

BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI

BIO G512 (Molecular Mechanism of Gene Expression)

COMPREHENSIVE EXAMINATION (OPEN BOOK)

TIME: 3.0 Hrs

21.12.2022

Maximum Marks:40

Note:

- (1) Each question carries 4.0 Marks
- (2) Provide precise answer to the questions

Q.1 A bacterium is isolated that contain an inducible operon whose genetic products metabolize oil. Further studies demonstrate that the operon is under positive control and that there is a *X* gene whose product interacts with an operator region (*O*) to regulate the structural gene designated *Y*.

In an attempt to understand how the operon functions, a constitutive mutant strain and several partial diploid strains were isolated and tested with the results shown here:

Host chromosome	F' factor	Phenotype
Wild type	None	inducible
Wild type	<i>X</i> gene from the mutant strain	Inducible
Wild type	Operon from mutant strain	constitutive
Mutant strain	<i>X</i> gene from the wild type	constitutive

- (a) Draw all possible conclusions about the mutation as well as the nature of regulation of the operon.
- (b) Is the constitutive mutation in the trans-acting *X* element or in the cis-acting *o* operator element?

Q2. Construct a merozygote of the *trp* operon in *E.coli* with two forms of first gene (*e* gene; *e*₁ *e*₂) in the operon. Describe the types of *cis* and *trans* effects that are possible, give mutants of any components of the operon. Can this repressible system work for any type of operon other than those control amino acid synthesis?

Q3. You have isolated a mutant that makes a temperature sensitive rho molecule; rho functions normally at 30⁰ C, but not at 40⁰C. If you grow this strain at both temperatures for a short period of time and isolate a newly synthesized RNA, what relative size RNA do you expect to find in each case?

Q4. T4, T7 and λ all have life cycles in which a large fraction is devoted to late transcription. Why is the duration of time allotted to late transcription greater than the time for early transcription?

P.T.O.

- Q5. An operon involved in utilizing a sugar X is regulated by a gene called *b*. When X is added to the cells, Xase is made; otherwise it is not. If the *b* gene is deleted (denoted by Δb), no Xase can be made. The diploid $b^+/\Delta b$ is inducible. Point mutants of *b* are of two type; *b1* never makes Xase, *b2* is constitutive. The partial diploids $b^+ /b1$ and $b^+/b2$ are inducible and constitutive respectively. What is a likely mode of action of the protein encoded by the *b* gene?
- Q6. A gene is Transcribed and Translated in 100 μ l reaction. Into that reaction are added 2 μ l of 20 mCi/mL [35 S] methionine (2000 Ci/mmol). After incubation at 37°C for 60 minutes, a 4 μ l aliquot is TCA precipitated to determine incorporation of radioactive label. In the liquid scintillation counter, the filter gives off 1.53×10^6 cpm. A 2 μ l aliquot of the reaction is counted directly in the scintillation counter to determine total counts in the reaction. This sample gives 2.4×10^7 cpm. What percentage of the [35 S] methionine has been incorporated into polypeptide?
- Q7. The control of gene initiation by the acetylase and deacetylase implies that these enzymes are somehow targeted to specific gene promoters. How is this done?
- Q8. What is the fundamental difference between **Intron late** and **intron early** theories of evolution of genes?
- Q9. What is the experimental evidence that chromatin loosening is involved in activation of eukaryotic genes?
- Q10. Comparison of the promoter sequences of a family of mammalian genes reveals that all share a sequence of eight nucleotides. Outline how you would test experimentally the possible role of this octamer sequence in regulating the expression of these genes.

-----GOOD LUCK-----