Molecular Neuroscience: short form and fill in the blank questions and answers

1. **Understanding Gene Function:** Discuss the various approaches and techniques employed to understand the function of a gene or protein (KO, depletion, overexpression, interactions, sequence alignment).
2. **Gene Expression Review:** Gene expression works as DNA → mRNA → _____.
3. **Gene Depletion Strategies:** Elaborate on the different methods used to deplete a gene or protein, ranging from broad, untargeted approaches to more precise genome editing techniques.
4. **Challenges of Broad Gene Depletion:** Broadly depleting essential genes can cause _____.
5. **Cell-Type and Time-Specific Gene Depletion:** Detail the strategies that allow for the depletion or mutation of a gene/protein in a specific cell type or during a particular developmental stage.
6. **Forward vs. Reverse Genetics:** Differentiate between a "forward genetic screen" and a "reverse genetic screen."
7. **Overexpression of Genes:** Describe the principles and methods involved in overexpressing a gene or protein within a cell. How does overexpression contribute to understanding gene function, and what molecular tools are utilized?
8. **Protein Tags: Function and Application:** Define what protein tags are and explain their significance in molecular biology. Discuss at least two major applications of protein tags, providing examples.
9. **Fluorescent Protein Tags:** Discuss the impact of fluorescent protein tags, such as GFP, on biological research. Explain how they are used to label proteins, cells, and even entire animals.
10. **Introducing a Foreign Gene:** To introduce a foreign gene in the animal of your interest, which steps are required and which techniques?
11. **Brainbow Technique:** Describe the "Brainbow" technique. Explain its purpose and the underlying genetic principles that allow it to label individual neurons with distinct fluorescent protein combinations.
12. **Delivery of Transgenes:** Explain the primary methods used to deliver transgenes into a cell. Focus on the workhorse method and its mechanism for integrating genetic material.
13. **Making Transgenic Organisms:** Outline the general steps involved in creating a transgenic organism. What is the fundamental goal of this process, and how does it allow for the study of gene function in vivo?
14. **Optogenetics: Principles:** Channelrhodopsin _____ neurons with light,

while Halorhodopsin _____ them.

15. **Optogenetics: Circuit Investigation:** Discuss how optogenetics is utilized to investigate neural circuits and individual neurons. Provide examples of how light stimulation can either activate or inhibit neurons.
16. **Investigating Synaptic Function and Connectivity:** Describe at least two distinct molecular approaches for investigating synaptic function and connectivity.
17. **cFOS Reporter for Activity:** The cFOS reporter system uses an _____ to label recently activated neurons.
18. **CamKII Reporter:** Describe the CamKII reporter system. How does it function as a calcium sensor, and how can its expression be targeted to specific cell types?
19. **Memory Reporter System:** Detail the "Memory reporter" system described in the lecture. Explain how it allows for the labeling of neurons activated during a specific behavioral task, including the role of tamoxifen.
20. **Visualizing Small Proteins:** For visualizing a very small protein, a(n) _____ tag is more appropriate than GFP.
21. **Model Organisms for Disease:** Discuss the utility of model organisms in studying human neurological disorders. What are the advantages of using them, and what challenges might arise?
22. **Antibodies in Molecular Biology:** Explain why antibodies are considered invaluable tools in molecular biology, based on their general utility in research.
23. **Visualizing Active Neurons (General):** If you want to visualize neurons active during a specific task, what general components and strategies would you need to employ?
24. **Cell-Type-Specific Reporters:** How are cell-type-specific reporters made? Explain the key molecular component that enables this specificity.
25. **Optogenetics for Behavioral Causality (Necessity):** Design an optogenetic experiment to test if neuron X's activity is required (necessary) for antennal grooming in fruit flies, given it's active during grooming.
26. **Optogenetics for Behavioral Sufficiency:** If stimulating neuron X leads to antennal grooming, can you conclude that its activity is required (necessary) for grooming? Justify your answer.
27. **Halorhodopsin Experiment:** If stimulating halorhodopsin in grooming fruit flies' X neurons stops grooming, explain what happens in X neuron and whether X neuron activity is now concluded as necessary for grooming.
28. **Strengths of Optogenetics and Model Systems:** Summarize the key strengths of optogenetics compared to other techniques for testing causality. Additionally, discuss why different experimental model systems are essential,

highlighting their main advantages and disadvantages.

