**Chapter 10 Study Questions**

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

**Author’s Note:** The Case Study on spindle morphogenesis in yeast provides a very complete analysis of suppression, non-allelic non-complementation, and synthetic enhancer within the context of a well-studied biological process. Students are encouraged to work through that example as a way to understand how gene interactions can be used productively to study a complicated biological process.

1. In a typical screen for suppressor mutations, most geneticists will begin by mutagenizing a missense mutant. In a typical screen for synthetic enhancer mutations, most geneticists will begin with a null mutant allele.
2. Explain the goals of doing a screen for suppressor and synthetic enhancers. What are some of the challenges associated with such screens?
3. Explain why suppressor screens usually begin with a missense mutant. Are there situations when an investigator may want to begin with a null mutant instead?
4. Explain why synthetic enhancer screens usually begin with a null mutant. Are there situations when an investigator may want to begin with a missense mutant?
5. How has our ability to do genome-wide mutant screens (as discussed in Chapter 9) affected the process and feasibility of suppressor and synthetic enhancer screens? Does this change any of the “rules” laid out in this chapter?
6. Some researchers believe that non-allelic non-complementation has been an under-utilized tool for identifying and characterizing gene interactions.
   1. Using one of the Arabidopsis herbicide resistant mutants identified in your mutant hunt in Chapter 4, describe how you might use non-allelic non-complementation to search for other genes involved in this process. Be sure to include some important characteristics of the starting mutation.
   2. Based on what is postulated about the biological mechanisms by which non-allelic non-complementation occurs, what might be some of the limitations of your mutant hunt?

Questions 3-5. Suppressor mutations have been widely used in *C. elegans*, and many types of suppressors have been found; there has also been extensive work with synthetic enhancers. The next several questions are based on some such screens in worms, although similar examples can be found in other organisms. In *C. elegans*, a gene name typically consists of three letters followed by a number; alleles are indicated by a one or two letter designation followed by a number. Thus, *let-2* is the name of the gene while *mn153* and *mn139* are different mutant alleles of *let-2*.

1. A mutation in the gene *sup-5* was tested for its ability to suppress several different lethal alleles in a number of different genes. Some of the results are summarized below.

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| **Genotype** | **Phenotype** |
| *let-2 (mn153)/let-2 (mn153)* | dead at the egg stage |
| *sup-5/sup-5; let-2 (mn153)/let-2 (mn153)* | alive and fertile adults |
| *let-2 (mn139)/let-2 (mn139)* | dead at the egg stage |
| *sup-5/sup-5; let-2 (mn139)/let-2 (mn139)* | dead at the egg stage |
|  |  |
| *let-12 (mn121)/let-12 (mn121)* | dead at the L1 stage |
| *sup-5/sup-5; let-12 (mn121)/let-12 (mn121)* | alive and fertile adults |
| *let-12 (mn123)/let-12 (mn123)* | dead at the L1 stage |
| *sup-5/sup-5; let-12 (mn123)/let-12 (mn123)* | alive and fertile adults |

