**Chapter 11 Study Questions**

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

1. Outline some of the strengths and possible limitations of constructing a biological pathway based primarily on epistasis. Which of the limitations would make you especially cautious about interpreting the results of epistasis?
2. A one-color microarray experiment was done to determine the transcriptional targets of two different transcription factors, TxF1 and TxF2. The mutations inactivating TxF1 and TxF2 affected their DNA binding domains but their genes were not deleted. Twelve features from the microarray are shown below. A dark circle indicates that the gene is being transcribed at a high level, while an open circle indicates that the gene is not being transcribed; light gray circles indicate a low level of transcription.



1. Draw the pathway of regulation by TxF1 for each of the twelve genes in the microarray, using the symbols from the chapter.
2. Draw the same pathway for TxF2.
3. In a hypothetical plant, the ***b*** gene encodes an enzyme that makes a blue pigment; ***b+\_*** plants have blue flowers while ***bb*** plants have white flowers. The transcription of the ***b*** gene is regulated by the ***t*** gene; ***t+\_*** plants transcribe the ***b*** gene at high levels, while ***tt*** plants do not. Thus, ***tt*** plants also have white flowers. The genes are not linked.
4. A plant of genotype ***tt; b+b+*** is crossed to one that is ***t+t+ bb***. What color are the flowers of the F1 plant? If the F1 plant is allowed to self-fertilize, what will be the phenotypic ratios of flower colors among the F2 plants?
5. PCR primers were designed to amplify part of the transcript from the ***b*** gene. RNA was extracted from wild-type blue plants (***t+t+; b+b*+),** from each of the two white parents **(*t+t+; bb*** and ***tt; b+b+),*** and from three white plants of unknown genotype from the F2 generation. The RNA was used for RT-PCR, and the products were run on an agarose gel. The results are shown below. (Note: as conventional, larger products are towards the top and smaller products are towards the bottom of the gel.)



1. Based on this gel, what might be the molecular defect in the ***b*** gene that eliminates its function?
2. What are the likely genotypes of the three F2 white plants, labeled white #1, white #2, and white #3?
3. In *C. elegans*, there are many genes that control the formation of the vulva. Recessive mutations in the gene *lin-3* cause the worms to make no vulva (Vulvaless, or Vul). Recessive mutations in the gene *lin-1* cause the worm to make multiple vulvae (Muv). A double mutant is made between *lin-3* and *lin-1*. The *lin-1; lin-3* double mutant worm is Muv.
   1. What is the wild-type function of the *lin-1* gene, based on its mutant phenotype?
   2. Would you consider *lin-1* and *lin-3* to be in a positive or negative relationship with each other?
   3. Draw the pathway that illustrates the relationship between *lin-1* and *lin-3*.
4. Recessive mutations in *lin-45* also result in a Vul phenotype, whereas a dominant hypermorphic mutation in *lin-45* results in a Muv phenotype. What double mutants would you make to determine where *lin-45* acts in the pathway, and what phenotype would you expect to see if
5. *lin-45* acts upstream of *lin-3*
6. *lin-45* acts downstream of *lin-1*
7. *lin-45* acts downstream of *lin-3* but upstream of *lin-1*

Questions 6-12 are based on somatic sex determination in Drosophila. This example is also used in Chapter 4, and some of the mutants are introduced there. In normal flies, 1X embryos (typically XY) develop as males. 2X embryos (typically XX) develop as females. The chromosomal signal for sex determination is assessed by a gene called *Sex-lethal* (*Sxl*) which then acts on several downstream genes that carry out the sex determination developmental program in somatic cells. *Sxl* is X-linked. In *Sxl* loss-of-function mutations, XY flies are normal but XX flies develop as males; however, because dosage compensation is also affected these flies usually die unless particular alleles of *Sxl* or conditions are used. For these questions, the lethality of *Sxl* will be ignored, and the sex transformation phenotype will be considered. The phenotypes of recessive mutations in several autosomal genes that act downstream of *Sxl* genes are summarized in the table. The phenotype of *dsx/dsx* flies is identical in XX and XY genotypes, and shows aspects of both male and female sexual development, referred to as an intersex. The phenotype of *ix/ix* XX flies is also intersexual, but XY *ix/ix* flies are normal males. Many of these questions are similar to experiments done by Baker and Ridge, 1980 Genetics 94: 383-423, McKeown, Belote, and Boggs 1988 Cell 53: 887-895, or Nagoshi, et al. 1988 Cell 53: 229-238 although some results have been simplified.

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| **Genotype** | **XY Phenotype** | **XX Phenotype** |
| wild-type | males | females |
| *Sxl* | normal males | males if survive |
| *tra-2/tra-2* | males but sterile | male |
| *tra/tra* | males and fertile | male |
| *dsx/dsx* | intersexes | Intersexes |
| *ix/ix* | Male | Intersex |
| *dsx/dsx; tra-2/tra-2* | intersex | Intersex |
| *dsx/dsx; tra/tra* | intersex | intersex |
|  |  |  |

1. What is the inferred role for each of these five genes in normal Drosophila sex determination? This needs to be answered carefully to avoid confusion in the remaining questions. Does the phenotype of the mutant suggest that the gene is needed in one sex or in both sexes? Can you tell if it is needed to turn on the development of one sex or to turn off the development of the other sex?
2. Based on the phenotypes of the *dsx; tra-2*, and the *dsx; tra* double mutants, construct a pathway by which these genes interact.
3. Is it possible, based on these results, to place *tra-2* and *tra* in a pathway with respect to each other? Would a double mutant between them be informative? Why or why not?
4. *dsx* and *ix* have nearly identical phenotypes, but the *dsx* phenotype is seen in both XY and XX flies while the *ix* phenotype is seen only in XX flies. Would a double mutant between them be informative? Why or why not?
5. In order to understand the molecular basis for the interactions among these genes, each of the genes was cloned, and the transcription pattern for each gene was determined. One of the striking results was that most of the genes are transcribed in both sexes, but the activities of the genes are regulated by alternative splicing. (This example is introduced in Chapter 1, and is one of the best studied examples of alternative splicing.) The transcripts for the wild-type *tra* gene are shown in the gel below. Note that *tra* has two transcripts in XX flies, but only one in XY flies. The gel shows the splicing pattern for *tra* in several mutant flies; the color of the genotype indicates the somatic sex of the fly, with red meaning female, blue meaning male, and purple meaning intersexual. Explain these results in light of the pathways that you drew in questions 7 and 8.



1. A similar set of experiments was done to examine the effects of some of these genes on the splicing of the *dsx* gene. The results are shown in the gel below. *dsx* has different transcripts in XX and XY flies, due to alternative splicing. (A minor additional transcript found in XY flies has been omitted for simplicity.) The splicing pattern of *dsx* was examined in various mutant flies, in both XX and XY flies. Explain these results in light of the pathways that you drew in questions 7 and 8, including any additional information that can be inferred based on these results on the results in question 10.



1. Do these experiments show that any of these genes directly regulates the splicing of one of the other genes or is additional information needed to make that conclusion?