Question 1. (a) Explain the following statements (Any FOUR) :

(i) Steroid hormones can act as transcription regulators.

- Steroid hormones are lipid-soluble molecules that can readily pass through the plasma membrane into the cytoplasm.
- Once inside the cell, they bind to specific intracellular receptor proteins, forming a hormone-receptor complex.
- This complex then translocates into the nucleus, where it binds to specific DNA sequences called hormone response elements (HREs) in the promoter region of target genes.
- Binding of the hormone-receptor complex to HREs can either activate or repress the transcription of these genes, thereby regulating the synthesis of specific proteins and ultimately influencing cellular functions.

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(ii) Mutation in KDEL sequence of a resident ER protein may lead to its loss from the cell.

- The KDEL sequence (Lys-Asp-Glu-Leu) is a specific four-amino acid C-terminal motif found on many resident proteins of the endoplasmic reticulum (ER).
- This sequence acts as an ER retention signal, ensuring that these proteins are retrieved from the Golgi apparatus and returned to the ER if they accidentally escape.
- The retrieval mechanism involves KDEL receptors in the Golgi that bind to the KDEL sequence and package the proteins into COPI-coated vesicles for retrograde transport back to the ER.
- A mutation in the KDEL sequence would prevent the resident ER protein from binding to its receptor, leading to its inability to be retrieved from the Golgi.

- Consequently, the protein would continue through the secretory pathway and eventually be secreted from the cell or delivered to other organelles, leading to its loss from the ER.

(iii) Cancer patients undergoing chemotherapy often need bone marrow transplantation.

- Chemotherapy drugs are designed to kill rapidly dividing cells, which include cancer cells.
- However, these drugs are not specific to cancer cells and also target other rapidly dividing healthy cells in the body.
- Bone marrow is a site of continuous production of blood cells (red blood cells, white blood cells, and platelets) from hematopoietic stem cells, making its cells highly proliferative.
- Chemotherapy often damages or destroys the hematopoietic stem cells in the bone marrow, leading to a severe reduction in the production of blood cells (myelosuppression).
- This myelosuppression results in conditions like anemia (low red blood cells), increased susceptibility to infections (low white blood cells), and bleeding problems (low platelets).
- To restore the healthy blood cell production and allow patients to recover from the damaging effects of chemotherapy, a bone marrow transplantation is often necessary to replace the damaged stem cells with healthy ones.

(iv) DAG and IP3 act as second messengers.

- Second messengers are intracellular signaling molecules that relay signals from receptors on the cell surface to target molecules within the cell, amplifying the initial signal.
- Diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) are two important second messengers generated upon the activation of

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phospholipase C (PLC) by various cell surface receptors, such as G-protein coupled receptors (GPCRs).

- Upon activation, PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) into DAG and IP3.
- DAG remains embedded in the plasma membrane and activates protein kinase C (PKC), which then phosphorylates various target proteins, leading to diverse cellular responses.
- IP3 is water-soluble and diffuses into the cytoplasm, where it binds to IP3 receptors on the endoplasmic reticulum (ER), triggering the release of calcium ions (Ca^{2+}) from the ER stores into the cytoplasm.
- The released Ca^{2+} then acts as another second messenger, activating various calcium-dependent enzymes and processes.

(v) Pre-sequences of mitochondrial proteins are positively charged whereas the transit peptides of chloroplast proteins are not.

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- Mitochondrial precursor proteins destined for import into the mitochondria typically have N-terminal pre-sequences that are rich in basic (positively charged) amino acids such, as arginine and lysine, and poor in acidic amino acids.
- This positive charge is crucial for their interaction with the negatively charged outer mitochondrial membrane and the import receptors (TOM complex) during the initial stages of translocation. It also helps in guiding the protein through the membrane channels.
- In contrast, chloroplast precursor proteins contain N-terminal transit peptides that are typically uncharged or have a net neutral charge.
- These transit peptides are rich in hydroxylated amino acids (serine and threonine) and often lack acidic amino acids, but they do not exhibit the strong positive charge characteristic of mitochondrial pre-sequences.

- The distinct characteristics of these targeting sequences reflect the different recognition and translocation mechanisms employed by mitochondria and chloroplasts for protein import.

Question 1. (b) Discuss the contribution of following scientists :

(i) Tim Hunt

- Tim Hunt is a British biochemist known for his significant contributions to the understanding of cell cycle regulation.
- In 1982, while studying sea urchin embryos, he discovered cyclins, a class of proteins whose concentrations oscillate during the cell cycle, rising during interphase and falling sharply at mitosis.
- He demonstrated that these cyclins are crucial for activating cyclin-dependent kinases (CDKs), which in turn drive the progression through different phases of the cell cycle.
- His work, along with that of Paul Nurse and Leland Hartwell, revolutionized the understanding of cell cycle control, leading to their shared Nobel Prize in Physiology or Medicine in 2001.

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(ii) Gunter Blobel

- Gunter Blobel was a German-American biologist who made groundbreaking discoveries in the field of protein targeting and cellular compartmentalization.
- In the 1970s, he proposed the "signal hypothesis," which explained how newly synthesized proteins are targeted to their correct destinations within the cell, such as the endoplasmic reticulum, mitochondria, or chloroplasts.
- He postulated that proteins destined for secretion or insertion into membranes carry specific "signal sequences" at their N-terminus that direct them to the appropriate cellular compartment.

- His work laid the foundation for understanding protein trafficking and translocation across membranes, for which he was awarded the Nobel Prize in Physiology or Medicine in 1999.

(iii) Yoshio Masui and Clement Markert

- Yoshio Masui and Clement Markert are recognized for their pioneering work on the maturation promoting factor (MPF).
- In the early 1970s, Masui and Markert, working independently, demonstrated the existence of a cytoplasmic factor in amphibian oocytes that could induce meiotic maturation when transferred to immature oocytes.
- This factor, later named Maturation Promoting Factor (MPF), was shown to be responsible for initiating and driving the oocyte through meiosis.
- Their discovery of MPF was a crucial step in understanding the universal mechanisms that control cell cycle progression, particularly the entry into M-phase (mitosis or meiosis). MPF was later identified as a complex of CDK1 and cyclin B.

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Question 1. (c) Write the biological functions of the following proteins :

(i) SNARE

- SNARE (Soluble N-ethylmaleimide-sensitive factor Attachment protein Receptor) proteins are a large family of integral membrane proteins that play a central role in membrane fusion events within eukaryotic cells.
- They mediate the specific docking and fusion of vesicles with their target membranes (e.g., vesicles with the plasma membrane during exocytosis, or vesicles with specific organelles).
- SNAREs ensure the precise delivery of cargo by forming a stable, four-helix bundle complex between the vesicle (v-SNARE) and target

membrane (t-SNARE), pulling the membranes together and facilitating lipid bilayer fusion.

- They are critical for processes such as neurotransmitter release, hormone secretion, and intracellular trafficking.

(ii) PDE

- PDE stands for Phosphodiesterase.
- Phosphodiesterases are a family of enzymes that break down cyclic nucleotides, specifically cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), into their inactive 5'-monophosphate forms.
- By hydrolyzing cAMP and cGMP, PDEs regulate the concentration of these important second messengers within the cell.
- This regulation is crucial for modulating a wide array of cellular processes, including signal transduction pathways, muscle contraction, nerve impulse transmission, and inflammation. Many drugs target PDEs to influence these processes.

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(iii) STAT

- STAT stands for Signal Transducers and Activators of Transcription.
- STAT proteins are a family of transcription factors that play a critical role in mediating the signaling of cytokines and growth factors from the cell surface to the nucleus.
- Upon activation of cytokine receptors, Janus kinases (JAKs) phosphorylate STAT proteins.
- Phosphorylated STATs then dimerize, translocate to the nucleus, and bind to specific DNA sequences in the promoter regions of target genes, thereby regulating gene expression.

- STAT pathways are involved in various biological processes, including cell proliferation, differentiation, immune response, and apoptosis.