Answers

1. **Understanding Gene Function:** Gene function is understood through:
- **Bioinformatics (sequence analysis):** This involves using tools like BLAST or UniProt to compare gene or protein sequences, predict protein domains, and infer potential functions based on known homologous proteins.
- **Gene expression pattern analysis:** Investigating where and when a gene is expressed (its expression pattern) provides clues about its role in specific tissues or cell types.
- **Genetic manipulations:**
    - **Knockout experiments:** Removing or inactivating a gene to observe the resulting phenotypic changes, thereby revealing its necessity for certain processes.
    - **Overexpression:** Increasing the levels of a gene or protein to see if it can induce or enhance specific cellular behaviors.
- **Protein visualization:** Visualizing the subcellular location of the protein provides insights into its site of action within the cell.
- **Interaction studies:** Identifying its interaction partners helps uncover molecular pathways it participates in. If it binds to DNA, determining where it binds (e.g., via ChIP-seq) can reveal its role in gene regulation.

2. **Gene Expression Review:** protein.

3. **Gene Depletion Strategies:** Gene depletion methods range from broad (random mutagenesis) to precise. Targeted approaches include RNA interference (RNAi) for mRNA knockdown, and engineered nucleases like ZFNs and TALENs for DNA cleavage. CRISPR-Cas9/12a is the most precise modern method, causing targeted DNA breaks. Degrons are used for specific protein degradation.

4. **Challenges of Broad Gene Depletion:** embryonic lethality.

5. **Cell-Type and Time-Specific Gene Depletion:** Precision is achieved via: 1) **Cell-Type-Specific Promoters** (e.g., hSyn for neurons) that activate tools only in target cells, and 2) **Inducible Expression Systems** (e.g., doxycycline) that

allow temporal control by drug presence.

6. **Forward vs. Reverse Genetics:** Forward genetics starts with random mutations to find phenotypes, then identifies the responsible gene. Reverse genetics starts with a known gene, alters it, then observes resulting phenotypes to uncover its function.

7. **Overexpression of Genes:** Overexpression increases gene/protein levels to observe if higher amounts induce or enhance cellular behaviors, revealing function. Methods involve placing the gene under **strong promoters**, delivering via **viral vectors**, and using **inducible systems** for temporal control.

8. **Protein Tags: Function and Application:** Protein tags are short peptide sequences fused to a protein of interest, not altering its function, but serving as a detectable marker.

    1. **Subcellular Localization:** Fluorescent tags (e.g., GFP) visualize protein distribution in cells.
    2. **Purification and Interaction Partner Identification:** Tags (e.g., His, HA) bind the protein to a matrix for purification and identification of interacting partners.
9. **Fluorescent Protein Tags:** Fluorescent protein tags, like GFP, enable visualization in living systems. They are genetically fused and emit light, used to label proteins (for real-time dynamics), cells (for lineage/migration), and entire animals (for *in vivo* structure visualization, like Brainbow for circuits).

10. **Introducing a Foreign Gene:** Steps include preparing the gene construct, delivering it (e.g., via pronuclear microinjection into zygotes or viral vectors), screening for successful integration, and breeding founder animals to establish stable transgenic lines.

11. **Brainbow Technique:** Brainbow labels individual neurons with unique fluorescent protein combinations to trace neural connectivity. It uses the **Cre-lox recombination system** with multiple fluorescent protein genes. Random Cre-mediated recombination in neurons creates a distinct color code for each cell, allowing precise tracing of neural pathways.

12. **Delivery of Transgenes:** Primary methods are **viral vectors** (e.g., lentiviruses, AAVs) and non-viral methods (e.g., lipofection). The "workhorse" lentiviral

vector stably integrates DNA into the host cell's genome via viral integrase, ensuring long-term gene expression in both dividing and non-dividing cells.

13. **Making Transgenic Organisms:** The goal is to study gene function *in vivo*, create disease models, or produce valuable proteins by stably integrating a foreign gene into an animal's germline. Steps involve preparing the construct, microinjecting it into zygotes, implanting, screening offspring, and breeding to establish stable lines.

14. **Optogenetics: Principles:** activates; inhibits.

15. **Optogenetics: Circuit Investigation:** Optogenetics causally manipulates neuronal activity to investigate circuits. Activating specific neurons (e.g., striatal neurons with Channelrhodopsin) can reveal their downstream effects (e.g., inhibiting substantia nigra neurons). Inhibiting neurons (e.g., with Halorhodopsin) tests if their activity is necessary for a specific behavior, establishing causal links.

