

GS351 Fall 2006

Preparatory Questions for Thursday October 5th

**October 5** Transcriptional repression.

*Assigned readings:*

Gilbert W and B Müller-Hill (1966) Isolation of the Lac Repressor. (1966) PNAS 56:1891-1898.

Lewis et al. (1996) Crystal Structure of the Lactose Operon Repressor and Its Complexes with DNA and Inducer. Science 271:1247-1254.

*Optional background readings:*

Gilbert W and B Müller-Hill (1967) The Lac Operator is DNA. PNAS 58:2415-2421.

1. How was the  $\text{lacI}^t$  (tight-binding) mutant isolated?
2. Describe the method used by Gilbert and Müller-Hill to purify the lac repressor.
3. What negative controls were done to demonstrate that what was purified was the product of the lacI gene?
4. What is the size of the lac operator? What is its structure? What does the structure of the operator suggest about how the lac repressor binds to it?
5. What are the functional motifs present in the lac repressor? How were these originally identified?
6. Draw a ball-&-stick model of the lac repressor bound to its operator similar to that shown in Figure 10.
7. Describe the location of the  $\text{lacI}^s$  mutations and discuss how the crystal structure supports the mechanism of action of the mutations.
8. Many of the  $\text{lacI}^d$  mutations cluster in the region coding for the amino terminus of the repressor. These mutations can be suppressed by nonsense suppressing tRNAs. What is nonsense suppression and how does it work?
9. Given what you know about the structural motifs present in the lac repressor, suggest a mechanism for the action of the  $\text{lacI}^d$  mutations.
10. The lac operator has dyad symmetry, yet the repressor is a tetramer. Discuss the model for the function of the tetramer.
11. Discuss the mechanism of how the lac repressor is likely to inhibit transcription.