(iv) Caspases

- Caspases are a family of cysteine-dependent aspartate-directed proteases.
- They are central executioners of programmed cell death, primarily apoptosis (but also involved in some forms of pyroptosis).
- Caspases are synthesized as inactive zymogens (procaspases) and are activated by proteolytic cleavage in response to pro-apoptotic signals.
- Once activated, they cleave a wide range of cellular substrates at specific aspartate residues, leading to the systematic dismantling of the cell, DNA fragmentation, and the formation of apoptotic bodies, without inducing inflammation.
- Caspases are divided into initiator caspases (e.g., caspase-8, -9, -10) and executioner caspases (e.g., caspase-3, -6, -7).

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Question 2. (a) Explain the structure of G-protein coupled receptors and their mechanism of action with an example. How does intake of caffeine affect GPCR signaling?

- Structure of G-protein Coupled Receptors (GPCRs):
    - GPCRs are a large and diverse family of integral membrane proteins that respond to a wide variety of extracellular signals, including hormones, neurotransmitters, light, and odorants.
    - They are characterized by a common structural motif: a single polypeptide chain that traverses the plasma membrane seven times, forming seven transmembrane α-helices.

- These seven helices are connected by extracellular loops (involved in ligand binding) and intracellular loops (involved in G-protein coupling).
- The N-terminus of the receptor is located extracellularly, and the C-terminus is located intracellularly.
- The intracellular loops, particularly the third intracellular loop and the C-terminal tail, are crucial for interacting with and activating heterotrimeric G-proteins.

- Mechanism of Action (with example - Glucagon receptor signaling):
  - GPCRs mediate their effects by interacting with heterotrimeric G-proteins, which consist of three subunits: alpha (α), beta (β), and gamma (γ). In an inactive state, the Gα subunit is bound to GDP and is associated with the G$\beta\gamma$ dimer.
  - When an extracellular ligand (e.g., glucagon) binds to the GPCR (e.g., glucagon receptor), it induces a conformational change in the receptor.
  - This conformational change activates the receptor, enabling it to act as a guanine nucleotide exchange factor (GEF) for the associated heterotrimeric G-protein.
  - The activated receptor promotes the dissociation of GDP from the Gα subunit and the binding of GTP in its place.
  - GTP binding causes the Gα subunit to dissociate from the receptor and the G$\beta\gamma$ dimer, leading to the activation of both the Gα-GTP complex and the G$\beta\gamma$ dimer.
  - These activated subunits then diffuse along the membrane and interact with specific effector proteins, such as adenylyl cyclase (AC) or phospholipase C (PLC), initiating a signaling cascade.

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- For example, in glucagon signaling, the activated Gα subunit (specifically Gα_s) stimulates adenylyl cyclase, leading to the production of cyclic AMP (cAMP) from ATP.
- cAMP acts as a second messenger, activating protein kinase A (PKA), which then phosphorylates various target proteins, ultimately leading to glucose mobilization (e.g., breakdown of glycogen in the liver).
- The Gα subunit possesses intrinsic GTPase activity, which eventually hydrolyzes the bound GTP back to GDP, leading to its re-association with the G$\beta\gamma$ dimer and the receptor, thus returning the G-protein to its inactive state and terminating the signal.

- How intake of caffeine affects GPCR signaling:
  - Caffeine primarily exerts its stimulant effects by acting as an antagonist of adenosine receptors, which are a type of GPCR.
  - Adenosine is a neuromodulator that, when bound to its receptors (especially A1 and A2A receptors in the brain), generally promotes drowsiness and inhibits neuronal activity.
  - Caffeine has a similar chemical structure to adenosine and can bind to adenosine receptors without activating them.
  - By binding to and blocking adenosine receptors, caffeine prevents adenosine from binding and exerting its inhibitory effects.
  - This blockade of adenosine receptors leads to increased neuronal activity and the release of excitatory neurotransmitters like dopamine and norepinephrine, resulting in increased alertness, reduced fatigue, and other stimulant effects.
  - Therefore, caffeine indirectly affects GPCR signaling by competitively inhibiting the binding of an endogenous ligand (adenosine) to its specific GPCRs.

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Question 2. (b) Comment on the following :

(i) Conventional chemotherapeutic drugs usually target all dividing cells, leading to common side effects like hair loss, nausea, and vomiting whereas oncogene-targeted drugs specifically act against cancer cells.

- Conventional chemotherapeutic drugs are broadly cytotoxic agents that primarily function by interfering with fundamental cellular processes essential for cell division, such as DNA replication, transcription, or mitosis. Because cancer cells are characterized by uncontrolled and rapid proliferation, these drugs are more damaging to them. However, many healthy cells in the body also divide rapidly, including those in the bone marrow (responsible for blood cell production), hair follicles, gastrointestinal lining, and immune system.
- The indiscriminate targeting of all rapidly dividing cells by conventional chemotherapy leads to a range of systemic side effects. Hair loss occurs because hair follicle cells are rapidly dividing. Nausea and vomiting result from the damage to the rapidly proliferating cells lining the gastrointestinal tract. Myelosuppression (bone marrow suppression) leads to a weakened immune system and anemia.
- In contrast, oncogene-targeted drugs (also known as molecularly targeted therapies) are designed to specifically inhibit the activity of proteins encoded by oncogenes that are aberrantly activated or overexpressed in cancer cells, or to block specific signaling pathways that are crucial for cancer cell growth and survival but less critical for normal cells. These drugs exploit the unique molecular vulnerabilities of cancer cells.
- For example, drugs like Herceptin target the HER2 receptor, which is overexpressed in certain breast cancers, while Imatinib targets the Bcr-Abl fusion protein found in chronic myeloid leukemia. Because these drugs aim at specific molecular targets predominantly found or hyperactive in cancer cells, they generally spare healthy cells that do

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not possess these targets or rely on these pathways to the same extent.

- This targeted approach leads to significantly fewer and less severe side effects compared to conventional chemotherapy, as the damage to normal, healthy cells is minimized. However, even targeted therapies can have side effects, as the targeted pathways might still play some role in normal physiology, or cancer cells can develop resistance over time.

(ii) Treatment of cells with a drug that makes membranes permeable to protons, affect the function of lysosomes.

- Lysosomes are acidic organelles within eukaryotic cells, crucial for the degradation of waste materials, cellular debris, and macromolecules. Their acidic internal environment (pH 4.5-5.0) is maintained by a vacuolar H+-ATPase (V-ATPase) pump, which actively pumps protons (H^+) from the cytoplasm into the lysosomal lumen, consuming ATP.

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- This low pH is essential for the optimal activity of the lysosomal hydrolases (e.g., proteases, lipases, nucleases), which are acid-dependent enzymes responsible for breaking down various substrates.

- If a drug makes membranes permeable to protons, it means that protons can freely diffuse across the lysosomal membrane, rather than being actively pumped and retained. This would lead to the leakage of protons out of the lysosome or prevent their accumulation inside.

- The net effect would be an increase in the intralysosomal pH, making the lysosome less acidic or even neutral.

- Consequently, the lysosomal hydrolases, which function optimally at acidic pH, would become inactive or significantly less efficient at a higher pH. This inactivation would impair the lysosome's ability to

degrade cellular waste and foreign materials, leading to the accumulation of undigested substances within the cell.

- Such accumulation can lead to cellular dysfunction, toxicity, and various lysosomal storage disorders. Therefore, disrupting the proton gradient across the lysosomal membrane profoundly affects lysosomal function.

Question 2. (c) Describe the events by which APC/c promotes the separation of sister chromatids at anaphase.

- The Anaphase-Promoting Complex/Cyclosome (APC/C) is a ubiquitin ligase that plays a crucial role in regulating the metaphase-anaphase transition during mitosis. Its activation is triggered by the full alignment of chromosomes at the metaphase plate and the satisfaction of the spindle assembly checkpoint (SAC).

- Events by which APC/C promotes the separation of sister chromatids:
    - **Activation of APC/C:** At metaphase, once all chromosomes are properly attached to the spindle microtubules and aligned at the metaphase plate, the spindle assembly checkpoint (SAC) is satisfied. This satisfaction leads to the dissociation of SAC proteins (like Mad2 and BubR1) from Cdc20, a co-activator of APC/C. The release allows Cdc20 to bind to and activate APC/C.
    - **Ubiquitination of Securin:** Activated APC/C, in conjunction with Cdc20, targets a protein called securin for ubiquitination. Securin is a key regulatory protein that binds to and inhibits separase, an enzyme responsible for cleaving cohesin.
    - **Proteasomal Degradation of Securin:** The ubiquitination of securin by APC/C/Cdc20 marks it for degradation by the 26S proteasome. As securin levels rapidly drop, separase is released from its inhibition.

