

**Cell fate determination in *C. elegans*: analysis of signaling pathways using epistasis (and mosaic analysis)**

Reading: these notes; 8e: pp. 162-163, 9e: p. 99-100

**Learning Goals**

- Predict the interactions of genes that act in a pathway, based on their phenotypes when mutant
- Interpret the results of epistasis tests in determining the order of genes in a pathway.
- [Predict the outcomes of mosaic analysis experiments, and explain how these outcomes can be used to determine how a gene product acts (within a cell, or on neighboring cells)]

When several mutations are discovered in different genes that all affect the same process, one usually guesses that these genes work together in a pathway, for example: gene A encodes a signaling molecule that binds to a receptor (coded by gene B), which in turn activates a transcription factor (coded by gene C), which acts to specify a particular cell as a muscle cell, for example. Often, both loss-of-function (*lf*, usually recessive) and gain-of-function (*gf*, usually dominant) mutations have been isolated in these genes and shown to result in opposite phenotypes, suggesting that these genes function as *switches* that can normally be regulated so that they are either on or off in the same signaling pathway. When a mutation locks them into one or the other state, the results are opposite phenotypic effects.

To discover whether genes interact, and what the order of their switching functions is (which ones are “upstream” and “downstream” in a series of switches), geneticists commonly carry out an *epistasis* test. You learned about epistasis in Genetics (MCDB 2150): the word means “to mask expression of”. Sometimes if two genes work together in the same pathway, a mutation in one gene can mask the expression of the other. One example you may remember from genetics is that of Labrador retrievers. Two genes are involved in the enzymatic synthesis of pigment. One gene is required for deposition of the pigment, the other gene encodes the pigment gene itself. The gene required for deposition of the pigment is epistatic to the pigment gene because if you can't deposit pigment, it doesn't matter what your genotype is for the pigment itself. This makes sense when analyzing genes that work together in a metabolic or enzymatic pathway. The protein product of one gene is required for the synthesis of the protein in the pathway.

However, in developmental biology, we are almost always discussing signaling pathways, not enzymatic pathways. In these pathways, the protein products interact with each other, but there aren't intermediary protein products or phenotypes. This is because a signaling pathway is essentially like a series of switches. If a protein activates transcription of the next gene in the pathway, then if the first protein is not active (off), the next protein will also not be active (off)

**Why do epistasis tests?**

Scientists generally perform epistasis tests in the laboratory to see if they can order gene products into a sequential functional signaling pathway. Which gene encodes the starting signal of the pathway? Which gene encodes the receptor? Which gene encodes the kinase activated by the activate receptor? Etc. Loss of function mutations in these genes may all produce the same phenotype; so, without some additional genetic analysis, it could be hard to figure out the pathway.

For example, one might begin with two separate mutants that have been isolated that appear to be involved in the cell death pathway. One mutant has no cell death; the other mutant has excess cell death. If the mutations are both loss of function, then one might predict that the protein products of these two genes might inhibit each other in some way to affect whether or not a particular cell dies. To analyze the order in which these genes act, a double mutant is made by crossing the two mutant strains with each other. If the genes do actually function in the same pathway (ie, interact with each other directly, or through another gene product), then the result of such a cross will be that one phenotype will “win” (mask the expression of the other phenotype). The mutant gene whose phenotype “wins” is said to be *epistatic* to the other gene. If the two genes

work in separate, parallel pathways, one usually gets some kind of an intermediate phenotype from the double mutant—this is a sign that one needs to isolate additional mutants and test them as well, since two parallel pathways could be converging. This is a more complicated situation, so we'll stick with the result where the double mutant has the same phenotype as one of the original mutants. These types of tests have been done extensively in analysis of gene function in *C. elegans*, and were particularly useful in identifying all the different components of the often used Ras signaling pathway that is involved in determining the development of the vulva, as well as the cell death (apoptotic) pathway, and others.

