**Chapter 9 Study Questions**

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

1. The Study Questions for Chapter 2 asked about performing a genomic analysis for a little-studied organism known as Placozoa. This set of questions will ask about conducting a mutant analysis for Placozoa. It will be helpful to read a bit about them for background. The key biological features are that the organisms are multicellular but only a few cell layers thick. Recombination has been reported for one group, which suggests that they can carry out sexual reproduction, but most of their reproduction appears to be asexual by budding. They are composed of epithelia sheets of cells, with some mesenchymal cells between the epithelium. They crawl along surfaces (such as aquarium glass or the ocean floor) using cilia. There are many different and competing views of their evolutionary relationships since they have some but not all molecular, morphological, and cellular features of various other eukaryotic taxa. Assume that the genome has been sequenced and that annotation is on-going, as discussed in the Chapter 2 Study Questions. You could also assume that transgenic organisms can be made and that genes modified in vitro can be stably introduced into the organism, although not necessarily targeted by recombination.
2. Based on your background reading, what is one mutant phenotype that might be of interest for your analysis? There are numerous possibilities, but you will use this phenotype in the remainder of these questions.
3. Assuming that the species you are working with can reproduce sexually, outline a traditional genetic screen of the type discussed in Chapter 4 to find mutants that affect the phenotype you chose.
4. If the species cannot reproduce sexually, could a traditional genetic screen be done? Why or why not?
5. For a “reverse genetic” analysis of the type outlined in Chapters 7 and 8, it is necessary to define a gene that encodes a polypeptide (or ncRNA) that is a good candidate for affecting the phenotype in question. What gene would you choose to carry out a reverse genetic analysis and why would you choose this gene? Outline how you would carry out this analysis and what you might expect to find if your hypothesis about the role of this molecule is correct.
6. You also would like to carry out a genome-wide mutant analysis of the types described in this chapter. What method would you choose for this analysis and why would you choose this method?
7. Faced with the opportunity to do a traditional genetic analysis for your phenotype, a reverse genetic analysis, and a genome-wide genetic analysis, which approach would you choose to do? Why would you choose this method?
8. Three techniques are described in the chapter that can be used to disrupt the function of every gene in a genome-wide mutant screen. Compare the relative advantages and possible limitations of using targeted gene disruptions, RNAi, and CRISPR-Cas9 screens.
9. Genome-wide mutant screens have consistently shown that many genes do not have a mutant phenotype in single mutant strains. Yet genetics has thrived as an experimental approach by studying single genes that do have mutant phenotypes.
   1. What do you think are some of the properties of genes that have a mutant phenotype in single mutant strains versus those genes that do not have a mutant phenotype in single mutants?
   2. Have we been misled about the nature of genes and their biological roles by focusing on those that had a mutant phenotype? Why or why not?
10. Zhang et al. (2018 G3 8:3005-3018) carried out an extensive screen using RNAi in the Drosophila germline to identify proteins that are regulated by phosphorylation during egg activation. This set of questions is based upon that paper.
    1. The authors tested 207 RNAi clones targeting 189 genes for their effects on this process, so the screen was not in fact “genome-wide”. However, it seems likely that nearly all of the relevant genes were tested in this analysis. What was the evidence that indicated to the authors that they could focus on a smaller set of genes and proteins rather than doing a full genome-wide test of every gene?
    2. The authors focused on a germline-specific RNAi system to test these genes. What aspect of their screening method ensured that the dsRNA used for RNAi would be expressed in the germline? Why did they use germline-specific expression rather than testing the entire organism, which would have been easier?
    3. How did their primary and secondary screens differ, and why did they carry out both? (It is always important to ensure that results are reproducible, but they had an additional rationale as well.)
    4. Briefly explain how the phenotypes observed led them to classify the genes into six classes in Table 1.
    5. What was some other evidence that this RNAi screen was working as expected, in addition to the mutant phenotypes observed?
    6. Section 9.8 in the chapter posits two lessons that have been learned from genome-wide screens. How do the results of this screen illustrate these two lessons?
    7. Table 1 identifies 108 genes for which no mutant phenotype was observed by RNAi. Briefly discuss why these genes might have no mutant phenotype.
    8. The authors chose to carry out this screen using RNAi rather than CRISPR, although CRISPR techniques for Drosophila are well-developed. In light of what is known about the processes involved in oogenesis and early development in Drosophila, what was the importance of using RNAi for this analysis? It may also be helpful to consider the molecular effects of both RNAi and CRISPR.
11. A review article by Doench (Nature Review Genetics 2018 19:67-80) presents a thorough and readable discussion of the considerations for carrying out a genome-wide CRISPR screen, with an emphasis on conducting the screen in mammalian cells.
    1. Briefly discuss how and why pooling is done in such screens.
    2. What role does bar-coding play in some in vivo models and why is it important?
    3. The choice of the sgRNA and its target is one of the most important in any CRISPR screen. Figure 3B shows a standard diagram of a eukaryotic gene, and identifies some good and poor potential target sites within this gene. Discuss each of these sites in turn, commenting on what would make it a good or poor target site for a gene knockout screen.
    4. Suppose that your screen wants to focus particularly on the ncRNAs in a genome. What might be your preferred method of analysis, and why?
    5. Imagine that you have done an initial screen and identified 16 candidate genes that appear to affect your phenotype of interest. Outline how you might pursue these candidate genes, taking into account that some of them might be due to off-target effects.