General instructions: Be sure that your name is on every sheet. Clearly state your reasoning; if we can understand what you are saying during the grading, there is a greater chance that we will give you partial credit. Total points = 100.

1. (67 points) A mutagenesis for touch-insensitive (tin) mutants in C. elegans gave the following results (Note: gene tin-6 is in quotes because the single mutation has not been well studied. It’s phenotype is recessive, and it complements the other recessive mutations.)

<table>
<thead>
<tr>
<th>Gene</th>
<th>recessive alleles</th>
<th>dominant alleles</th>
<th>temperature sensitive alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>tin-1</td>
<td>24</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>tin-2</td>
<td>19</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>tin-3</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>tin-4</td>
<td>16</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>tin-5</td>
<td>20</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>“tin-6”</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>tin-7</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>tin-8</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>tin-9</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>tin-10</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a. (6) For which of the first six genes can you deduce the null phenotype (list these in number order)? What is the null phenotype in each case? Explain your answer.

*tin-1, tin-2, tin-4, and tin-5* are genes that have probably been mutated to a null phenotype. All have a large number of alleles. The null phenotypes are respectively: recessive touch insensitivity, dominant touch insensitivity, recessive temperature-sensitive touch insensitivity, and recessive touch insensitivity.

b. (4) How could you determine whether the null phenotype you predict in part a is the correct null phenotype for the first two genes on your list? (Note: you will probably have to do two different experiments.)

*For tin-1* look over a deletion and see if the phenotype becomes more severe (it should not). Then look for more non-complementing mutations. *For tin-2* look for the phenotype of a heterozygote deletion for the region.

c. (6) Give three explanations for the results in the table for the gene(s) for which you cannot predict a null phenotype.

small gene, small region of a gene, redundant gene (synthetic mutations)
d. (6) For each of the explanations in c describe an experiment that would allow you to determine the null phenotype.

For the first two one would do the same experiment as for tin-1 in part b. In the first case, no new phenotype would be obtained, for the second it would. For synthetic mutations: mate to wild-type and see if the resulting heterozygotes give _ or 1/16 mutant progeny.

e. (2) How do you explain the temperature-sensitive allele of tin-1?

Hypomorphic mutation

f. (3) How do you think that the dominant mutations in tin-5 were shown genetically (not molecularly) to be in the gene?

Revert balanced heterozygotes to wild-type and then look to see if they give recessive alleles that can be used in a complementation test.

g. (3) Mutation of tin-3 animals results at high frequency (i.e, for a similarly sized experiment as above 17 mutants were found) in animals with a wild-type phenotype. Explain this result.

The null phenotype is wild type, indicating redundancy. The dominant allele is making an abnormal interfering (antimorphic) product.

h. (3) Mutation of tin-7 animals results at high frequency (14 mutants) in animals defective in chemosensation. Explain this result.

The null phenotype is a defect in chemosensation. Since the original dominant mutation caused a touch sensitivity defect, this is an example of (spatial) misexpression.
i. (4) What part of the *tin-7* gene would you expect to be mutated to give this phenotype? Where in the gene would you look for the defect?

One would expect to see a mutation in an enhancer element. One would not expect the mutation to be in the coding region.

j. (3) Mutation of *tin-8* animals results at high frequency (19 mutants) in animals that were supersensitive to touch. The new mutations are recessive. Explain this result.

The loss of function is supersensitivity; the original dominant mutation was a hypermorph.

k. Doubly mutant animals with a *tin-1* mutation and one of the recessive *tin-8* mutations in part j are touch insensitive. Doubly mutant animals with a recessive *tin-5* mutation and one of the recessive *tin-8* mutations are touch supersensitive.

i. (4) Define epistasis and tell which genes are epistatic to which other genes.

Epistasis is when one gene’s phenotype predominates over that of another; *tin-1* is epistatic to *tin-8* and *tin-8* is epistatic to *tin-5*.

ii. (4) Based on these data derive a genetic pathway for these for genes.

*tin-5* \[\rightarrow *tin-8\] \[\rightarrow *tin-1* \] 6 touch sensitivity

l. The dominant mutations in *tin-5* cause the touch sensing cells to branch extensively. This phenotype is not seen with the recessive alleles. In addition the phenotype is prevented (suppressed) by one and only one of the recessive mutations of *tin-9*. This *tin-9* mutation does not suppress the activity of any other *tin* mutations or mutations in any other gene.

i. (2) What sort of mutation is the dominant allele of *tin-5*? Explain your answer.

antimorph (interfering); the mutation is a gain of function in the same cells.

ii. (2) What type of mutation is the suppressing allele of *tin-9*? Explain your answer.
hypomorphic (not null)

iii. (2) What type of suppressor is the suppressing allele of tin-9? Explain your answer.

physiological suppressor because it is gene (and allele) specific.

iv. (2) What would you hypothesize about the relationship of the wild-type products of the tin-5 and tin-9 genes from these results?

Because of the allelic specificity, one might hypothesize that the products physically interact with (bind to) each other.

m. (3) Mutations in tin-10 prevent the expression of several of these genes. How could this result be demonstrated (assume that you have cloned several of the other tin genes)?

GFP fusions for the genes; Northern blots.

n. (3) tin-10 is cloned and shown to encode a leucine zipper protein. How does this result explain the observation in part m?

tin-10 is likely to encode a transcription factor, which would explain why the expression of several other tin gene is affected.

o. (4) What experiment could you do to support your hypothesis in part n and show molecularly that that effect is direct, i.e., not through another gene product? What would be the expected result?

DNase I footprints or gel shifts

2. (15 points) ii. (9) Name three aspects of gene regulation with regard to lactose metabolism and/or tryptophan biosynthesis that generally do not or could not occur in eukaryotes. Explain your answers.

The coordinate expression of genes in an operon by making a polycistronic mRNA; the use of attenuators; the use of operators that block polymerase progression.
i. (6) Name three cis dominant mutations (two with regard to lactose metabolism and a different one with regard to tryptophan biosynthesis) in *E. coli*. For each explain the consequences of the mutations.

**Mutations in p or o for the lac operon and in the attenuator for the trp operon.**

3. (18 points) Answer the following with regard to phage lambda.
   i. (4) What are the genetic differences between lysis through induction of a lysogen and lysis through infection of lambda phage?

   **Induction requires the removal of cl (by recA) and xis is made (along with int) to allow excision; the retroregulation involving sib does not take place.**

   ii. (4) What would be the effect of nutritional state on both of these events?

   **Nutritional state is important after infection because if high, it will lead to the destruction of cl; this control is not important for induction because recA eliminates cl so lysogeny does not occur.**

   iii. (4) What cis dominant mutations (name two) could prevent lysogeny?

   **P_{RE} and P_{Rmi}; see the problem set.**

   iv. (6) What gene could be mutated so that infection leads to the replication of lambda DNA, but the DNA is not packaged and no phage particle proteins are made? How does the mutation have its effect?

   **Q, antitermination does not take place and R (the gene needed for lysis) and the head and tail protein genes are not transcribed. O and P (the genes needed for lambda replication) are made, so the phage DNA is copied.**