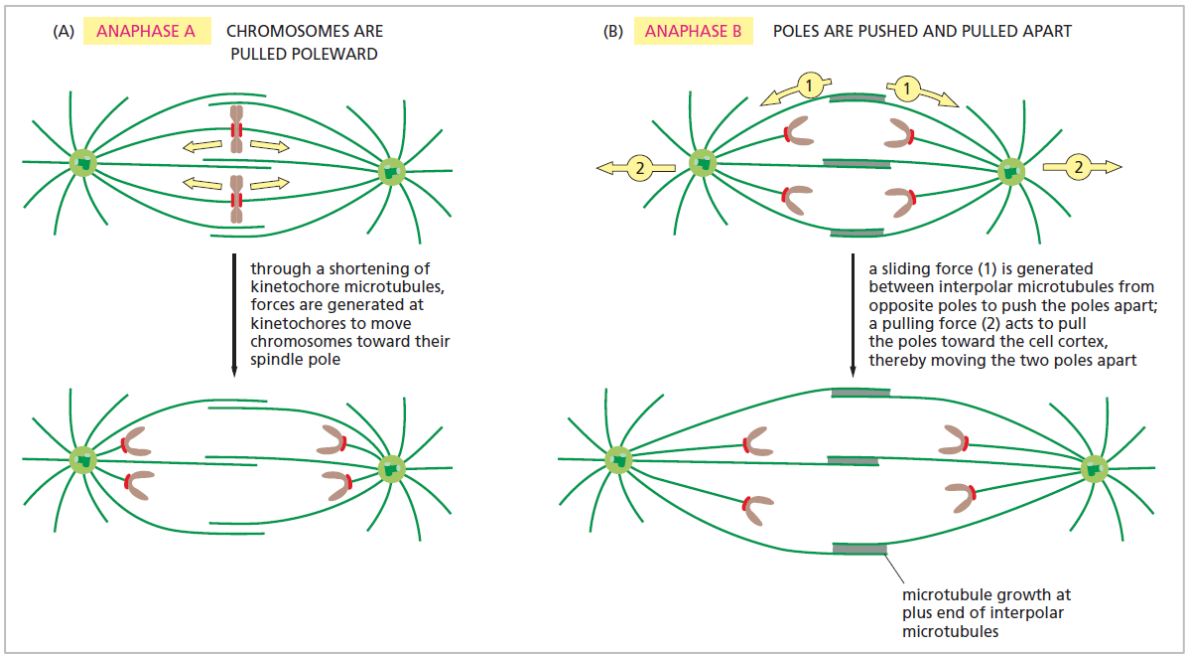
Question 13. What are the different functional classes of membrane proteins? Give an example of each. **[6 marks]**



Functional Class	Protein Example
Transporters	Na^+ pump
Ion channels	K^+ leak channel
Anchors	integrins
Receptors	platelet-derived growth factor (PDGF) receptor
Enzymes	adenylyl cyclase

OR

Explain the two processes mediated by microtubules that segregate daughter chromosomes at anaphase.



Question 14. Give one example in each of the following categories of extracellular signaling molecules, and also categorize them as short-range or long-range signals. **[1.5 marks each]**

(i) Hormone – Long range

(ii) Local mediator – Short range

(iii) Neurotransmitter – Long range

(iv) Contact-dependent signaling molecule – Short range

Refer to the following tables for examples:

Hormones
Adrenaline (epinephrine)
Cortisol
Estradiol
Insulin
Testosterone
Thyroid hormone (thyroxine)

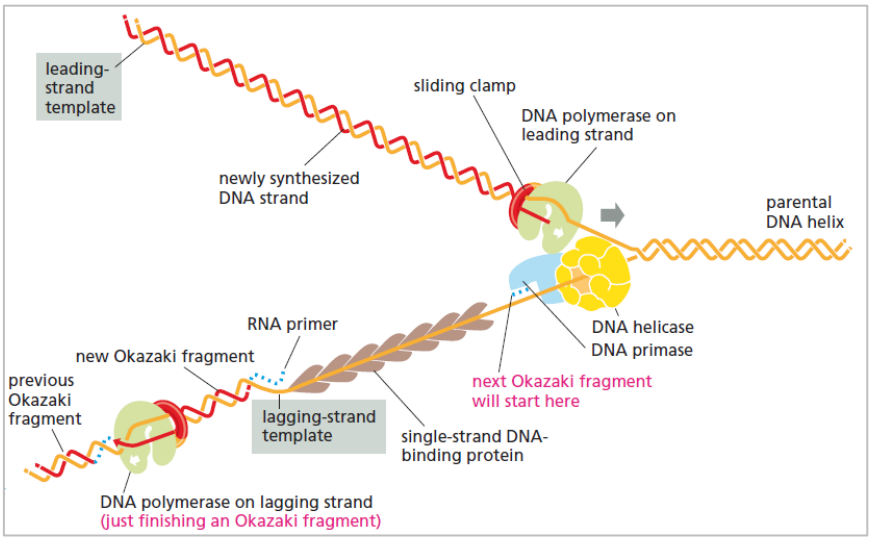
Local Mediators
Epidermal growth factor (EGF)
Platelet-derived growth factor (PDGF)
Nerve growth factor (NGF)
Histamine
Nitric oxide (NO)

Neurotransmitters
Acetylcholine
γ-Aminobutyric acid (GABA)

Contact-dependent Signal
Delta

Question 15. Draw a well labelled diagram for **any one** of the following: **[6 marks]**

(i) A replication fork illustrating the coordinated functioning all of the proteins involved in DNA replication process.