Since we have already discussed the general series of events that lead to differentiation of the gonad and the vulva, we can now look at how the many genes that were isolated and thought to be involved in these events were actually ordered into a complex pathway.

### Phenotypic and epistasis analysis of *lf* and *gf* mutations in genes involved in vulval development

As discussed previously, for most genes involved in development, both loss-of-function (*lf*, usually recessive) and gain-of-function (*gf*, often dominant) mutations are isolated. When the *lf* and *gf* phenotypes are opposite, and there are multiple genes with such opposing phenotypes, it is often the case that these genes function as *switches* that can normally be regulated so that they are either on or off. When a mutation locks them into one or the other state, the results are opposite phenotypic effects.

For example, an *lf* mutation in a gene called *lin-1* results in a Muv phenotype, while an *lf* mutation in a gene called *let-60* results in a Vul phenotype. When a double mutant (*lin-1;let-60*) is made by crossing the two mutant strains with each other, one of the phenotypes of the starting strains will “win” (mask the expression of the other phenotype) if the genes function in the same pathway. The mutant gene whose phenotype “wins” is said to be *epistatic* to the other gene. In a signaling pathway, the mutant gene that is epistatic will be the more downstream gene of the two. The reasoning goes something like this: the “double” mutant phenotype you see is the result of a series of genes products being inactivated. The last gene in the pathway is the gene who's state (“on” or “off”) determines the phenotype, regardless of what has happened to genes upstream. We'll work on a several pathways as examples in class to help you understand.

Sometimes establishing the order of genes in a pathway can be quite tricky. One can have a whole series of genes that activate each other (no inhibitory interactions), and thus a loss of function mutation in each of the genes yields the same phenotype. Because the mutants have the same phenotype, creating a double mutant will not yield any information. The only way to analyze order in such a pathway is to isolate gain of function mutations as well as loss of function mutations: then one can generate double mutants and compare phenotypes. Lastly, sometimes the relationship is still fuzzy after genetic analysis: at this point, genomic analysis (finding the sequence of the gene in a database) is the best bet for determining how the protein product acts.

Epistasis tests (and mosaic analysis, described below) allowed the dissection of the complete pathway of molecules involved in vulval fate determination. You've already seen the list below of genes involved; here it is again:

Once the genes involved in vulval formation were cloned, it was possible to identify the signaling pathways that control the various events suggested by ablation experiments, epistasis, and mosaic analysis (as described above). Many components of the famous Ras signaling pathway, conserved in virtually all organisms, were discovered by studying the formation of the vulva in *C. elegans*.

*lin-3*: encodes the AC signaling ligand, a growth factor of the TGF- $\alpha$  family related to EGF.

*let-23*: encodes a membrane protein homologous to the EGF receptor (a PTK receptor); it is the receptor for LIN-3.

*let-60*: encodes a homolog of the cellular oncogene Ras, which is mutated to cause loss of growth control in many mammalian cancers. In worms, it is activated by activated *let-23*.

*lin-1*: encodes a transcription factor which is inhibited by LIN-45 (another member of the pathway) and negatively regulates the initial choice between vulval and non-vulval fates of Pnp cells (see pathway diagram in PowerPoint slides). *lin-1* is active in cells that take on the non-vulval fate, and inactive in cells that take on the vulval fate.

The second pathway involved in determining the subtleties of which of the VPCs adopts the primary fate (at the center of the developing vulva) and which the secondary fates on each side is the juxtacrine signaling pathway Notch/Delta. The cell (normally P6p) that encounters the highest concentration of *lin-3* ligand and adopts the primary fate sends out a lateral inhibitory signal that is received by *lin-12*-encoded (Notch-like) receptors on the two neighboring cells (P5p and P7p), which therefore adopt secondary fates.

[We may or may not get to use of mosaic analyses using *C. elegans*. If we don't discuss it in this class period, I'll bring it up later when we get to the use of mouse as a model system. You will only be responsible for this section if we actually discuss it in class.

