CLASSIC EXPERIMENT 21.1

HUNTING DOWN GENES INVOLVED IN CELL DEATH

H. M. Ellis and H. R. Horvitz, 1986, *Cell* **91**:818

During the development of multicellular organisms, certain cells are destined to die. Scientists have directed much research toward understanding this process of programmed cell death. Some sought to understand why a cell would be destined to die during development, while others asked how a cell regulates this form of death. In 1986, H. Robert Horvitz provided clues by examining the genetics of cell death using a well-characterized model system, the nematode *Caenorhabditis elegans*.

Background

Developmental biologists have long noted that some cells die during the normal development of a multicellular organism. This process—called apoptosis or programmed cell death—remained a puzzling phenomenon for many years. Early research in the field concentrated on identifying cells that were fated to die. Until the middle of the 1980s, scientists knew little about the mechanism by which the cell controlled this process. At this time, Horvitz began his investigations into the genetics of programmed cell death in the nematode *Caenorhabditis elegans*.

C. elegans is a powerful model system for studying the genetics of complex developmental processes. It is a small, multicellular organism, made up of just more than 1000 cells, so scientists could trace the developmental lineage of each cell of the organism. Previous studies had defined precisely which cells in *C. elegans* were fated to die during development. When examined by Nomarski differential contrast microscopy, cells fated to die became highly refractile for a few minutes before cell death occurred. In the late 1970s, researchers isolated two cell death mutants of *C. elegans: ced-1* and *ced-2*. These mutants extend the life of a dying cell, causing it to remain refractile for hours rather than minutes (see Figure). Horvitz used these mutants to look for more genes involved in the control of programmed cell death.

The Experiment

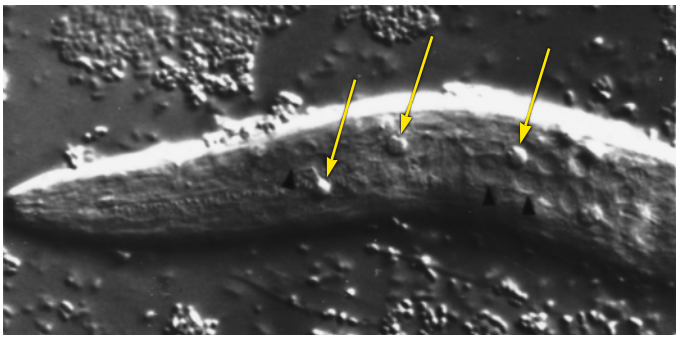
Geneticists analyze complex processes by looking for organisms that display traits or phenotypes that result from an alteration in normal function of a gene. To perform a genetic analysis of programmed cell death, a geneticist looks either for cells that escape programmed cell death or for cells that undergo programmed cell death when they should survive. Finding such mutant organisms in a naturally occurring population would be difficult because the overall mutation rate of an organism is rather low. To facilitate the search, mutations are induced, often by treating individuals with mutagenic chemicals. This enriches the population for mutants. Because mutagenic chemicals will induce mutations in all genes, not just those involved in the process being studied, scientists carefully devise genetic screens to guide their studies.

To analyze the control of cell death in *C. elegans,* Horvitz designed a genetic screen for mutations that alter the process. In the screen described here, he looked for mutations that allowed cells that would normally die during development to survive. A second important part of the screen was the choice of organisms to study. Rather than look for mutant progeny of wild-type nematodes, Horvitz looked at the progeny of *ced-1* mutants. In *ced-1* mutants, cells that die by programmed cell death are not engulfed and immediately eliminated from the organism. This causes them to remain highly refractile under Nomarski optics for longer than would a cell in a wild-type organism. By using *ced-1* mutants in his studies, Horvitz had an increased time frame to look for mutant nematodes in which the cells normally fated to die escape programmed cell death.

