**Chapter 6 Study Questions**

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

With partial solutions

1. What is meant by the term “gene activity”? In an era when genomes are sequenced and many genes are cloned before they are analyzed, is this a useful concept? If so, how is it used? Why is it useful to consider both null mutants and hypomorphic mutants when discussing gene activity?
2. *C. elegans* has defined cell lineages so the timing of cell division and the location of each cell is known. The cell lineages generate a total of 1090 somatic cells (not including the germline). Of these cells, 131 undergo programmed cell death; 18 cells die after hatching and are the easiest to see. Programmed cell death is also known as apoptosis. H. Robert Horvitz and his students identified hundreds of mutants in twelve genes that affect these cell deaths, which provided the foundation for understanding apoptosis in other organisms. Most of these genes are called *ced* for “cell death”, and the different gene names are *ced-1, ced-2, ced-3 etc.*
3. wo different recessive mutants, referred to as *n1001* and *n2005*, have no cell deaths and all 131 cells survive rather than die. Both genes were mapped to chromosome 3. Very briefly describe how to determine if these two mutations are alleles of the same gene or if they are alleles of two different genes without sequencing the genomes of each mutant.

1. One of the recessive mutant alleles of the *ced-3* gene is temperature-sensitive, symbolized ced*-3(ts)* with the following phenotypes observed.

**Genotype Phenotype**

WT 131 cell deaths

*ced-3 (ts)* at 15° C 131 cell deaths

*ced-3 (ts)* at 25° C no cell deaths

1. Based on the mutant phenotype, what is the normal function of the wild-type *ced-3+* gene?

1. If this *ced-3 (ts)* allele is made heterozygous for a deletion of the *ced-3* gene in the genotype *ced-3 (ts)/ deletion*, no cell deaths are observed at any temperature. This type of genetic test defines this mutation to be what type of *ced-3* allele?
2. Do you predict that all or most mutations that are temperature-sensitive will also show this same effect with a deletion? Why or why not?
3. Most recessive mutations in the gene *unc-13* in *C. elegans* result in a worm that is paralyzed when homozygous. The wild-type gene for *unc-13+* was cloned and inserted onto a vector along with the wild-type allele for a gene known as *ncl-1+.* In a *ncl-1* mutant worm, the shape and size of the cell nucleus (which is easy to see in worm cells) is abnormal but the worm is otherwise normal. The vector with both *ncl-1+* and *unc-13+* was microinjected into the gonad of a worm that is mutant for both *unc-13* and *ncl-1,* so it is paralyzed and its nuclei are abnormal. The offspring are scored for their ability to move—**it is important to realize that only moving worms were examined**. Worms that could move (that is, that were Unc-13+) were then scored for the shape and size of their nuclei in both muscle cells and nerve cells. Among moving worms, 64% have normal nuclei in both muscle and nerve cells, 36% have normal nuclei in their nerves and abnormal nuclei in their muscles, and none have normal nuclei in their muscles and abnormal nuclei in their nerves. To confirm the result, 20 paralyzed worms were then examined—**it is important that paralyzed worms were examined in this experiment**. Of these 20 paralyzed worms, 12 had abnormal nuclei in both muscle and nerve cells and 8 had abnormal nuclei in nerves but normal nuclei in muscles. What cells need *unc-13+* for normal function? Explain briefly.

1. A few of the mutations in *Drosophila melanogaster* that affect sex determination were introduced in Chapters1 and 3, and will be considered again in Chapter 11. Recessive mutations in the genes *tra* or *tra-2* result in XX flies (normally females) showing male sex determination. In 1X flies, *tra* mutants are normal males, while *tra-2* mutants are sterile males but that effect on fertility is not relevant for these questions; for these questions, it can be considered that both 1X and 2X embryos develop as males in these mutants. An easily visible and reliable indicator of male development is the appearance of a sex comb on the foreleg; males have a sex comb whereas females do not.
   1. A temperature-sensitive allele of *tra* was used for temperature-shift experiments. In homozygotes for this allele, XX flies are female (and have no sex combs) when grown at 18C but male (and have sex combs) when grown at 28C. XX larvae homozygous for this mutation were grown at 28 C and shifted to 18C at various times before and after pupariation. Once shifted, they are kept at 18C. The results of these shift down experiments are shown.

Time of Shift to 18 C % with Male Sex Combs

72 hrs before pupation 13

60 hrs before pupation 11

48 hrs before pupation 92

36 hrs before pupation 89

18 hrs before pupation 90

0 hrs before pupation 93

8 hrs after pupation 95

no shift 97

A companion shift-up experiment was also done. XX larvae homozygous for this mutation were grown at 18C and shifted to 28C at various times before and after pupation. The results are summarized below.

Time of Shift to 28 C % with Male Sex Combs

90 hrs before pupation 94

66 hrs before pupation 96

56 hrs before pupation 93

42 hrs before pupation 13

30 hrs before pupation 9

15 hrs before pupation 8

8 hrs after pupation 6

no shift 5

What is the best estimate for the time at which the wild-type *tra* gene product is needed? Briefly explain your answer.

* 1. The wild-type copy of the *tra* gene was cloned into a P element vector in which its transcription is under the control of a heat-shock promoter. Thus, when the fly is heat-shocked at 35 C for three hours, the *tra* gene will be transcribed. This P element construct (symbolized **P *[hs-tra+])*** was integrated into flies, and the resulting flies were sexually normal under normal growth conditions. When XX larvae are heat-shocked, they development as females. More importantly, when XY larvae were heat-shocked, they also developed as females. Briefly explain this result.
  2. This part of the question anticipates topics that are covered in Chapter 11, so this is asking you to think ahead. A fly strain is made that has the **P [*hs-tra+*]** element but is a homozygous mutant for *tra-2/tra-2*. When the larvae of this strain are heat-shocked, the XY flies now develop as males. How can you explain this result?
  3. What do you predict will be the phenotype of XX flies from the experiment done in Part c?