#### Mosaic analysis: a genetic test for how and where genes act

Mosaic analysis can be tricky, and is not used as much anymore as it was before the advent of genome sequencing. Mosaic analysis serves two purposes: it can be used to ask where in the animal a particular gene product normally is required to function, and whether the gene product acts cell-autonomously or non-autonomously. The term *genetic mosaic* (also sometimes called a *chimera*) refers to an organism in which some cells are mutant, while the rest are wild type. This technique was used extensively before it was easy to simply look up and predict a protein's function based on its similarity to other proteins with such functions.

A gene or its protein product is said to act cell-autonomously if the phenotype of a cell it affects depends only on that cell's own genotype (mutant or wild-type for the gene in question). In other words, a mutation in the gene affects only cells in which the mutant gene is present. If a gene product functions cell-autonomously, it is unlikely to be a ligand (which would affect neighboring cells, not the ligand-producing cell). Autonomously functioning genes encode membrane-bound or intracellular proteins, such as receptors or transcription factors, which are required for normal cellular function. This is often confusing to students because "cell autonomous" seems to indicate that a cell would not need a signal. It is important to distinguish here between a cell having an autonomous cell fate, which means it does not require a signal, and a protein acting cell-autonomously. When a protein acts cell-autonomously, it means it functions in the cell that produces it (thus, it must NOT be released from the cell).

A gene or its product is said to act non-autonomously if it affects the phenotype of a neighboring cell. In other words, a mutant form of the gene will generally affect not the phenotype of the cell that carries it, but rather the phenotypes of neighboring cells. If a gene product functions non-autonomously, it probably encodes a signaling ligand whose receptors are on adjacent or nearby cells.

As an example, *lf* mutations in a gene called *lin-12* cause both of the AC/VU precursor cells in somatic gonad development to become AC cells. In mosaic animals in which one cell is mutant for *lin-12* and the other is wild type, the mutant cell always becomes the AC and the wild type cell becomes the VU. Therefore *lin-12* acts cell-autonomously and is likely to be a receptor (or downstream of a receptor) that recognizes a ligand produced by the VU cell. Conversely, *lf* mutations in the *lin-3* gene cause a vulvaless phenotype, similar to that seen when the AC is ablated. Mosaics in which the AC is mutant for *lin-3* and the vulval equivalence group cells are wild type do not form a vulva, whereas in the converse mosaic, the vulva forms normally. Therefore, the *lin-3* gene acts non-autonomously and in fact encodes the EGF-like ligand by which the AC induces specification of the VPCs.

Analysis of mosaics can provide important information about gene functions that is difficult to obtain by any other means. The trick is how to create mosaic animals, and the techniques are different for different species. [In *C. elegans*, animals mosaic for a particular gene can be made starting with a strain homozygous for a recessive *lf* mutation in the gene. By crossing with males of a strain carrying a chromosomal fragment that includes a wild-type copy of the gene as a free duplication, hermaphrodites with one wild-type copy on the duplication and two chromosomal mutant copies are generated. Because the mutations are recessive, these animals should be phenotypically wild type. However, free duplications are occasionally lost during meiosis or mitosis. A loss during mitosis will lead to a mosaic animal, because some cells in the animal will still carry the duplication (and thus have wild type function of the gene), while others will have lost the duplication (and thus will be mutant for the gene). Because the cell lineage is completely determined, the genotype of every cell in

the animal can be deduced from identification of a few mutant cells in the adult.]

***Review Questions***

- 1) How do you test whether a mutation in one gene is epistatic to a mutation in another gene? What can such a test tell you?
- 2) Be able to draw a pathway of genes that interact based on their mutant phenotypes.
- 3) How and why is mosaic analysis used in worms? What type of questions can you answer using this method?
- 4) What outcome do you expect in mosaic analysis if the product in question acts as a signaling molecule?

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