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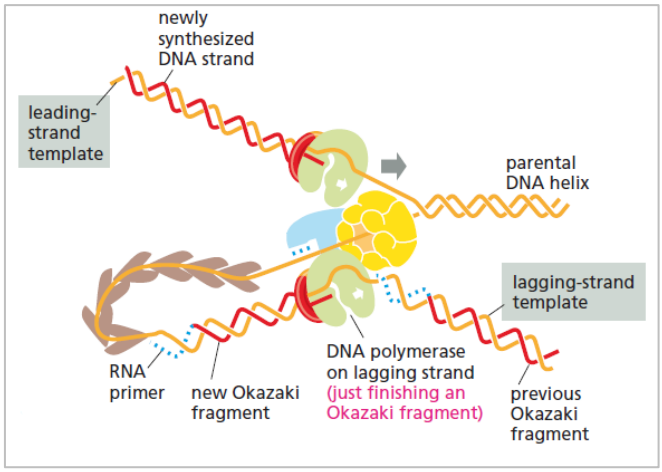
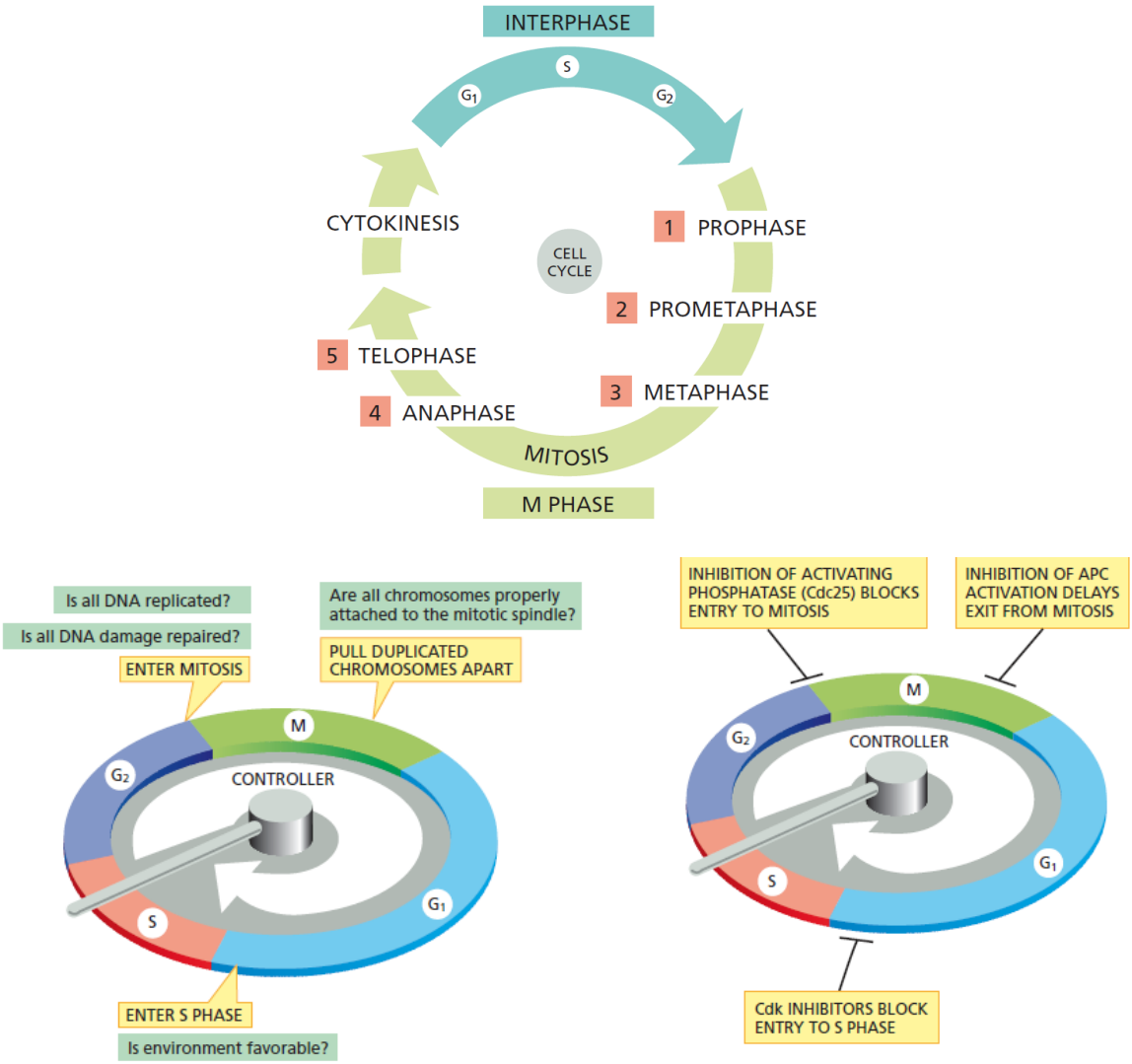


(or something similar)

(ii) A cell division cycle with all the different phases in sequential order, including the details of M phase. Also mark the three main check-points in the cell-cycle along with the important players of the cell-cycle control system active during that phase.



Question 16. The equilibrium constant, K_{eq} for the following reaction ATP → ADP + Pi is 2 X 10^5 M. If the measured cellular concentrations at an instance of time are [ATP] = 5 mM, [ADP] = 2.5 mM, and [Pi] = 6 mM, is the reaction at equilibrium in living cells? Justify your answer. **[4 marks]**

K_{eq} = [ADP][Pi]/[ATP]

For the given concentrations [ADP][Pi]/[ATP] = (2.5mM) (6mM)/(5mM) = 3 mM = $3x10^{-3}$ M

This value is far from the equilibrium constant of the reaction ($2x10^5$M); therefore, the reaction is also far from the equilibrium. [ATP] is much higher and [ADP] is much lower that is expected at the equilibrium.