**Chapter 12 Study Questions**

*Genetic Analysis: Genes, Genomes, and Networks in Eukaryotes*

1. The complete genome sequence of an organism has often been compared to compiling a “parts list”. By contrast, an organism has “some assembly required.” Let’s think through this analogy, reflecting on what is found when you open a box of parts that require some assembly to make a functional object.
   1. Discuss some of the genomic and molecular information that is needed to think of the genome sequence as an accurate and complete parts list.
   2. What are some of the techniques and strategies by which the information about the parts in 1a can be obtained, ideally as quickly and readily as possible?
   3. What additional information is needed to assemble the parts of a non-biological object into something functional?
   4. What is the corresponding information that is needed to “assemble the genomic parts list” to produce a biological function?
   5. What are some of the techniques and strategies to obtain the information needed in question 1d, even if only partial assembly instructions are feasible?
   6. Of the information needed for question 1d, which do you think will be the most difficult to obtain, and why?
2. Transcription factor networks for gene regulation are among the best studied molecular biological systems. Contrast the goals and strategies of transcription factor networks that are
   1. Protein-based.
   2. Gene-based.
   3. Genome-based.
3. Gene regulatory hierarchies such as those shown in Figures 12-7, 12-8, and 12-9 and on the ENCODE and modENCODE web sites are often used to display the interactions of transcription factor with each other and their target genes.
   1. Compare the expected effects of a mutation that affects a gene in the top tier of such a hierarchy with ones that affect a gene at the lowest tier of the hierarchy.
   2. Suppose a gene regulatory hierarchy such as the one in Figure 12-7 for *Drosophila melanogaster* could also be constructed for a related Drosophila species. Which parts of the hierarchy would you expect to be the most similar and which parts might you expect to be the most different between two closely related species? This has not yet been done and the answer is not known, in part because of the effort to construct even one hierarchy, so it is more important to think through your reasoning rather than attempt to “get the right answer”.
4. The evolution of transcription factor networks can be done by comparing specific circuits in closely related species. While no one paper will provide a full picture of the evolution of transcription factor networks, Sorrells et al (Nature 2015 523: 361-365, plus supplemental information) is an excellent and clear analysis of one specific network in three different species of yeast. The following questions are based on that paper, and will provide some ways to think about the evolutionary changes governing other transcription factor networks.
   1. Define the concept of historical contingency, and discuss how this affects our thinking and analysis of transcription factor networks.
   2. What is the particular transcription factor network being analyzed in this paper and what role does it play in the biology of these three species of yeast?
   3. The results indicate that general pheromone response genes in all three species have an enrichment of Ste12 binding sites in their upstream regions. The authors write that “this enrichment was expected”. Discuss why this enrichment was expected to be similar in all three species.
   4. In contrast to the general pheromone response genes, **a-**specific genes differ in their enrichment of Ste12 binding sites in their upstream regions among the three different clades, with only the Saccharomyces clade showing a significant enrichment. Three different models are proposed that could account for this lack of enrichment in *K. lactis*. Explain each of these three models using appropriate diagrams.
   5. Among the methods used to distinguish among these three models was a ChIP assay for Ste12. For each of the three models proposed in question 4d, what are the anticipated results of this ChIP assay?
   6. Explain the evidence that supported the second model. There were three lines of evidence supporting Model 2.
   7. By inferences from ancestral species, **a**-specific binding sites were gained in the Saccharomyces clade. Discuss the experiments and the underlying rationale that address how and why these **a**-specific binding sites were gained.
   8. What is the evidence that the presence of **a**-specific binding sites is the result of selection in Saccharomyces rather than the result of genetic drift? This question may require a definition of purifying selection and genetic drift, if these terms are not a part of your current genetics vocabulary.
   9. Mating type-specific gene expression in yeasts is among the best studied transcriptional circuits in eukaryotes. What are some of the lessons to be learned from the analysis of this circuit in this paper that might be applied to thinking about transcriptional regulation in other biological processes in other eukaryotes?