16. **Investigating Synaptic Function and Connectivity:**

    1. **Fluorescent Protein Complementation at Synapses:** Detects synapse formation when two fluorescent protein halves (on pre- and post-synaptic proteins) come together and fluoresce.
    2. **cFOS Driven Fluorescent Reporter:** Labels recently active synapses/neurons. The cFOS promoter (activity-induced) drives a fluorescent reporter, marking active cells.

17. **cFOS Reporter for Activity:** immediate early gene (IEG)

18. **CamKII Reporter:** The CamKII reporter system senses calcium; GFP fluoresces when M13 binds to Calmodulin upon calcium sensing. Its expression is targeted to specific cell types using **Cre lines**, where the reporter mouse (Cre-dependent) is crossed with a Cre-expressing mouse under a cell-type-specific promoter (e.g., for excitatory neurons).

19. **Memory Reporter System:** This system labels neurons active during a specific behavioral task. The cFOS promoter drives tamoxifen-inducible Cre-ERT2. When active neurons express Cre-ERT2 and **tamoxifen is administered**, active Cre excises a "STOP" cassette from a reporter gene (e.g., mCherry), permanently labeling those neurons.

20. **Visualizing Small Proteins:** HA.

21. **Model Organisms for Disease:** Model organisms are vital for studying human neurological disorders due to genetic manipulability, controlled environments, and ability to dissect mechanisms. Challenges include potential phenotypic differences (milder/hard to interpret due to redundancy or species differences), limited complexity for human diseases, and ethical concerns.

22. **Antibodies in Molecular Biology:** Antibodies are invaluable due to their high specificity and affinity for targets. They are widely used for: **detection and quantification** (e.g., Western blot), **localization** (e.g., immunohistochemistry), and **purification** (e.g., immunoprecipitation) of proteins. Antibodies were critical in discovering and mapping the precise locations of **neurotransmitters receptors** and other key proteins in the brain, which fundamentally advanced our understanding of neuronal communication and neurological disorders. They allowed researchers to pinpoint where these proteins were expressed, whether they were active, and what other molecules they interacted with, essentially creating a detailed molecular map of brain cells.

23. **Visualizing Active Neurons (General):** To visualize neurons active during a task, use an **activity-dependent fluorescent reporter system** (e.g., cFOS promoter driving mCherry). This system requires **genetic targeting** (e.g., Cre-lox) and **temporal control** (e.g., tamoxifen with Cre-ERT2) to ensure labeling only during the specific behavioral task.

24. **Cell-Type-Specific Reporters:** Cell-type-specific reporters are made by placing a reporter gene (e.g., GFP) under the control of a **cell-type-specific promoter**. This promoter is the key molecular component, as it's a DNA sequence naturally active only in the target cell type (e.g., synapsin for neurons).

25. Optogenetics for Behavioral Causality (Necessity):
    Design: Introduce Halorhodopsin (inhibitory opsin) specifically into neuron X. Induce grooming. While grooming, deliver light to inhibit X neurons.
    Expected Outcome: If X is required, flies should stop or significantly reduce grooming.

26. **Optogenetics for Behavioral Sufficiency:** No, if stimulating neuron X causes

grooming, you **cannot conclude necessity**. This only proves **sufficiency** (X *can* cause grooming). Other pathways may exist, and necessity requires showing grooming *cannot* occur without X's activity (i.e., by inhibiting X).

27. Halorhodopsin Experiment:
    What happens in X neuron: Halorhodopsin pumps chloride ions into the neuron, causing hyperpolarization, which inhibits or silences the X neuron.
    Conclusion on Necessity: Yes, since inhibiting X neurons stops grooming even when stimulated, X neuron activity is now concluded as required (necessary) for antennal grooming.

28. Strengths of Optogenetics and Model Systems:
    Optogenetics Strengths: Offers temporal precision, cell-type specificity, reversibility, and is relatively non-invasive, enabling precise causal testing.
    Model Systems are Essential: They offer complementary strengths (e.g., flies for genetics, mice for complex behaviors), allow study of conserved mechanisms, are cost-effective, and facilitate experiments not possible in humans. However, they face challenges like translational gaps and species-specific differences.