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- **Activation of Separase and Cohesin Cleavage:** Once active, separase cleaves the Scc1 subunit (also known as Rad21 in some organisms) of the cohesin complex. Cohesin is a multi-protein complex that encircles and holds sister chromatids together along their entire length from S phase until anaphase.
- **Separation of Sister Chromatids:** The cleavage of cohesin by separase dismantles the "glue" holding the sister chromatids together. This allows the sister chromatids to be pulled apart towards opposite poles of the cell by the shortening kinetochore microtubules, marking the onset of anaphase.
- **Ubiquitination of Cyclin B:** In parallel, APC/C/Cdc20 also targets mitotic cyclins (specifically Cyclin B, which is part of the MPF complex with CDK1) for ubiquitination and proteasomal degradation. The degradation of Cyclin B inactivates CDK1, leading to dephosphorylation of CDK substrates and exit from mitosis.

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Question 3. (a) Predict the effects of the following mutations on the ability of the cell to undergo apoptosis:

(i) Mutation in Bad such that it cannot phosphorylate protein kinase B.

- This statement appears to have a factual error. Normally, Protein Kinase B (PKB, also known as Akt) phosphorylates Bad, which inactivates Bad and promotes cell survival.
- Assuming the intended mutation is "Mutation in Bad such that it cannot be phosphorylated by protein kinase B" or "Mutation in protein kinase B such that it cannot phosphorylate Bad":
  - If Bad *cannot be phosphorylated* by protein kinase B (Akt), it would remain in its active, dephosphorylated state.
  - Active Bad promotes apoptosis by binding to and inhibiting anti-apoptotic Bcl-2 family proteins (like Bcl-2 and Bcl-XL), thereby allowing pro-apoptotic proteins (like Bax and Bak) to

oligomerize and permeabilize the mitochondrial outer membrane.

- Therefore, such a mutation would *enhance* the cell's ability to undergo apoptosis, as the pro-apoptotic signal from Bad would not be suppressed by Akt-mediated survival pathways.

(ii) Mutation in Bax such that it cannot form dimers.

- Bax is a pro-apoptotic protein of the Bcl-2 family.
- Upon apoptotic stimuli, Bax undergoes a conformational change and translocates from the cytosol to the mitochondrial outer membrane, where it oligomerizes (forms dimers and higher-order multimers).
- This oligomerization of Bax (and Bak) leads to the formation of pores or channels in the mitochondrial outer membrane, resulting in the release of pro-apoptotic factors (e.g., cytochrome c) from the intermembrane space into the cytosol.
- If Bax is mutated such that it cannot form dimers (and thus oligomers), it would be unable to permeabilize the mitochondrial outer membrane.
- This would prevent the release of cytochrome c and other pro-apoptotic factors, thereby *inhibiting or significantly reducing* the cell's ability to undergo intrinsic (mitochondrial) pathway apoptosis. The cell would become more resistant to apoptotic signals.

(iii) Mutation in adaptor proteins such that it cannot form dimers.

- Adaptor proteins are crucial for the assembly of various signaling complexes, including those involved in apoptosis.
- In the extrinsic (death receptor) apoptotic pathway, adaptor proteins like FADD (Fas-Associated Death Domain) are essential. FADD, through its death effector domain (DED), binds to the DED of pro-caspase-8, leading to the formation of the Death-Inducing Signaling

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Complex (DISC). This complex facilitates the dimerization and auto-activation of pro-caspase-8.

- In the intrinsic pathway, adaptor proteins like Apaf-1 (Apoptotic Protease Activating Factor-1) are crucial. Upon cytochrome c release, Apaf-1 undergoes a conformational change and oligomerizes to form the apoptosome. This complex then recruits and activates pro-caspase-9.
- If an adaptor protein (e.g., FADD or Apaf-1) is mutated such that it cannot form dimers (or higher-order oligomers required for complex assembly), it would disrupt the formation of the crucial signaling platforms (DISC or apoptosome).
- This disruption would prevent the dimerization and activation of initiator caspases (caspase-8 or caspase-9).
- Consequently, downstream executioner caspases would not be activated, leading to a significant *inhibition or complete block* of apoptosis. The cell would become highly resistant to apoptotic signals.

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(iv) Overexpression of Bcl-2.

- Bcl-2 is a major anti-apoptotic protein of the Bcl-2 family.
- Its primary function is to inhibit apoptosis by binding to and sequestering pro-apoptotic Bcl-2 family proteins (like Bax and Bak), thereby preventing their oligomerization and the permeabilization of the mitochondrial outer membrane.
- Overexpression of Bcl-2 means that there will be an abnormally high level of this anti-apoptotic protein within the cell.
- This excess Bcl-2 would effectively "neutralize" or sequester a large number of pro-apoptotic proteins, making it very difficult for the cell to initiate the intrinsic (mitochondrial) pathway of apoptosis.

- Therefore, overexpression of Bcl-2 would *inhibit or suppress* the cell's ability to undergo apoptosis, making the cell more resistant to cell death signals and potentially contributing to cancer development.

Question 3. (b) Explain the process of N-linked glycosylation of a secretory glycoprotein. What is the role of Dolichol phosphate in the synthesis of membrane glycoproteins?

- Process of N-linked Glycosylation of a Secretory Glycoprotein:
    - N-linked glycosylation is the most common type of protein glycosylation in eukaryotes, where an oligosaccharide (glycan) is attached to the nitrogen atom of an asparagine (Asn) residue within the consensus sequence Asn-X-Ser/Thr (where X is any amino acid except proline). This process occurs in the endoplasmic reticulum (ER) and is crucial for protein folding, stability, and cellular recognition.
    - **Oligosaccharide Synthesis on Dolichol Phosphate:** A pre-formed, branched oligosaccharide precursor (consisting of 14 sugar residues: 2 N-acetylglucosamine (GlcNAc), 9 mannose (Man), and 3 glucose (Glc) units) is synthesized in the ER on a lipid carrier molecule called dolichol phosphate. The synthesis begins on the cytoplasmic face of the ER membrane, where GlcNAc and Man residues are added. The dolichol-oligosaccharide then "flips" to the ER lumen. More Man and Glc residues are added on the luminal side.
    - **Transfer to Protein:** Once the complete 14-sugar oligosaccharide is assembled on dolichol phosphate in the ER lumen, it is transferred *en bloc* (as a single unit) to the asparagine residue of a nascent polypeptide chain. This transfer is catalyzed by the enzyme oligosaccharyl transferase, which is associated with the protein translocon. This occurs co-translationally (while the protein is being synthesized and translocated into the ER lumen).

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- **Trimming and Quality Control:** After transfer, the newly glycosylated protein undergoes a series of trimming steps. First, the three terminal glucose residues are sequentially removed by glucosidases. This glucose trimming is important for quality control in the ER, as it allows chaperones like calnexin and calreticulin to bind to the monoglucosylated protein and assist in its proper folding. If the protein does not fold correctly, it may undergo re-glucosylation and re-enter the folding cycle. If persistently misfolded, it will be targeted for degradation via ER-associated degradation (ERAD).
- **Further Modifications in Golgi:** After proper folding in the ER, the glycoprotein is transported to the Golgi apparatus. In the Golgi, the oligosaccharide chain undergoes further extensive modifications, including the removal of some mannose residues and the addition of various other sugar residues like GlcNAc, galactose (Gal), and sialic acid (Neu5Ac), resulting in the diversity of complex N-linked glycans.

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- Role of Dolichol phosphate in the synthesis of membrane glycoproteins:
  - Dolichol phosphate is a long-chain polyisoprenoid lipid embedded in the ER membrane.
  - Its crucial role is to serve as the lipid carrier for the synthesis and transfer of the pre-formed oligosaccharide chain during N-linked glycosylation.
  - Since the initial sugar residues are added on the cytoplasmic side of the ER membrane, and the final transfer to the protein occurs in the ER lumen, dolichol phosphate facilitates the "flipping" or translocation of the growing oligosaccharide chain from the cytoplasmic leaflet to the luminal leaflet of the ER membrane.