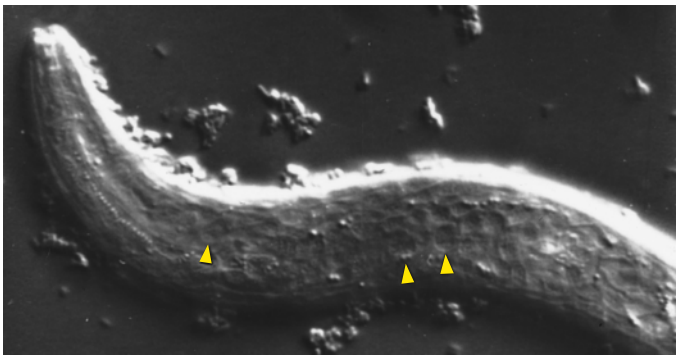
In his initial screen for genes involved in programmed cell death, Horvitz treated *ced-1* mutant nematodes with a mutagenic chemical and allowed them to reproduce for two generations, producing a genetically defined line that carries mutations on both copies of the chromosome. Because the progeny are homozygous for all mutations, he could now observe the phenotype of mutations that are recessive. Once the organisms were bred to homozygosity, he analyzed the second generation for mutations that affected cell death. Specifically, he compared the highly refractile dying cells in *ced-1* nematodes to the same cells in mutagenized progeny. These cells displayed the same phenotype in the majority of larvae examined. In a small number of larvae, including those that harbored mutations in genes that control programmed cell death, these cells do not die, and hence do not become refractile. This initial screen uncovered several recessive mutants that mapped to a single gene that he called *ced-3* (see Figure).

Horvitz went on to characterize the phenotype of the *ced-3* mutants, taking advantage of the fact that the identity of all cells destined to undergo programmed cell death was known. By examining the fate of cells that normally undergo programmed cell death, he showed that mutation in *ced-3* blocked this process completely (see Table). He then followed these surviving cells through the *C. elegans* life cycle. When compared with wild-type nematodes, *ced-3* mutants reproduced normally and showed no behavioral abnormalities. The primary

(a)



(b)



Screening for *C. elegans* genes involved in programmed cell death were observed using Nomarski differential contrast microscopy. (a) Newly hatched larva carrying a mutation in the *ced-1* gene. Because mutations in this gene prevent engulfment of dead cells, highly refractile dead cells accumulate, facilitating their visualization. The arrows indicate three highly refractile cells. (b) Newly hatched larva with mutations in both the *ced-1* and *ced-3* genes. Using the nuclei indicated by the arrowheads as orientation points, one can compare panels a and b. In panel b, notice that the three highly refractile cells seen in panel a are not observed. The absence of refractile dead cells in these double mutants indicates that no cell deaths occurred. Thus *ced–3* was identified as a gene involved in programmed cell death. [From H. M. Ellis and H. R. Horvitz, 1986, *Cell* **91:**818. Courtesy of Hilary Ellis.]

difference appeared to be the extra cells present in *ced-3* mutants due to the absence of programmed cell death. Horvitz proceeded to analyze a number of mutations within the *ced-3* gene. Each allele resulted in the identical phenotype, survival of cells that should be destined to die. This suggested to Horvitz that mutations in *ced-3* resulted in the loss or decreased expression of an essential gene in the programmed cell death pathway. He had isolated the first gene required for control of programmed cell death in *C. elegans*.

Discussion

The isolation of the *ced-3* mutant was merely the first step in Horvitz's efforts to dissect the genetics of programmed cell death in *C. elegans*. In addition to *ced-3*, he uncovered two other essential genes in the pathway, as well as 10 other genes that are involved in this process. These genes control all aspects of the process of cell death, from the initial decision to die, to the killing, engulfment, and degradation of the dead cell. As is often seen, these genes that control cell death in *C. elegans* have counterparts in higher organisms, including humans. The importance of these genes in the regulation of cell growth in humans has become apparent. The human homologues of two genes isolated by Horvitz have been implicated in cancer.

Mutations in *Ced-3* Eliminates Cell Death

GENOTYPE	AVERAGE NUMBER OF CELL DEATHS OBSERVED	
	FIRST LARVAL STAGE	POSTEMBRYONIC
Wild type	ND	13
ced-1	28	11.23
ced-3	0.3	0.04

ND = Not determined
[Data adapted from H. M. Ellis and H. R. Horvitz, 1986, *Cell* **44:**819.]