1. The biological functions of *let-2* and *let-12* are not related to one another. Based on these experiments, what type of suppressor is *sup-5*, and what information do we learn about the lethal mutations (either these alleles or these genes) from these results?
2. Several years after the suppression analysis, the *sup-5* gene was cloned by position, and the genomic region was sequenced. The region that included *sup-5* had three predicted genes in it: one was predicted to be a membrane spanning protein; one was predicted to be a G-protein involved in signal transduction; and the third was predicted to be a tRNA molecule. Which of this is most likely to be *sup-5* and why?
3. What evidence would be the most conclusive about these three predicted genes was in fact *sup-5*? Try to be as specific about the expected results as you can.
4. In the original mutant screen, several alleles were found of a gene named *let-7.* Although tested, none of these alleles was suppressed by *sup-5*. After the *let-7* gene was cloned, its functional gene product was found to be a non-translated microRNA. Would it have been possible to find an allele of *let-7* that is suppressed by *sup-5*? Why or why not?
5. (These experiments and results are slightly modified from the original experiments.) Worms homozygous for a missense mutation called *e444* in the gene *unc-52* are paralyzed. Moerman et al. identified suppressors of *unc-52 (e444)* by treating *e444/e444* homozygotes with a mutagen and looking for worms that could move. A number of suppressor mutations were found and mapped. One group of suppressor mutations all mapped to the same location and failed to complement each other for suppression of *unc-52 (e444)*; this group defined the gene *sup-4*. The various *sup-4* mutant alleles also suppressed many different alleles of *unc-52* in addition to *e444*. One other suppressor called *mn472* mapped to a different location than either *unc-52* or *sup-4* and was found to be an allele of the gene *mec-8*. Other alleles of *mec-8* did not suppress *unc-52* *(e444*), nor did *mec-8 (mn472*) suppress other alleles of *unc-52*. Upon molecular and cellular analysis, the *unc-52* gene encodes a protein secreted by the muscle cells that anchors them to the body wall. *mec-8* encodes a protein found in the extracellular matrix of the skin cells of the body wall.
6. What type of suppressor is *sup-4*?
7. What type of suppressor is defined by *mec-8 (mn472)*?
8. Suppose that one did a yeast two-hybrid screen using the wild-type UNC-52 protein as the bait. Which of the following results do you predict would be found and why?
9. The wild-type MEC-8 protein and the wild-type SUP-4 proteins will be both found as one of the prey genes
10. The wild-type MEC-8 protein will be found as one of the prey proteins, but the wild-type SUP-4 protein might or might not be found
11. A mutant MEC-8 protein will be found as a prey protein, but the wild-type MEC-8 protein will not be found. No prediction can be made regarding SUP-4.
12. Neither a wild-type MEC-8 nor a mutant MEC-8 will be found as a prey, but no prediction can be made regarding SUP-4 protein.
13. The wild-type SUP-4 protein will be found as one of the prey proteins, but no prediction can be made regarding MEC-8 mutant or wild-type.
14. Based on these results, postulate a biological model involving the genetic interactions of *mec-8* and *unc-52*.
15. Does your model predict an interaction between *mec-8* and *sup-4*? How would you modify it if *sup-4* also suppresses *mec-8 (mn472*)?
16. (These experiments and results are slightly modified from the original experiments.) Many genes that affect the cell lineages that give rise to the vulva have been described in *C. elegans*. One of the mutant phenotypes seen in some mutants is the presence of two vulvae, a phenotype known as Bi-vulva or Biv. Green et al. (Cell 2008) focused on four of these genes and included the following table in their paper (slightly simplified). The phenotypes of the four single mutants are listed first, and the phenotype of some of the appropriate double mutant strains are listed next. All of the mutations are recessive and all of the strains examined and compared were homozygotes.

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| **Genotype** | **Phenotype (% Biv animals)** |
| Wild-type | 0 |
| *lin-18/lin-18* | 43% Biv |
| *lin-17/lin-17* | 72% Biv |
| *lin-44/lin-44* | 0% Biv |
| *mom-2/mom-2* | 1% Biv |
| *lin-17/lin-17; lin-18/lin-18* | 100% Biv |
| *lin-17/lin-17; lin-44/lin-44* | 67% Biv |
| *lin-17/lin-17; mom-2/mom-2* | 96% Biv |
| *lin-18/lin-18; lin-44/lin-44* | 88% Biv |
| *lin-18/lin-18; mom-2/mom-2* | 44% Biv |

**Note:** The percentage of Biv animals in the *lin-17; lin-44* double mutant (67%) is not significantly different from the percentage of Biv animals in the *lin-17* single mutant (72%). Likewise, the percentage of Biv animals in the *lin-18* single mutant (43%) and the *lin-18; mom-2* double mutant (44%) are not significantly different. The other percentages are significantly different when single and double mutants are compared.

1. What is the correct terminology for the nature of the genetic interaction between *lin-18* and *lin-17?*
2. Why is there no interaction effect between *lin-17* and *lin-44*, and no interaction effect between *lin-18* and *mom-2*
3. The data from the *lin-44; mom-2* double mutant was intentionally omitted from the table. What do you predict will be the phenotype of this double mutant and why? Although it is not possible to assign an expected percentage of Biv animals from the double mutant, do you expect the percentage of Biv animals in the double mutant to be less than 10% or more than 10%?
4. Molecular analysis has shown that the LIN-17 protein and the LIN-18 protein are probably membrane-bound proteins, whereas the LIN-44 and the MOM-2 proteins are expected to be secreted or extracellular proteins. Propose a model for the signaling pathway(s) involving these four genes. *lin-17* and *lin-44* are in the same pathway.
5. Concisely describe a molecular or biochemical experiment that could directly confirm part of your model in part D. What result would be expected if your model is correct and what result(s) would contradict your model?