- This lipid acts as an anchor and transporter, allowing the sequential addition of sugar units and ensuring that the fully assembled oligosaccharide is correctly presented to the nascent protein within the ER lumen for transfer, thus playing an indispensable role in the synthesis of all N-linked glycoproteins, including membrane glycoproteins.

Question 3. (c) Explain the molecular mechanism that leads to cancer when Rb protein and p53 protein are inactivated by mutation.

- Both Retinoblastoma (Rb) protein and p53 protein are crucial tumor suppressor proteins, often referred to as "guardians of the genome." Their inactivation by mutation (loss of function) is a common event in the development of many cancers.
- **Inactivation of Rb Protein and Cancer:**
  - **Function of Rb:** Rb protein is a key regulator of the cell cycle, specifically controlling the G1 to S phase transition. In its active, hypophosphorylated state, Rb binds to and inactivates E2F transcription factors. E2F transcription factors are responsible for promoting the expression of genes required for DNA replication and cell cycle progression (e.g., genes for DNA polymerase, thymidine kinase).
  - **Mechanism of Inactivation in Cancer:**
    - **Mutation:** In many cancers, the gene encoding Rb (RB1) is mutated or deleted, leading to a non-functional or absent Rb protein.
    - **Viral Oncoproteins:** Certain oncogenic viruses (e.g., HPV, adenovirus, SV40) produce oncoproteins (e.g., E7, E1A, large T antigen) that directly bind to and inactivate Rb.
    - **Hyperphosphorylation:** In normal cells, Rb is hyperphosphorylated by active cyclin-dependent kinases

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(CDK4/6-cyclin D and CDK2-cyclin E) during late G1, which releases E2F and allows S-phase entry. In cancer, aberrant activation of CDKs or overexpression of cyclins can lead to constitutive hyperphosphorylation and inactivation of Rb, even without a direct mutation in the Rb gene.

- **Consequence for Cancer:** When Rb is inactivated (by mutation, deletion, or binding to viral proteins), it can no longer bind to and inhibit E2F transcription factors. This leads to the constitutive activation of E2F, causing uncontrolled transcription of S-phase genes and forcing the cell to continuously enter S phase, regardless of external signals or DNA integrity. This uncontrolled cell proliferation is a hallmark of cancer.

- **Inactivation of p53 Protein and Cancer:**
  - **Function of p53:** p53 is a "master tumor suppressor" that plays a central role in maintaining genomic integrity. It functions as a transcription factor, responding to various cellular stresses, particularly DNA damage. When activated (e.g., by phosphorylation), p53 induces the expression of genes involved in:
    - **Cell Cycle Arrest:** Such as p21, which inhibits CDK-cyclin complexes, leading to G1, S, or G2/M phase arrest, allowing time for DNA repair.
    - **Apoptosis:** Such as Puma, Noxa, and Bax, which promote programmed cell death if DNA damage is irreparable.
    - **DNA Repair:** Genes involved in DNA repair mechanisms.
  - **Mechanism of Inactivation in Cancer:**
    - **Mutation:** The TP53 gene (encoding p53) is the most frequently mutated gene in human cancers (mutated in

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over 50% of all cancers). Most mutations are missense mutations in the DNA-binding domain, rendering p53 unable to bind to DNA and activate its target genes.

- **MDM2 Overexpression:** MDM2 is an E3 ubiquitin ligase that targets p53 for proteasomal degradation. In some cancers, MDM2 is overexpressed, leading to excessive degradation of p53.
- **Viral Oncoproteins:** Certain viral oncoproteins (e.g., HPV E6, SV40 large T antigen) directly bind to and promote the degradation or inactivation of p53.

- **Consequence for Cancer:** When p53 is inactivated by mutation or other mechanisms, the cell loses its critical safeguard against DNA damage and uncontrolled proliferation. The cell can no longer effectively trigger cell cycle arrest to allow for DNA repair, nor can it initiate apoptosis if the damage is too severe. This leads to:
  - **Accumulation of Mutations:** Cells with damaged DNA continue to divide, accumulating further genetic alterations, which can drive oncogenic transformation.
  - **Uncontrolled Proliferation:** Loss of cell cycle checkpoints allows cells to divide irrespective of DNA damage or other inhibitory signals.
  - **Resistance to Apoptosis:** Cells become resistant to programmed cell death, allowing damaged or aberrant cells to survive and proliferate, forming tumors.

- **Synergistic Effect:** The inactivation of both Rb and p53 in a cell creates a particularly dangerous combination for cancer development. Rb inactivation removes the brake on cell division, pushing cells into uncontrolled proliferation, while p53 inactivation removes the mechanism to detect and eliminate these rapidly dividing, often genetically unstable, cells. This dual loss of tumor

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suppressor function accelerates tumor progression and contributes to the aggressive nature of many cancers.

Question 4. (a) Elaborate on the four major mechanisms of regulation of CDK activity during the cell cycle.

Cyclin-dependent kinases (CDKs) are central regulators of the eukaryotic cell cycle, driving progression through different phases by phosphorylating specific target proteins. Their activity is tightly regulated by several mechanisms:

1. **Cyclin Binding:**
    - CDKs are inactive as monomeric enzymes. Their activity is absolutely dependent on binding to a regulatory protein called a cyclin.
    - Different cyclins are expressed and degraded at specific phases of the cell cycle (e.g., cyclin D for G1, cyclin E for G1/S, cyclin A for S/G2, cyclin B for M).
    - Binding of a specific cyclin to its corresponding CDK induces a conformational change in the CDK active site, making it accessible to substrates. This allows the CDK to become partially active.
    - The specificity of CDK-cyclin complexes (e.g., CDK4/6-cyclin D, CDK2-cyclin E, CDK2-cyclin A, CDK1-cyclin A, CDK1-cyclin B) dictates which substrates are phosphorylated at different cell cycle stages.
2. **CDK Phosphorylation and Dephosphorylation:**
    - While cyclin binding confers partial activity, full activation of most CDK-cyclin complexes requires phosphorylation on a specific threonine residue (e.g., Thr161 in CDK1 and CDK2) in the "T-loop" or "activation loop" of the CDK subunit.

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- This phosphorylation is carried out by a dedicated CDK-activating kinase (CAK), which is itself a CDK (CDK7-cyclin H). Phosphorylation by CAK further enhances the catalytic activity of the CDK-cyclin complex.
- Conversely, phosphorylation at inhibitory sites (e.g., Thr14 and Tyr15 in CDK1 and CDK2) by Wee1 kinase and Myt1 kinase inhibits CDK activity. These phosphorylations prevent ATP binding or substrate binding.
- Dephosphorylation of these inhibitory sites by Cdc25 phosphatases (Cdc25A, B, C) removes the inhibitory phosphates, leading to a burst of CDK activity. This activation by Cdc25 is a critical positive feedback loop, especially for entry into mitosis.

3. **CDK Inhibitor (CKI) Proteins:**

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- CKIs are a family of proteins that directly bind to and inhibit the activity of CDK-cyclin complexes. They act as "brakes" on the cell cycle.
- There are two main families of CKIs:
  - **INK4 family (Inhibitors of Kinase 4):** These include p16, p15, p18, and p19. They primarily inhibit CDK4 and CDK6 by binding to the CDK subunit and preventing cyclin D binding. This regulation is crucial for controlling the G1 phase.
  - **Cip/Kip family (CDK-interacting protein/Kinase inhibitory protein):** These include p21, p27, and p57. They can inhibit a broader range of CDK-cyclin complexes (e.g., CDK2-cyclin E, CDK2-cyclin A, CDK1-cyclin B) by binding to both the CDK and cyclin subunits, often forming a ternary complex that blocks the active site. p21 is often induced by p53 in response to DNA damage, leading to cell cycle arrest.

- By binding to and inhibiting CDKs, CKIs prevent phosphorylation of CDK substrates, thereby arresting the cell cycle at specific checkpoints.

