FOB-II

Rubric for Quiz 1 Total Marks- 36

Subjective Questions (Attempt one question between Q1 and Q2; and one question from Q3 and Q4). Each question = 10 marks

Question1: The partnership between a G-protein-coupled receptor (GPCR) and its G-protein is fundamental to signaling. Critically analyze the statement: "The physical separation of the ligand-binding function (in the GPCR) from the signal-transducing function (in the G-protein) is a masterstroke of evolutionary design." Discuss the advantages this modular, two-part system provides in terms of signal amplification, diversification, and integration.

Answer 1.

The Modular Masterstroke: Analysis of GPCR-G-protein Signaling

The statement that the physical separation of the ligand-binding function in a G-protein-coupled receptor (GPCR) from the signal-transducing function in a G-protein is a masterstroke of evolutionary design is accurate. This two-part, modular system provides a sophisticated and highly flexible framework for cellular signaling, offering profound advantages in terms of signal amplification, diversification, and integration.

Signal Amplification

The modularity of the GPCR-G-protein partnership is the foundation of its remarkable capacity for signal amplification. A single ligand-binding event can lead to the activation of hundreds of G-protein molecules, as the activated GPCR remains in an active state for a sufficient duration to interact with multiple G-proteins sequentially. Each of these activated G-proteins can then go on to activate an enzyme, such as adenylyl cyclase, which in turn can generate thousands of second messenger molecules, such as cyclic adenosine monophosphate (cAMP). This chain reaction, a hallmark of signal transduction cascades, means that a minimal extracellular signal can elicit a massive and widespread intracellular response. For example, the binding of a single epinephrine molecule to a receptor can lead to the breakdown of millions of glycogen molecules, a testament to the power of this amplification system.

Signal Diversification

The physical separation of the receptor and the G-protein allows for an immense degree of signal diversification. Cells possess a limited number of GPCRs and a distinct set of G-protein families (e.g., , Gi, Gq). A single type of GPCR can couple with different G-protein families depending on the cell type or intracellular context. This allows the same ligand to trigger different signaling pathways and cellular responses in different tissues. For instance, a hormone might bind to a receptor that activates a Gs protein in one cell, leading to increased cAMP, while in another cell, the same receptor is coupled to a Gq

protein, leading to the activation of phospholipase C and the production of inositol trisphosphate (IP3) and diacylglycerol (DAG). This modularity provides an elegant mechanism for context-dependent cellular behavior.

Signal Integration

Furthermore, the two-part system is critical for signal integration. A cell is constantly bombarded with multiple signals, and its response must be a coordinated product of all these inputs. GPCRs and G-proteins enable this by serving as points of convergence and divergence. Different GPCRs can activate the same G-protein, allowing for the integration of multiple distinct extracellular signals into a single pathway. Conversely, a single G-protein can influence multiple downstream effectors, allowing a single signal to trigger a complex, multifaceted response. This ability to integrate signals from various sources is essential for coordinating intricate physiological processes like metabolism, growth, and cellular differentiation.

Question2 : Cells use small, non-protein second messengers like cAMP and Ca2+ to relay signals internally. Evaluate the strategic purpose of using these molecules instead of relying solely on a direct protein-to-protein cascade. Compare and contrast the generation, properties, and downstream effects of the cAMP and the IP3/Ca2+ second messenger systems.

Answer: The use of small, non-protein second messengers like cAMP and is a brilliant strategy for cellular signaling. Rather than relying on a simple protein-to-protein cascade, these molecules provide a strategic advantage that is essential for a cell's ability to respond to its environment with speed, scale, and specificity.

The strategic purpose of second messengers is to:

1. **Amplify Signals:** Second messengers allow for a massive amplification of the initial signal. A single activated receptor can lead to the production of thousands of second messenger molecules, each of which can activate a downstream protein. This creates a cascade effect, ensuring a large and robust cellular response to a minimal external stimulus.
2. **Increase Speed and Diffusibility:** Being small and diffusible, second messengers can rapidly spread throughout the cell, activating multiple targets simultaneously in different cellular compartments. This is much faster and more efficient than a linear protein-to-protein cascade, which is limited by the physical diffusion and binding of large protein molecules.
3. **Provide a Point of Integration:** Different signaling pathways can converge on the same second messenger, allowing the cell to integrate multiple signals and produce a coordinated response. For example, several different GPCRs can all lead to an increase in cAMP, allowing the cell to sum up various external stimuli into a single, cohesive signal.
4. **Enable Complex Regulation:** The concentration of second messengers can be tightly and dynamically regulated, providing a mechanism for fine-tuning the cellular response. Enzymes that produce and degrade these molecules are themselves under cellular control, allowing for intricate feedback loops.

Comparison of cAMP and /Ca2+ Second Messenger Systems

The cAMP and IP3/Ca2+ systems are two of the most well-known second messenger pathways, each with unique mechanisms, properties, and downstream effects.

