C. elegans Genetics and Apoptosis

Most of what we have covered in the course to date is classical genetics; in particular, transmission genetics or how genes behave in crosses. But a lot of modern genetics is the use of genetics as a tool to understand biological problems. Just as biochemistry is used as a tool to understand aspects of cell signaling, genetics can be used as a complementary and equally effective tool to address this problem. Researchers use genetics as a tool to address outstanding issues in cell biology, development and neurobiology. The argument can be made that the most important contribution of genetics to our understanding of biology has been in development. Without basic research in *Drosophila* and *C. elegans*, much less would be known about the development of more complicated organisms, including humans. What we will be doing in the next several lectures is discussing the use of model organisms, and how they are used to study a development.

C. elegans Genetics

C. elegans is a small free living soil nematode that is easily propagated in the laboratory. It has a short life cycle (3.5 days) and can be maintained on petri plates that have been seeded with *E. coli*, a *C. elegans* food.

There are two sexes: self fertilizing hermaphrodites and males. Sex is determined by the X to autosome ratio as in flies. Hermaphrodites have two X chromosomes and a ratio of 1 and males have a single X chromosome (and no Y chromosome) and a ratio of 0.5. Ratios between 0.5 and 1.0 cn lead to intersexual animals.

A single hermaphrodite makes approximately 1000 oocytes and 300 sperm and produces 300 progeny by self fertilization. Males are produced spontaneously at a frequency of 1/2000 progeny by X chromosome nondisjunction. A hermaphrodite that has been mated to a male will produce two types of progeny: progeny from self fertilization and progeny from cross fertilization. The self progeny will be all hermaphrodites and half of the cross progeny will be male. To distinguish between self and cross progeny hermaphrodites, the hermaphrodites can be marked. For example, crossing a wild-type male with a Dumpy (Dpy) hermaphrodite (homozygous for a recessive mutation that causes the worms to be short and fat) results in two types of hermaphrodites: self progeny that are Dpy and cross progeny that are not Dpy.

Apoptosis

Apoptosis plays a prominent role in development. In some parts of the developing vertebrate CNS, more than half of the neurons die, a process thought to weed out neurons that haven't made appropriate connections.

Apoptosis also plays an important role in removing cells with DNA damage, and the loss of this function can lead to cancer.

C. elegans development

C. elegans develops by an almost completely invariant lineage. In other words, the pattern of divisions and the cells that are produced are the same from animals to animal. The hermaphrodite consists of 959 somatic cells. During development, 131 cells undergo programmed cell death. *A priori*, these cell deaths could have been suicide or murder. In suicide, the cell is programmed to die, possibly by inheriting determinants for this fate from the mother cell. Murder would result if a nearby cell killed the cell programmed to die. For years it was thought that these cells committed suicide, but more recent work indicates that nearby cells play a role, perhaps more like assistant suicide. Work by the Horvitz lab at MIT defined many of the genes involved in apoptosis, and their homologs regulate apoptosis in organism as diverse as nematodes and humans.

Choice of mutagens

When designing a screen the first question that we must address is what type of mutations do we want? Here, the investigators wanted point mutations that disrupted specific genes involved in cell death, so they selected a chemical mutagen. In *C. elegans*, the mutagen EMS has been used extensively; for genes where mutagenesis rate of EMS has been measured, it mutates these genes at a rate of 1/2000 haploid genomes screened. From mutagenized parents, the progeny of each F1 represent two mutagenized genomes, so if you screen the F2 progeny of a single F1, you have screened two haploid genomes.

Screens for cell death mutants

Mutants defective in programmed cell death fall into two groups: those defective in apoptosis and those defective in engulfment. Engulfment is the process where the cell corpse is phagocytosed by nearby cells. The first mutants were engulfment mutants identified by Ed Hedgecock. Hedgecock screened the F2 (for zygotic mutants) and the F3 (for maternal effect mutants) progeny of mutagenized parents for mutants with persistent cell corpses. The corpses persist in these mutants because they are inefficiently phagocytosed. Hedgecock identified two genes in these screens, *ced-1* and *ced-2*. Additional screens for engulfment mutants in the Horvitz lab isolated both zygotic and maternal-effect mutants that defined the genes *ced-5, ced-6, ced-7, ced-8, ced-10* and *ced-12*.

The Horvitz lab took advantage of the engulfment phenotype to identify genes involved in apoptosis. Mutagenizing *ced-1* mutants and screening the F2 progeny for animals that lacked the persistent corpses

identified mutants that never produced corpses because cell death never occurred. These mutants contained 131 extra cells, those cells than normally died in wild-type animals. These genes are required for apoptosis. In other words they normally promote apoptosis.

One of the genes identified was *ced-9*, and it was originally defined by a single dominant allele *n1950*. Animals heterozygous for a deficiency of this locus do not have a cell death phenotype indicating that *n1950* is a gain-of-function mutation. Using some genetic tricks that we won't discuss *n1950* was used to identify loss-of-function alleles of *ced-9*. Loss of *ced-9* results in a recessive lethality caused by excessive cell death; cells that normally survive now die in the mutant. Thus *ced-9* normally inhibits cell death and genes like *ced-3* promote cell death.

Ordering genes

Since *ced-3* promotes cell death and *ced-9* inhibits it, how do the genes act together to control cell death. There are three possibilities. They could act separately or in parallel to control cell death, or they could act in a linear pathway. If they act in a pathway, *ced-9* could inhibit *ced-3*, which could then promote cell death. Alternatively, *ced-3* could inhibit *ced-9*, which could inhibit cell death. Analysis of *ced-9(lf); ced-3(lf)* double mutants shows that they look exactly like *ced-3* single mutants. All 131 cells normally destined to die survive. Using genetic terminology, we would say that *ced-3* is epistatic to *ced-9*. This result is consistent with the linear pathway where *ced-9* inhibits *ced-3*. In regulatory pathways, the epistatic gene functions downstream. These types of genetic interactions can be used to order genes into pathways. We will talk more about epistasis and pathways later in the course.