4. **Targeted Proteolysis of Cyclins:**

   - The oscillating levels of cyclins are achieved primarily through precisely timed ubiquitination and degradation via the ubiquitin-proteasome system.
   - **SCF complex (Skp1-Cullin1-F-box protein):** This ubiquitin ligase complex targets G1 cyclins (e.g., cyclin D, cyclin E) and CKIs (e.g., p27) for degradation, allowing progression from G1 into S phase. Its activity is often regulated by phosphorylation of its substrates.
   - **APC/C (Anaphase-Promoting Complex/Cyclosome):** This is a key ubiquitin ligase that becomes active in mitosis. It targets mitotic cyclins (e.g., cyclin B) and securin for ubiquitination.
     - Degradation of mitotic cyclins by APC/C leads to the inactivation of CDK1, which is essential for exit from mitosis, dephosphorylation of mitotic substrates, and cytokinesis.
     - Degradation of securin by APC/C releases separase, leading to the cleavage of cohesin and the separation of sister chromatids at anaphase.
   - The controlled degradation of cyclins ensures the unidirectional progression of the cell cycle and the timely inactivation of CDK activity after each phase.

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Question 4. (b) Write the mechanism of action of the following drugs/inhibitors:

A. Chemotherapeutic drugs :

(i) Herceptin (Trastuzumab)

- **Target:** Herceptin is a monoclonal antibody that specifically targets the Human Epidermal Growth Factor Receptor 2 (HER2), also known as ErbB2. HER2 is a receptor tyrosine kinase that is overexpressed in approximately 15-20% of breast cancers and some other cancers.
- **Mechanism of Action:**
    - **Blocks Ligand Binding:** Although HER2 does not have a known direct ligand, its activity relies on dimerization with other HER family receptors. Herceptin binds to the extracellular domain of HER2, physically blocking its ability to dimerize with other HER receptors (e.g., HER1, HER3) and thus preventing downstream signaling that promotes cell growth and proliferation.
    - **Inhibits Receptor Signaling:** By binding to HER2, Herceptin inhibits the constitutive activation of HER2-mediated signaling pathways, such as the Ras/MAPK pathway (involved in cell proliferation) and the PI3K/Akt pathway (involved in cell survival).
    - **Antibody-Dependent Cell-mediated Cytotoxicity (ADCC):** Herceptin's Fc region can bind to Fc receptors on immune effector cells (like Natural Killer cells). This binding recruits immune cells to the tumor, which then recognize and kill the HER2-overexpressing cancer cells through ADCC.
    - **Anti-angiogenic Effects:** It may also inhibit angiogenesis (new blood vessel formation) in tumors.
    - **Increases Chemosensitivity:** Herceptin can also make cancer cells more sensitive to conventional chemotherapy.

(ii) Imatinib (Gleevec)

- **Target:** Imatinib is a small molecule tyrosine kinase inhibitor that primarily targets the Bcr-Abl fusion protein, a constitutively active tyrosine kinase that is characteristic of Chronic Myeloid Leukemia

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(CML) and some other leukemias. It also inhibits other tyrosine kinases like c-Kit (involved in gastrointestinal stromal tumors, GIST) and PDGF receptor (PDGFR).

- **Mechanism of Action:**
    - **Competitive Inhibition:** Imatinib acts as a competitive inhibitor by binding to the ATP-binding site of the Bcr-Abl kinase (and c-Kit, PDGFR).
    - **Prevents Phosphorylation:** By occupying the ATP-binding pocket, Imatinib prevents ATP from binding to the kinase. Since ATP is required as a phosphate donor for phosphorylation, Imatinib blocks the phosphorylation of downstream signaling proteins.
    - **Inhibits Downstream Pathways:** This inhibition of phosphorylation effectively shuts down the oncogenic signaling pathways (e.g., Ras/MAPK, PI3K/Akt, STAT pathways) that are aberrantly activated by Bcr-Abl and drive the proliferation and survival of cancer cells.
    - **Induces Apoptosis/Cell Cycle Arrest:** By blocking these essential survival and growth signals, Imatinib leads to cell cycle arrest and apoptosis in cancer cells that depend on the activity of the targeted kinases, without significantly affecting normal cells that rely on different signaling pathways.

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B. Inhibitors of intracellular signaling :

(i) Sildenafil (Viagra)

- **Target:** Sildenafil is a phosphodiesterase-5 (PDE5) inhibitor.
- **Mechanism of Action:**
    - **Inhibition of PDE5:** Sildenafil specifically inhibits the enzyme PDE5, which is predominantly found in the smooth muscle cells

of the corpus cavernosum in the penis, as well as in the pulmonary vasculature and other tissues.

- **Prevents cGMP Degradation:** In response to sexual stimulation, nitric oxide (NO) is released, which activates guanylyl cyclase to produce cyclic guanosine monophosphate (cGMP). cGMP causes relaxation of smooth muscle by decreasing intracellular calcium levels, leading to vasodilation and increased blood flow. PDE5 is responsible for breaking down cGMP into inactive 5'-GMP, thus terminating the signal.
- **Enhances cGMP Levels and Vasodilation:** By inhibiting PDE5, sildenafil prevents the degradation of cGMP, leading to higher and sustained levels of cGMP in the smooth muscle cells.
- **Erectile Function:** The prolonged elevation of cGMP promotes greater and more sustained smooth muscle relaxation and vasodilation, facilitating increased blood flow into the penis, which results in an erection.
- **Pulmonary Hypertension:** In pulmonary hypertension, Sildenafil works by relaxing the smooth muscle cells in the pulmonary arteries, reducing pulmonary arterial pressure.

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(ii) Phorbol esters

- **Target:** Phorbol esters are a class of natural compounds (e.g., phorbol 12-myristate 13-acetate, PMA) that are potent activators of Protein Kinase C (PKC).
- **Mechanism of Action:**
  - **Mimic DAG:** Phorbol esters structurally resemble diacylglycerol (DAG), an endogenous second messenger that activates PKC.
  - **Direct PKC Activation:** They directly bind to the C1 domain of conventional and novel PKC isoforms. This binding occurs at the plasma membrane, where PKC is recruited.

- **Sustained Activation:** Unlike DAG, which is rapidly metabolized, phorbol esters are not metabolized by the cell, leading to prolonged and sustained activation of PKC.
- **Disruption of PKC Regulation:** This chronic activation can lead to a variety of cellular effects, including changes in cell proliferation, differentiation, and gene expression. However, prolonged activation can also lead to downregulation of PKC over time due to proteasomal degradation or cellular adaptation, which complicates their cellular effects.
- **Tumor Promotion:** Historically, phorbol esters have been studied as tumor promoters because their sustained PKC activation can disrupt normal cell growth control and contribute to cell proliferation, making them valuable tools in cancer research, but not therapeutic agents for human use due to their toxic and carcinogenic properties.

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Question 4. (c) Explain the role of CDK2/cyclin-A complex in ensuring that the DNA is replicated only once per cell cycle in the S-phase.

The CDK2/cyclin A complex plays a critical role in controlling DNA replication, specifically in preventing re-replication of the genome within a single cell cycle. This ensures that each chromosome is duplicated exactly once.

- **Licensing of Origins of Replication (G1 Phase):**
  - Before DNA replication can begin, origins of replication on the DNA must be "licensed." This licensing occurs during the G1 phase and involves the assembly of the pre-replicative complex (pre-RC).
  - Key components of the pre-RC include the origin recognition complex (ORC), Cdc6, Cdt1, and the MCM (minichromosome maintenance) helicase complex.

- In G1, CDK activity (including CDK2/cyclin A) is low. This low CDK activity is essential for Cdc6 and Cdt1 to load the MCM helicase onto the origins, forming the pre-RC, thereby "licensing" the origins for replication.

- **Initiation of Replication (S Phase):**
  - As the cell progresses into S phase, CDK2/cyclin E and then CDK2/cyclin A activities rise.
  - CDK2/cyclin A, along with DDK (Dbf4-dependent kinase), phosphorylates components of the pre-RC, particularly the MCM helicases, leading to the activation of the helicase and the unwinding of DNA.
  - This phosphorylation also recruits other replication factors, leading to the initiation of DNA synthesis at the licensed origins.
- **Preventing Re-replication (The Role of CDK2/cyclin A):**
  - The crucial role of CDK2/cyclin A (and other CDKs) in preventing re-replication lies in its ability to *inactivate* components of the pre-RC after the origins have fired. This ensures that once an origin has initiated replication, it cannot be re-licensed until the next G1 phase.
  - Specifically, active CDK2/cyclin A phosphorylates:
    - **Cdc6:** Phosphorylation of Cdc6 targets it for ubiquitination by SCF (Skp1-Cullin-F-box) complex and subsequent degradation by the proteasome. Alternatively, phosphorylated Cdc6 can also be exported from the nucleus to the cytoplasm.
    - **Cdt1:** Phosphorylation of Cdt1 promotes its binding to Geminin, an inhibitor that directly binds to Cdt1 and prevents its interaction with MCM helicase. Cdt1 can also be targeted for degradation.

