1. The cis and trans elements regulating the lac operon were first identified by Jacob and Monod through their genetic screens for mutants with defective lac operon expression. Their model for lac operon regulation, which held for many decades, had lac repressor binding to a single operator element in the lac promoter. Why do you suppose the two auxiliary operators were missed for all these years? Were Jacob and Monod sloppy scientists? Were they oversimplifying?

2. In the right hand column of Fig. 2, the authors express “repression” by single numbers (1300, 440, etc.). What do these numbers really mean, and how did the authors determine them? (How can a beta-galactosidase assay for enzyme activity provide information about promoter activity, i.e. rate of transcription?)

3. What is the purpose of gel-shift experiment in Fig. 4? Are the authors demonstrating that the lac promoter forms loops in vitro? In this figure the authors assert that one of the gel band represents a looped DNA molecule bound to a single lac repressor tetramer; the supporting paper they cite, Kramer et al., refers to this assignment as an assumption. What results in Fig. 4 are consistent with this assumption. Can you think of additional ways to support this assignment?

4. Why should too high a concentration of tetrameric lac repressor prevent loop formation? What is the basis for arguing that such a concentration is reached in B but not A of Table 1?

5. The repression factor in Table 1 was calculated slightly differently than in Fig. 2. What was this difference and why was the method used in Fig. 2 preferable?

6. Have the authors convinced you that DNA loops really form at the lac promoter in vivo? Can you summarize the key findings in this paper that support this idea? Can you think of any other (reasonable) interpretations of the data? Are there additional experiments that might strengthen the authors’ looping model?