

GS351 Fall 2006

Preparatory Questions for Tuesday October 3rd

*Assigned readings:* Jacob, F and J Monod (1961) Genetic Regulatory Mechanisms in the Synthesis of Proteins. J Mol Biol 3:318-356.

*Optional background readings:*

Alberts, 4<sup>th</sup> edition, Molecular Biology of the Cell, Chapter 7

Hartwell et al. 2<sup>nd</sup> edition, Genetics – From Genes to Genomes, Chapter 16.

Describe methods for identifying where transcription initiates in vitro and in vivo. What must be considered when comparing eukaryotes and prokaryotes?

Describe a method for determining where a protein interacts with a DNA molecule.

What is an F' and an Hfr?

How was a gene identified and defined in 1961? How was it shown that mutations in different genes cause the same phenotype?

Describe the phenotype of each of these mutants.

lacZ<sup>-</sup>

lacY<sup>-</sup>

lacI<sup>-</sup>

Describe a method to isolate lacZ and lacY mutants.

Describe a method to isolate lacI mutants.

Describe the phenotype of each of these mutants.

lacI<sup>d</sup>

lacI<sup>s</sup>

Discuss how two-factor crosses were used to determine the genetic size of the lacZ gene.

β-galactosidase and acetylase have similar induction kinetics. How was it shown that these two enzymes are encoded by separate genes?

Jacob and Monod (and others) were uncertain if permease and acetylase were encoded by the same gene. Their uncertainty stemmed from the fact that most of the mutations in lacY were polar on lacA. What is a polar mutation? Give a few examples of how polar mutations act.

Describe the phenotype of lacO<sup>c</sup> mutants. Describe two ways to isolate lacO<sup>c</sup> mutants.

Describe the properties of  $\text{lacO}^{\circ}$  mutants (Table 3 line 10). Suggest a molecular explanation for the mutation.

Discuss the evidence that repression works via a cytoplasmic substance expressed by  $\text{lacI}$ .

Jacob and Monod considered the possibility that the  $\beta$ -galactosidase enzyme interacted directly with the inducer. What is the evidence against this hypothesis?

The  $\text{lac}$  repressor could act by directly repressing  $\text{lacZ}$  and  $\text{lacA}$  or, by indirectly inhibiting an activator of  $\text{lacZ}$  and  $\text{lacA}$ . What evidence favored the direct repression model?

Discuss the evidence that  $\beta$ -galactosides induce a short-lived intermediate (mRNA) responsible for the synthesis of  $\beta$ -galactosidase activity.

Discuss the evidence for or against  $\text{lacO}$  functioning at the genomic, posttranscriptional and posttranslational level.