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- By inactivating Cdc6 and Cdt1 through phosphorylation, CDK2/cyclin A effectively removes the essential components needed to load new MCM helicases onto origins.
- This active suppression of origin re-licensing ensures that once DNA replication has begun at an origin, that specific origin (and thus that segment of DNA) cannot be re-replicated until the CDK activity drops again in the subsequent G1 phase, following mitosis. This stringent control prevents gene amplification and maintains genomic stability.

Question 5. (a) With the help of diagram, explain the following :

(i) Role of BiP in post-translational translocation of protein into the ER lumen *Please note: As per your instructions, I cannot create schematic diagrams. I will explain the process verbally.*

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- **Post-translational Translocation:** This mechanism is primarily used for proteins that are destined for the ER lumen but are fully synthesized in the cytoplasm before being imported into the ER. It is more common in yeast, but also occurs in mammalian cells for a subset of proteins.
- **Role of BiP (Binding immunoglobulin Protein):** BiP is a major Hsp70 chaperone protein located in the lumen of the Endoplasmic Reticulum (ER). It is an ATP-dependent molecular chaperone and plays a crucial role in protein folding and translocation.
- **Steps involving BiP:**
    a. **Cytoplasmic Synthesis and Targeting:** The precursor protein, often lacking a cleavable signal peptide but containing internal targeting signals, is synthesized on free ribosomes in the cytoplasm. It might be kept unfolded by cytoplasmic chaperones.
    b. **Interaction with Translocon:** The nascent or fully synthesized polypeptide then associates with the Sec61 translocon complex

in the ER membrane. In post-translational translocation, auxiliary proteins (e.g., Sec62/63 complex, Sec71/72 complex in yeast) guide the protein to the translocon.

c. **ATP-dependent Pulling by BiP:** Once a segment of the polypeptide enters the ER lumen through the translocon channel, BiP (in its ATP-bound state) rapidly binds to hydrophobic regions of the incoming polypeptide.

d. **Hydrolysis and Conformational Change:** BiP then hydrolyzes ATP to ADP, which causes a conformational change in BiP, leading it to bind tightly to the polypeptide. This binding acts like a "ratchet," preventing the polypeptide from sliding back out of the translocon into the cytoplasm.

e. **Sequential Binding and Pulling:** As more of the polypeptide enters the ER lumen, BiP molecules sequentially bind to newly exposed hydrophobic segments. Each binding event, coupled with ATP hydrolysis and the conformational change, effectively "pulls" the polypeptide into the ER lumen.

f. **Release and Folding:** Once the entire protein has been translocated into the ER lumen, BiP releases the polypeptide upon ATP binding. Other ER chaperones then assist in the proper folding of the newly imported protein within the ER lumen. If the protein has a signal peptide, it might be cleaved by signal peptidase after translocation.

- In essence, BiP acts as a molecular motor, harnessing the energy from ATP hydrolysis to unidirectionally pull and drive the polypeptide chain through the translocon channel into the ER lumen during post-translational translocation.

(ii) Nitric oxide signaling cascade leading to vasodilation *Please note: As per your instructions, I cannot create schematic diagrams. I will explain the process verbally.*

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- **Nitric Oxide (NO) Signaling:** Nitric oxide is a gaseous signaling molecule that plays a crucial role in various physiological processes, including vasodilation (relaxation of blood vessels).
- **Steps in the Cascade:**

    g. **Acetylcholine Release and Endothelial Receptor Binding:** The process often begins with the release of acetylcholine from nerve endings or other stimuli. Acetylcholine binds to specific G-protein coupled receptors (GPCRs) on the surface of endothelial cells, which line the blood vessels.

    h. **Activation of Phospholipase C (PLC):** Binding of acetylcholine activates the GPCR, which in turn activates a Gq protein. The activated Gq protein then activates Phospholipase C (PLC).

    i. **Production of IP3 and DAG:** PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) into two second messengers: inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG).

    j. **Calcium Release:** IP3 diffuses into the cytoplasm and binds to IP3 receptors on the endoplasmic reticulum (ER), triggering the release of stored calcium ions (Ca^{2+}) into the cytoplasm.

    k. **Activation of eNOS:** The increase in cytoplasmic Ca^{2+} levels leads to the activation of Calmodulin, which in turn binds to and activates Endothelial Nitric Oxide Synthase (eNOS).

    l. **NO Synthesis:** Activated eNOS then catalyzes the conversion of L-arginine to L-citrulline and, importantly, produces nitric oxide (NO).

    m. **NO Diffusion to Smooth Muscle Cells:** Being a small, lipophilic gas, NO can readily diffuse across the plasma membrane of the endothelial cells and into the adjacent vascular smooth muscle cells.

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n. **Activation of Guanylyl Cyclase:** In the smooth muscle cells, NO binds to and activates a soluble enzyme called Guanylyl Cyclase (sGC).

o. **cGMP Production:** Activated sGC catalyzes the conversion of Guanosine Triphosphate (GTP) into cyclic Guanosine Monophosphate (cGMP).

p. **Activation of PKG and Smooth Muscle Relaxation:** cGMP acts as a second messenger, activating cGMP-dependent protein kinase (PKG, also known as protein kinase G). PKG then phosphorylates various target proteins in the smooth muscle cells, leading to:

- Decreased intracellular Ca^{2+} levels (e.g., by promoting Ca^{2+} uptake into the ER or efflux from the cell).
- Activation of potassium channels, leading to hyperpolarization.
- Dephosphorylation of myosin light chain, leading to relaxation of the smooth muscle fibers.

q. **Vasodilation:** The relaxation of vascular smooth muscle cells results in the widening of the blood vessel (vasodilation), increasing blood flow.

Question 5. (b) How do ATR and ATM proteins regulate the DNA damage checkpoint of the cell cycle?

- **ATR (ATM-Rad3-related) and ATM (Ataxia Telangiectasia Mutated) proteins** are two critical serine/threonine protein kinases that serve as the master regulators of the DNA damage checkpoints in the cell cycle. They recognize different types of DNA damage and activate distinct signaling cascades to halt cell cycle progression, activate DNA repair mechanisms, and in some cases, induce apoptosis.
- **ATM: Sensor of DNA Double-Strand Breaks (DSBs):**

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- **Damage Recognition:** ATM is primarily activated by DNA double-strand breaks (DSBs), which are highly dangerous forms of DNA damage. These breaks are often recognized by the Mre11-Rad50-Nbs1 (MRN) complex, which recruits and activates ATM.
- **Activation:** In response to DSBs, ATM undergoes autophosphorylation and dissociates from its inactive dimer form into active monomers.
- **Substrate Phosphorylation:** Activated ATM then phosphorylates a wide range of downstream target proteins. Key targets include:
    - **Chg2 (Checkpoint Kinase 2):** Phosphorylation of Chk2 by ATM activates Chk2.
    - **p53:** ATM phosphorylates p53, leading to its stabilization and activation.
    - **BRCA1, H2AX (histone H2A variant):** Phosphorylation of these proteins facilitates DNA repair processes.
- **Cell Cycle Arrest:** Activated Chk2 and p53 mediate cell cycle arrest by:
    - **G1/S Checkpoint:** p53 induces the expression of p21, a CKI that inhibits CDK2/cyclin E, arresting the cell in G1.
    - **S-phase Checkpoint:** ATM directly or indirectly inhibits origin firing, slowing down DNA replication.
    - **G2/M Checkpoint:** Chk2 and p53 inhibit the activity of Cdc25 phosphatases (Cdc25A, C), preventing the activation of CDK1/cyclin B, thus arresting the cell in G2 before mitosis.
- **DNA Repair & Apoptosis:** ATM also promotes homologous recombination and non-homologous end joining for DSB repair.

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If damage is extensive, ATM-p53 pathway can trigger apoptosis.

