# BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI

## I Semester 2023-24

### BIO G512 (Molecular Mechanism of Gene Expression)

### COMPREHENSSIVE EXAMINATION (OPEN BOOK)

**TIME: 3.0 Hrs** **11.12.2023** **Maximum Marks:40**

---

Note: (1) Each question carries 5.0 Marks

(2) Provide precise answer to the questions

---

Q 1. In phage T4, the genes rIIA and rIIB lie adjacent to each other on the T4 chromosome. During the early phase of infection, rIIA and rIIB products are present in equimolar amounts. In the late phase of infection, the amount of rIIB protein is ten to fifteen times higher than that of rIIA protein. Nonsense mutations (mutations to a stop codon) in rIIA eliminate early but not late rIIB transcription. In the mutants that contain small deletions near the end of rIIA, the amount of rIIA product is always equal to the amount of rIIB product, regardless of the time of infection. Based on this information, devise a map of the rII region. Include the location(s) of the promoter(s).

Q 2. You are studying the regulation of a key gene that controls circadian rhythm in mice, called Early to Bed (ETB). Using mutational mapping of the promoter and *in vivo* analysis of mutant promoters you have identified the binding sites for two key transcriptional activators that are responsible for inducing sleep in mice. You call these factors SNZ1 and SNZ2 You purify and clone SNZ1 and SNZ2 to determine how they stimulate transcription. Using gel shift assays you find that they each bind independently to the EBT promoter and do not stimulate each other binding to DNA.You first test their ability to activate transcription *in vitro* by adding the purified activators to a plasmid containing the ETB promoter and the purified RNA Pol II and auxiliary factors (TFIIA,TFIIB,TFIID,TFIIE,TFIIF,and TFIIH).You get the following results:

| Activator | *In vitro* Transcription Units |
|---|---|
| None | 50 U |
| SNZ1 | 2000 U |
| SNZ2 | 50 U |
| SNZ1+ SNZ2 | 2000 U |

(a) Propose two distinct mechanisms for SNZ2 action in vivo that would explain SNZ2 ,s inability to activate transcription in the *in vitro* assay? Assume that the SNZ2 you purified is properly folded and competent to bind DNA and perform all its normal functions.

(b) To address why SNZ2 is not activating in your *in vitro* reactions, you decide to map the activation domain of SNZ2 *in vivo*. Describe the experimental approach you would take to accomplish this goal.

Q 3 (a) What mating-type phenotype would you expect from a diploid cell of genotype MATa/MATα with a mutation in α in which the α2 gene product functions normally in turning off the a-specific genes but is unable to combine with the a1 product?

(b) What amino acids can be inserted at the site of the UGA codon that is suppressed by a suppressor tRNA?

**P.T.O**

Q 4 The histidine operon is negatively regulated and contains ten structural genes for the enzymes needed to synthesize histidine. The repressor protein is also coded within the operon—that is, in the polycistronic mRNA molecule that codes for the other proteins. Synthesis of this mRNA is controlled by a single operator regulating the activity of a single promoter. The co-repressor of this operon is tRNAHis, to which histidine is attached. This tRNA is not coded by the operon itself. A collection of mutants with the following defects is isolated. Determine whether the histidine enzymes would be synthesized by each of the mutants and whether each mutant would be dominant, cisdominant only, or recessive to its wild type allele in a partial diploid.

(a) The promoter cannot bind with RNA polymerase.

(b) The operator cannot bind the repressor protein.

(c) The repressor protein cannot bind with DNA.

(d) The repressor protein cannot bind histidyl-tRNAHis .

(e) The uncharged histRNA (that is, without histidine attached) can bind to the repressor protein.

Q 5. A $lacI^+$ $lacO^+$ $lacZ^+$ $lacY^+$ Hfr strain is mated with an F lacI $lacO^+$ lacZ lacY strain. In the absence of any inducer in the medium, β-galactosidase is made for a short time after the Hfr and $F^-$ cells have been mixed. Explain why it is made and why only for a short time.

Q 6. A mutant strain of *E. coli* is found that produces both β-galactosidase and permease constitutively (that is, whether lactose is present or not).

(a) What are two possible genotypes for this mutant?

(b) A second mutant is isolated that produces no active β-galactosidase at any time but produces permease if lactose is present in the medium. What is the genotype of this mutant?

(c) A partial diploid is created from the mutants in parts (a) and (b): When lactose is absent, neither enzyme is made, and when lactose is present, both enzymes are made. What is the genotype of the mutant in part (a)?

Q 7. Several eukaryotes are known in which a single effector molecule regulates the synthesis of different proteins coded by distinct mRNA molecules—say, X and Y. At what level in the process of gene expression does regulation occur in each of the following situations?

(a) Neither nuclear nor cytoplasmic RNA can be found for either X or Y.

(b) Nuclear but not cytoplasmic RNA can be found for both X and Y.

(c) Both nuclear and cytoplasmic RNA can be found for both X and Y, but none of it is associated with polysomes.

Q 8. (a) Mention the individual codons that shows four way pairing in archetypal genetic code.

(b) How the mutational biases influence the codon usage bias in *Mycoplasma*? Explain with example the induction of valine during translation.