Practice Problems

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1 Dauer and Aging

C. elegans larvae, when experiencing stressful conditions, go into a condition known as dauer arrest. Many mutations have been identified in the pathways regulating dauer formation. Most of them fall into two classes: dauer constitutive (larvae automatically enter dauer) and dauer defective (larvae are unable to enter dauer). Dauer formation has been connected to aging and longevity as well.

1. *daf-2(lf)* and *age-1(lf)* mutations cause constitutive dauer formation as well as extended lifespan in *C. elegans*. You identify a mutation that suppresses both loss-of-function mutations. How can you confirm that it is extragenic to both *daf-2* and *age-1*?

2. *daf-2* has multiple mutant alleles: *daf-2(e1370)* which has a missense mutation in an exon, *daf-2(tm1236)* has a 561 bp deletion in an exon, and *daf-2(m631)* which has a nonsense mutation. All of these alleles have the same mutant phenotype. Do you expect the worms carrying these mutations to be dauer constitutive or dauer defective? How does each allele affect the function of the gene?

3. You isolate the extragenic suppressor mutation found above, which maps to a gene on chromosome I called *daf-16*. You notice that this suppressor mutation can suppress all three *daf-2(e1370)*, *daf-2(cxTi9878)* and *daf-2(m631)* mutations. What sort of extragenic suppressor is your mutation? What can you conclude about its interaction with *daf-2* and *age-1*?

4. The single *daf-16* suppressor mutation turns out to be a recessive loss-of-function allele. What sort of phenotype do you expect it to have?

5. Assuming that *daf-2* and *age-1* are in the same pathway, how would you test to see which gene is upstream of the other?

6. Knowing that *age-1(lf)* and *daf-2(lf)* mutations lead to an extended lifespan phenotype, how do you expect *daf-16(lf)* mutations to affect lifespan?

7. Certain *akt-1(lf)* mutations show a constitutive dauer phenotype at 27° C. *akt-1(lf) daf-16(lf)* mutants are dauer defective. What model explains the interaction between *akt-1* and *daf-16*? *age-1(lf) akt-1(gf)* mutants are dauer defective. What model explains the interaction between *akt-1* and *age-1*?

8. Write out the full pathway involving *daf-2*, *age-1*, *daf-16* and *akt-1*.

9. The *akt-1(null)* mutant, as said above, shows a conditional mutant phenotype. Researchers mutagenized *akt-1(null)* worms and screened for worms with constitutive dauer phenotype that occurred nonconditionally. They identified three genes, *eak-4*, *sdf-9* and *eak-6*. What can you say about these genes? Are they likely to function in the same or parallel pathway to *akt-1*?

2 Answers

1. Test to see if your suppressor is extragenic by crossing the double mutant (either *daf-2(lf); sup* or *age-1(lf); sup* back to wild-type and seeing if you obtain single *daf-2(lf)* or *age-1(lf)* mutants. (You can do a complementation test only if you already had the single suppressor mutation isolated.)

2. All the alleles cause loss-of-function and thus a dauer constitutive phenotype. *daf-2(e1370)* disrupts the structure of the protein by substituting a different amino acid. *daf-2(tm1236)* removes a large number of amino acids, although it does not cause a frameshift mutation. *daf-2(m631)* results in a truncated protein product by introducing a premature stop codon.

3. The temptation may be to think that the suppressor mutation in *daf-16* is non-gene-specific, because it suppresses mutations in both *daf-2* and *age-1*. However, it suppresses multiple alleles of *daf-2*, which implies that it's non-allele-specific. Informational suppressors are allele-specific and can suppress mutations in many unrelated genes; however *daf-2* and *age-1* are clearly related since they cause the same mutant phenotype. Thus, we can conclude that *daf-16* is acting as a bypass suppressor.

4. If a loss-of-function mutation suppresses another loss-of-function mutation, the suppressor gene probably creates the opposite phenotype from the gene it suppresses. Hence, *daf-16(lf)* will have the opposite phenotype as *daf-2(lf)* and *age-1(lf)*: dauer defective. We can conclude that *daf-2* and *age-1* inhibit *daf-16*.

5. Create "gain-of-function" mutants by making constructs with the gene downstream of a highly expressed promoter (P_{high}*::daf-2* and P_{high}*::age-1*). Look at P_{high}*::daf-2 age-1(lf)* and $P_(high)$*::age-1 daf-2(lf)* strains. If

P_{high}*::daf-2* can suppress *age-1(lf)*, then *age-1* is upstream of *daf-2*. If P_{high}*::age-1* can suppress *daf(lf-2(lf)*, then *daf-2* is upstream of *age-1*.

6. *daf-16(lf)* mutants will have shortened lifespans and will age quickly.

7. *akt-1* $\dashv$ *daf-16* and *age-1* $\rightarrow$ *akt-1*.

8. *daf-2* $\rightarrow$ *age-1* $\rightarrow$ *akt-1* $\dashv$ *daf-16*.

9. These genes enhance the mutant phenotype of *akt-1(null)*, so they are enhancers. Since they enhance a null mutation, they are likely to function in a parallel pathway to *akt-1*.