- **ATR: Sensor of Single-Stranded DNA (ssDNA) and Replication Stress:**
    - **Damage Recognition:** ATR is primarily activated by the presence of single-stranded DNA (ssDNA) regions, which typically arise from:
        - Stalled or collapsed replication forks (replication stress).
        - UV-induced DNA damage.
        - Nucleotide excision repair (NER) intermediates.
    - **Activation:** The RPA (Replication Protein A) complex binds to ssDNA, which then recruits ATR and its binding partner ATRIP (ATR-interacting protein) to the damage site, leading to ATR activation.
    - **Substrate Phosphorylation:** Activated ATR then phosphorylates a different set of downstream targets, although there is some overlap with ATM targets. Key targets include:
        - **Chk1 (Checkpoint Kinase 1):** Phosphorylation of Chk1 by ATR activates Chk1.
        - **p53:** ATR can also phosphorylate p53.
    - **Cell Cycle Arrest:** Activated Chk1 mediates cell cycle arrest by:
        - **S-phase Checkpoint:** Chk1 inhibits origin firing and stabilizes stalled replication forks, preventing the progression of replication stress.
        - **G2/M Checkpoint:** Chk1 inhibits Cdc25 phosphatases (Cdc25A, B, C), preventing the activation of CDK1/cyclin B, leading to G2 arrest.

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- **DNA Repair:** ATR signaling is crucial for stabilizing replication forks and facilitating DNA repair pathways like nucleotide excision repair and translesion synthesis.

- **Interplay and Overall Regulation:**
  - Both ATM and ATR ensure genomic integrity by coordinating cell cycle arrest, DNA repair, and sometimes apoptosis.
  - They act as master kinases that initiate a complex signaling network to respond appropriately to different types of DNA damage.
  - While ATM primarily responds to DSBs, and ATR to ssDNA/replication stress, there can be cross-talk and redundancy between their pathways, especially in response to complex damage.
  - Their proper function is vital for preventing cancer, as mutations in ATM or ATR lead to genomic instability and predispose individuals to cancer (e.g., Ataxia Telangiectasia in ATM mutations).

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Question 5. (c) What is oncogene addiction? Why is this concept important for selecting molecular targets for cancer therapy?

- **Oncogene Addiction:**
  - Oncogene addiction, also known as oncogene dependence, is a phenomenon observed in cancer cells where the continuous activity of a single oncogene (or a small set of oncogenes) is essential for the maintenance of the malignant phenotype. Despite the accumulation of numerous genetic mutations and alterations in cancer cells, they become "addicted" to or highly dependent on the sustained activity of a specific oncogene for their survival, proliferation, and overall growth.
  - If the activity of this specific oncogene is inhibited or blocked, the cancer cell undergoes cell cycle arrest, differentiation, or

apoptosis, while normal cells are largely unaffected. This is counterintuitive, as one might expect that a cell with multiple genetic aberrations would be robust and adaptable. However, oncogene addiction suggests that the constant activation of a particular oncogenic pathway creates a critical vulnerability for the cancer cell.

- **Importance for Selecting Molecular Targets for Cancer Therapy:**
    - The concept of oncogene addiction is profoundly important for the development and selection of molecular targets for cancer therapy, particularly in the realm of precision medicine and targeted therapies.
    - **Specific and Potent Therapeutic Targets:** Oncogene addiction highlights specific molecular targets (the "addicted" oncogene or its downstream pathway components) that, when inhibited, can lead to a disproportionate and often dramatic therapeutic response. This allows for the development of highly specific drugs that target these addicted pathways.
    - **Minimizing Side Effects:** Since normal cells do not typically exhibit this addiction to the same oncogene, inhibiting it selectively affects cancer cells. This leads to targeted therapies with a much higher therapeutic index and significantly reduced systemic side effects compared to conventional chemotherapy, which broadly targets dividing cells.
    - **Rational Drug Design:** Oncogene addiction provides a rational basis for designing drugs. Instead of broad cytotoxic agents, researchers can develop small molecule inhibitors or monoclonal antibodies that specifically block the function of the addicted oncogene or its downstream effectors (e.g., kinases, transcription factors).
    - **Personalized Medicine:** This concept is at the heart of personalized or precision medicine in oncology. By identifying

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the specific oncogene(s) that a patient's tumor is addicted to (often through genomic sequencing or biomarker testing), clinicians can select the most appropriate targeted therapy. For example, lung cancers with EGFR mutations are addicted to EGFR signaling and respond well to EGFR inhibitors (e.g., gefitinib, erlotinib). Similarly, CML is addicted to Bcr-Abl, which is effectively targeted by imatinib.

- **Predictive Biomarkers:** The existence of oncogene addiction has driven the search for predictive biomarkers. Identifying these biomarkers in a patient's tumor allows for patient stratification, ensuring that therapies are given only to those patients who are most likely to benefit, thereby improving treatment outcomes and avoiding ineffective treatments.
- **Understanding Resistance:** Studying oncogene addiction also helps in understanding mechanisms of resistance when targeted therapies fail. Often, resistance arises from the emergence of new mutations that bypass the addiction or activate alternative survival pathways.

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Question 6. (a) Explain with a diagram, the steps involved in the progression of a genetically altered cell into a cancerous cell.

Please note: As per your instructions, I cannot create schematic diagrams. I will explain the process verbally.

The progression of a normal, genetically altered cell into a cancerous cell is a multi-step process driven by the accumulation of multiple genetic and epigenetic changes over time. It typically involves a series of sequential mutations in proto-oncogenes and tumor suppressor genes, leading to the acquisition of hallmark capabilities that define cancer.

- **Step 1: Initiation (First Genetic Alteration)**
  - A single normal cell acquires an initial genetic alteration (mutation). This can be caused by various factors, including

exposure to carcinogens (e.g., chemicals, radiation), viral infections, or errors during DNA replication.

- This initial mutation often occurs in a proto-oncogene (e.g., leading to a constitutively active Ras protein) or a tumor suppressor gene (e.g., inactivating one copy of Rb or p53).
- At this stage, the cell is not yet cancerous but might have a slight growth advantage or increased susceptibility to further mutations. It is often termed a "initiated cell."

- **Step 2: Promotion (Clonal Expansion and Further Mutations)**
  - The initiated cell undergoes clonal expansion, driven by continuous exposure to promoting factors (e.g., chronic inflammation, growth factors, hormones) that stimulate cell proliferation. This expansion leads to a population of cells carrying the initial genetic alteration.
  - During this phase, due to increased proliferation and often compromised DNA repair mechanisms (due to the initial mutation), additional mutations accumulate in this expanding clone. These secondary mutations further enhance the cell's proliferative capacity or survival.
  - This can lead to the inactivation of the second allele of a tumor suppressor gene (Loss of Heterozygosity, LOH) or additional activating mutations in oncogenes.
- **Step 3: Progression (Acquisition of Cancer Hallmarks)**
  - With the accumulation of several critical mutations, the cells acquire a set of "hallmark" capabilities that allow them to grow uncontrollably and spread. These hallmarks, as described by Hanahan and Weinberg, include:
    - **Sustaining Proliferative Signaling:** Cells become independent of external growth signals and proliferate

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autonomously (e.g., activated Ras, overexpressed growth factor receptors).

- **Evading Growth Suppressors:** Cells bypass natural brakes on cell division (e.g., inactivated Rb or p53).
- **Resisting Cell Death (Apoptosis):** Cells become resistant to programmed cell death (e.g., overexpressed Bcl-2, inactivated p53).
- **Enabling Replicative Immortality:** Cells acquire the ability to divide indefinitely, often by maintaining telomere length (e.g., telomerase activation).
- **Inducing Angiogenesis:** Cells stimulate the formation of new blood vessels to supply nutrients and oxygen to the growing tumor.
- **Activating Invasion and Metastasis:** Cells gain the ability to detach from the primary tumor, invade surrounding tissues, and spread to distant sites.

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  - At this stage, the cells exhibit significant genomic instability, leading to rapid accumulation of more mutations and chromosomal aberrations. The tumor grows larger and becomes clinically detectable.