Feature	cAMP System	IP3/Ca2+ System
Generation	Generated from ATP by the enzyme adenylyl cyclase, which is activated by the G-protein Gs.	IP3 is generated from the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2) by the enzyme phospholipase C (PLC), which is activated by the G-protein Gq. Ca2+ is released from intracellular stores (primarily the endoplasmic reticulum) in response to IP3 binding to its receptor on the ER membrane.
Properties	Water-soluble, allowing it to diffuse freely throughout the cytosol to activate various targets.	IP3 is a water-soluble molecule, while Ca2+ is a cation with a very low cytosolic concentration, enabling it to act as a highly sensitive signal. The Ca2+ signal is often localized to specific regions of the cell.
Downstream Effects	Primarily activates Protein Kinase A (PKA), which phosphorylates a wide range of proteins, leading to changes in enzyme activity, gene expression, and other cellular functions. In some cases, it can directly bind to and regulate ion channels.	The release of Ca2+ from the ER is a critical step. Ca2+ then binds to and activates various proteins, most notably calmodulin. Calmodulin, in turn, activates other kinases (like CaMKs) and enzymes. IP3 also has downstream effects, like activating the IP3 receptors on the endoplasmic reticulum.
Signal Termination	The signal is terminated when phosphodiesterases (PDEs) hydrolyze cAMP into AMP. This rapid	The signal is terminated by the re-uptake of Ca2+ into the ER via SERCA pumps or its export from the cell. IP3 is degraded by various phosphatases.

degradation ensures the signal is short-lived and tightly controlled.

Question3: Cells possess two major pathways for repairing DNA double-strand breaks (DSBs): Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR). Critically evaluate the pros and cons of each pathway. Explain why NHEJ is considered "error-prone" yet is active throughout the cell cycle, while the high-fidelity HR pathway is predominantly restricted to the S and G2 phases.

Non-Homologous End Joining (NHEJ)

This pathway is the cell's most common and rapid response to DSBs. Its primary goal is to reconnect the broken DNA ends as quickly as possible to prevent more catastrophic chromosomal damage.

- **Pros:** The biggest advantage of NHEJ is its **speed and adaptability**. It can operate throughout the entire cell cycle, including the G0 and G1 phases when there is no sister chromatid to serve as a template. This makes it an essential "emergency" repair mechanism.
- **Cons:** NHEJ is considered **error-prone**. The process often involves the trimming of DNA ends before they are ligated together. This results in small deletions or insertions at the break site, which can disrupt a gene's function and lead to mutations.

Homologous Recombination (HR)

In contrast, the **HR** pathway is a precise, high-fidelity repair system that aims to restore the original DNA sequence without introducing errors.

- **Pros:** The main benefit of HR is its high accuracy. By using the undamaged sister chromatid as a template, it can perfectly repair the break and restore the original genetic sequence. This is critical for maintaining genomic stability.
- **Cons:** The major drawback of HR is that it is **restricted to the S and G2 phases** of the cell cycle. This is because the process fundamentally requires the presence of the replicated DNA (the sister chromatid) to serve as its template.

Why Their Roles Differ

The existence of these two distinct pathways is a strategic cellular choice. The error-prone NHEJ pathway is available throughout the cell's life because a flawed repair is better than an unrepaired break, which could lead to cell death. The high-fidelity HR pathway, however, is reserved for the specific window of the cell cycle when a perfect template is available, ensuring that the cell can correct the most dangerous lesions without introducing new mutations as it prepares to divide.

Question 4: Xeroderma Pigmentosum (XP) is a genetic disorder caused by defects in the Nucleotide Excision Repair (NER) pathway, leading to extreme UV sensitivity. Analyze the molecular link between the

failure of NER and the development of skin cancer in XP patients. Explain why the Base Excision Repair (BER) pathway cannot effectively compensate for the loss of NER function, despite both being excision repair mechanisms.

Answer: The core molecular link is that the **Nucleotide Excision Repair (NER)** pathway is the cell's primary defense against a specific type of damage: large, bulky DNA lesions that distort the double helix. The most common of these, and the ones directly relevant to XP, are **pyrimidine dimers** caused by exposure to ultraviolet (UV) radiation from sunlight.

In individuals with Xeroderma Pigmentosum, the genes encoding for key components of the NER pathway are mutated and non-functional. Consequently, the cell is unable to recognize and remove the helix-distorting pyrimidine dimers.

When a cell with a faulty NER pathway is exposed to UV light, these dimers are left unrepaired. If DNA replication proceeds before these lesions are fixed, the DNA polymerase can stall or make errors, leading to **mismatched bases, insertions, or deletions**. This accumulation of mutations in critical genes, especially tumor suppressor genes and proto-oncogenes, can eventually drive uncontrolled cell proliferation, leading to the development of skin cancer at a very early age.

Why BER Cannot Compensate for NER Failure

While both NER and BER are "excision repair" mechanisms, they are highly specific and operate on different types of DNA damage. The key reason BER cannot compensate is because it is not equipped to handle the type of damage caused by UV light.

- **Damage Specificity:** The **Base Excision Repair (BER)** pathway is a "small-scale" repair mechanism that is designed to correct common, non-helix-distorting lesions. Its enzymes, like DNA glycosylase, are specialized to recognize and remove single, damaged bases (e.g., uracil from a deaminated cytosine) but cannot detect the bulky, two-base pyrimidine dimers.
- **Lesion Size and Type:** NER, on the other hand, is a "large-scale" repair system. It is designed to recognize the physical distortion of the DNA helix itself, rather than a specific damaged base. The enzymatic machinery of NER excises a long segment of the DNA strand containing the bulky lesion, a job that the BER pathway's enzymes are simply not built to do.

In summary, the specific nature of the damage caused by UV radiation is what makes NER indispensable. BER's specialized tools are useless against the large, helix-distorting lesions that accumulate in XP patients, leaving them vulnerable to the carcinogenic effects of sunlight.

Objective Questions (Attempt all questions. Each question = 4 marks, more than one Answer is applicable, each wrong option = -2

Answer 1. → (c) The Gα subunit dissociates from the Gβγ dimer

Answer 2. → b) Phosphatidylinositol 4,5-bisphosphate (PIP2)

Answer 3. → c) DNA Photolyase

Answer 4. → d) A bulky DNA adduct caused by the carcinogen benzo[a]pyrene.