- **Step 4: Malignant Transformation and Metastasis**
  - The continuous accumulation of genetic and epigenetic changes leads to a fully malignant phenotype.
  - The cells acquire enhanced invasive properties, breaking through the basement membrane and invading surrounding stromal tissue.
  - They develop the ability to metastasize: intravasating into blood or lymphatic vessels, surviving in circulation, extravasating at

distant sites, and forming secondary tumors (metastases). This is the most life-threatening aspect of cancer.

- Throughout this multi-step process, selective pressure favors clones of cells that acquire increasingly aggressive malignant characteristics, leading to tumor heterogeneity and evolution.

Question 6. (b) Explain the differences between the process of autophagy and necrosis.

Feature	Autophagy (Autophagic Cell Death)	Necrosis
Nature of Process	Programmed (regulated), catabolic process; often a survival mechanism but can lead to cell death if severe or prolonged.	Unprogrammed, uncontrolled, pathological process; always leads to cell death.
Trigger	Cellular stress (e.g., nutrient starvation, organelle damage, hypoxia, infection), ER stress, certain signaling pathways.	Acute injury (e.g., trauma, extreme temperature, toxins, severe ischemia, infection).
Energy Dependence	ATP-dependent process; requires cellular energy for vesicle formation and protein synthesis.	ATP-independent (or rapid depletion of ATP).
Morphological Changes	Extensive vacuolization of cytoplasm; formation of autophagosomes (double-membraned vesicles engulfing cellular components). Nucleus typically remains intact initially.	Cell swelling (oncosis); rupture of plasma membrane; organelle swelling and disruption; nuclear changes (pyknosis,

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Feature	Autophagy (Autophagic Cell Death)	Necrosis
		karyorrhexis, karyolysis).
Plasma Membrane Integrity	Usually remains intact initially, until late stages of autophagic cell death.	Early loss of plasma membrane integrity, leading to leakage of cellular contents.
Cell Size	Shrinkage due to degradation of cytoplasm.	Swelling.
Inflammatory Response	Generally non-inflammatory or immunologically silent, as contents are contained within autophagosomes.	Triggers a strong inflammatory response due to release of DAMPs (Damage-Associated Molecular Patterns) from ruptured cells.
Lysosomal Involvement	Central role: Autophagosomes fuse with lysosomes (autophagolysosomes) for degradation of contents by lysosomal hydrolases.	Lysosomal enzymes are released into the cytoplasm and extracellular space due to membrane rupture, contributing to tissue damage.
Fate of Cellular Contents	Digested and recycled for cellular reuse.	Released indiscriminately into the extracellular space.

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Feature	Autophagy (Autophagic Cell Death)	Necrosis
Genetic Control	Genetically regulated by specific ATG (AuTophagy-related Genes) genes.	No specific genetic program; results from acute cellular damage.
Physiological Role	Cell survival, nutrient recycling, organelle quality control, host defense.	Pathological, leads to tissue damage and disease.
Examples	Self-digestion during starvation, removal of damaged mitochondria (mitophagy), clearing of protein aggregates.	Tissue infarction (e.g., heart attack, stroke), severe burns, acute infections.

Question 6. (c) Explain the Ras/MAPK signaling pathway and its activation. How does the dysregulation of this pathway lead to cancer?

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- **Ras/MAPK Signaling Pathway:**
    - The Ras/MAPK (Mitogen-Activated Protein Kinase) pathway is a fundamental and highly conserved signaling cascade in eukaryotic cells. It plays a critical role in relaying extracellular signals (such as growth factors, cytokines, and hormones) from the cell surface to the nucleus, regulating diverse cellular processes including cell proliferation, differentiation, survival, apoptosis, and motility.
    - The core of the pathway involves a sequential phosphorylation cascade: **Ras** → **Raf** → **MEK** → **ERK (MAPK)**.
- **Activation of the Ras/MAPK Pathway:**
    r. **Receptor Tyrosine Kinase (RTK) Activation:** The pathway typically begins with the binding of an extracellular growth factor (e.g., Epidermal Growth Factor (EGF), Platelet-Derived Growth

Factor (PDGF)) to its specific Receptor Tyrosine Kinase (RTK) on the cell surface.

s. **RTK Dimerization and Autophosphorylation:** Ligand binding causes RTKs to dimerize, leading to the activation of their intrinsic tyrosine kinase activity. This results in reciprocal phosphorylation of specific tyrosine residues in the cytoplasmic tails of the RTKs.

t. **Recruitment of Adaptor Proteins (Grb2):** The phosphorylated tyrosine residues serve as docking sites for adaptor proteins containing SH2 domains. Growth factor receptor-bound protein 2 (Grb2) is a key adaptor that binds to the phosphorylated RTK.

u. **Recruitment of GEF (SOS):** Grb2, in turn, recruits a guanine nucleotide exchange factor (GEF) called SOS (Son of Sevenless) to the plasma membrane.

v. **Ras Activation:** SOS interacts with and activates Ras, a small monomeric G-protein (part of the Ras superfamily). In its inactive state, Ras is bound to GDP. SOS promotes the exchange of GDP for GTP on Ras, converting Ras to its active, GTP-bound state.

w. **Raf Activation:** Active, GTP-bound Ras recruits and activates a serine/threonine kinase called Raf (also known as MAPKKK or MEK kinase).

x. **MEK Activation:** Activated Raf phosphorylates and activates another serine/threonine kinase called MEK (MAPKK or MAPK/ERK Kinase). MEK is unique because it is a dual-specificity kinase, phosphorylating both threonine and tyrosine residues.

y. **ERK (MAPK) Activation:** Activated MEK then phosphorylates and activates ERK (Extracellular signal-Regulated Kinase), which is the terminal kinase in this cascade (also known as MAPK).

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z. **Downstream Effects:** Activated ERK translocates from the cytoplasm to the nucleus, where it phosphorylates various transcription factors (e.g., Elk-1, c-Fos, c-Jun) and other effector proteins. This phosphorylation alters gene expression, leading to cellular responses such as cell proliferation, differentiation, and survival.

- **How Dysregulation of this Pathway Leads to Cancer:**
  - The Ras/MAPK pathway is one of the most frequently dysregulated signaling pathways in human cancers. Because it is a central regulator of cell proliferation and survival, its persistent activation provides a strong growth advantage to cells, contributing to malignant transformation.
  - **Oncogenic Mutations in Ras:**
    - Mutations in the *RAS* genes (HRAS, KRAS, NRAS) are found in approximately 30% of all human cancers, making them the most common oncogenes.
    - These mutations typically occur at specific "hotspot" codons (e.g., G12, G13, Q61) and render Ras constitutively active. Mutated Ras is unable to hydrolyze GTP to GDP effectively, meaning it remains locked in its active, GTP-bound state, regardless of external growth factor signals.
    - This leads to continuous, uncontrolled activation of the entire downstream MAPK cascade (Raf, MEK, ERK), resulting in persistent pro-growth and pro-survival signaling.
  - **Upstream Activation:** Overexpression or activating mutations in upstream components like RTKs (e.g., EGFR, HER2 amplification/mutation) can also lead to hyperactivation of the Ras/MAPK pathway.

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- **Mutations in Raf or MEK:** Less commonly, activating mutations can also occur directly in *BRAF* (a common oncogene in melanoma) or *MEK* genes, leading to constitutive activation further down the cascade, bypassing the need for Ras activation.
- **Consequences of Dysregulation in Cancer:**
  - **Uncontrolled Cell Proliferation:** Sustained activation of ERK leads to continuous phosphorylation of transcription factors that promote cell cycle progression, bypassing normal growth control mechanisms.
  - **Enhanced Cell Survival:** The pathway also activates genes and proteins that inhibit apoptosis, allowing potentially damaged or abnormal cells to survive.
  - **Increased Angiogenesis:** Dysregulated MAPK signaling can promote the production of pro-angiogenic factors (e.g., VEGF), supporting tumor growth by supplying nutrients and oxygen.
  - **Metastasis and Invasion:** The pathway contributes to epithelial-mesenchymal transition (EMT), increased cell motility, and protease production, facilitating tumor invasion and metastasis.
  - **Genomic Instability:** By promoting proliferation despite damage, dysregulated MAPK signaling can contribute to the accumulation of further mutations.
- Therefore, the constitutive activation of the Ras/MAPK pathway provides cancer cells with key "hallmarks of cancer," making it a critical driver of tumorigenesis and a major target for anti-cancer drug development.

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