It is an honor and a great pleasure today to introduce Dr. Robert Horvitz to you as the 1998 recipient of the Alfred Sloan Prize of the General Motors Cancer Research Foundation. Let me begin by telling you a little bit about Bob’s history.

Bob was an undergraduate at MIT, finishing, actually, with a double major in mathematics and economics in ’68. He did his graduate training in what I think of as the pressure cooker of the Harvard Bio Labs, working with Jim Watson and Wally Gilbert, and receiving his Ph.D. in 1974. His graduate work was on phage T4, and I would like to come back to that in a minute. After his graduate work, Bob went to the MRC Labs in Cambridge, England to join the then very new worm lab set up there by Sydney Brenner. Bob was thus one of the first generation of scientists to train with Brenner in his new genetic system, the soil nematode known as C. elegans. In 1978, he came back to the States to set up his own lab at MIT, then moved up through the ranks to his present position as Professor of Biology there in 1986. He has also been an Investigator of the Howard Hughes Medical Institute for the last ten years.

Bob has received numerous distinctions and awards, including the U.S. Steel Foundation Prize, a MERIT award from the NIH, the Rosenstiel Award, the Passano Award; and he is a member of the National Academy of Sciences. By all criteria, Bob has been a spectacularly successful scientist throughout his career.

Now, what has Bob done that is so important to us and to our understanding of cancer? Simply put, Bob showed us that there is a genetics of cell death. Bob showed us that cell death is a normal, genetically-determined part of development, that there is a predetermined program in which certain cells are destined to die in an orderly way. He was able to obtain mutants in C. elegans that were affected in this program. Thus, he could identify genes that were required to execute the death program or, alternatively, to inhibit that pathway. And a very important aspect of his work is that he could order these genes into specific pathways. Bob also helped to show that these genes were highly conserved, that you could, in fact, exchange them between organisms, and that there are vertebrate homologues of the genes, the same genes that we now know are often mutated in human cancers.

So, I will let Bob tell you about those genes that you have been hearing about and tell you the links between C. elegans and the vertebrate systems. Before I turn the podium over to him, I want to address just a couple of issues. I think it is worth contemplating whether there is anything we can see in Bob’s background that would help explain his scientific success. I would suggest that Bob’s early work on phage T4 is, in fact, a part of that story. Some of us may remember that early molecular biologists were attracted from other fields into biology specifically to work on phage, and the reason is quite simple. It is that phage were so incredibly convenient as a genetic system. You can do so much so fast with these organisms. And I think, very similarly, Sydney Brenner and people like Bob saw very early on that C. elegans was an equally powerful genetic system. They moved into this field with both feet and started applying the system to key questions of development. The genetic thinking that Bob applied to these problems of cell fate and cell death was really fundamental to the rapid progress that has been made in this field and this kind of thinking, this mentality, is a natural extension of the mentality of phage genetics that Bob really started out with.

Secondly, I would suggest that some of Bob’s success is due to his phenomenal ability to communicate and teach and inspire. He has trained and seeded the world with a very large number of scientists by now, many of whom are friends: Iva Greenwald, Victor Ambros, Leon Avery, Paul Sternberg, Cori Bargmann, Gary Ruvkun, Jim Thomas and many others. The list is very long and it is noteworthy that so many stellar scientists trained with Bob.

I can personally confirm something of Bob’s ability to teach and inspire. He probably won’t remember, but I first met Bob in the Harvard Bio Labs almost 30 years ago. At that time, I was a high school kid hanging out in the labs with my brother who was, like Bob, a graduate student in the Watson lab. As a naive kid, I would talk to everybody. And, Bob, I remember, explained what he was doing with T4 and the MOD and the ALT genes very clearly to me and made it fascinating. I am sure that Bob is, indeed, part of why I am in this business today and why I am doing science. I want to thank Bob for that and for teaching all of us in the years since so much good science.

So, it is with great personal pleasure that I introduce to you Robert Horvitz. The title of his talk is Genetic Control of Programmed Cell Death.
Abstract

Studies of the development of the nematode Caenorhabditis elegans established that programmed cell death involves specific genes and proteins and that these genes and proteins act within the cells that die. This finding revealed that cell death is a fundamental and active biological process, much like cell division and cell differentiation. The characterization of genes responsible for programmed cell death in C. elegans has defined a molecular genetic pathway. This pathway is conserved evolutionarily and provides a basis for understanding programmed cell death in more complex organisms, including humans. Knowledge of the mechanisms of programmed cell death should help lead to new methods for the diagnosis and treatment of human diseases characterized by too many or too few cell deaths, including cancer.

Introduction

"Programmed" or naturally occurring cell death, often referred to as apoptosis (1), is a major feature of animal development (2, 3). Abnormalities in programmed cell death have been implicated in a variety of human diseases, including cancer (e.g., Refs. 4–6). In addition, many chemotherapeutic agents as well as radiation therapy (e.g., Ref. 7) act by causing cells to die by a mechanism that seems to be identical to that used during developmental programmed cell death. For these reasons, an understanding of the mechanisms responsible for programmed cell death seems likely to be of central importance to an understanding of cancer.

To identify and characterize the genes and proteins involved in programmed cell death, we have been analyzing this process in the nematode Caenorhabditis elegans (Fig. 1). This roundworm is only 1 mm in length, and the adult hermaphrodite consists of only 959 somatic cells. C. elegans eats bacteria, and in the laboratory it feeds on Escherichia coli and is grown on Petri dishes. Its small size, cellular simplicity, easy handling, and rapid generation time (3 days) and a variety of other characteristics make C. elegans highly tractable for experimental analysis (8). Furthermore, because C. elegans is small and transparent, the deaths of individual cells can be observed in living individuals. For these reasons, C. elegans has proved to be exceptionally well suited for the study of programmed cell death.

Will a knowledge of the mechanisms responsible for programmed cell death in a microscopic roundworm help us understand how cell death is regulated, and misregulated, in human disease? Recent findings strongly suggest that the answer to this question is "yes." First, studies from many laboratories of basic cellular processes such as the cell cycle and signal transduction have revealed a striking biological universality: the same classes of genes and proteins function in organisms as diverse as yeast, nematodes, insects, and mammals. More specifically, as we discuss below, many of the genes that function in programmed cell death in C. elegans indeed have counterparts that function in apoptotic cell death in mammals, indicating that the molecular genetic mechanisms of programmed cell death are conserved from nematode to human.

Programmed Cell Death in C. elegans

During the development of the C. elegans hermaphrodite, 1090 somatic nuclei are generated from the fertilized egg by a completely described and essentially invariant pattern of cell divisions (9–11). Of these 1090 nuclei, 131 undergo programmed cell death. Similarly, during the development of the C. elegans male, 1179 somatic nuclei are generated, and 148 undergo programmed cell death. Cell death in C. elegans is rapid. Cells normally die within an hour of the time they are formed and, in most cases, before the onset of differentiation. As they undergo programmed cell death, cells have a characteristic flat, disc-like appearance when viewed with a light microscope using Nomarski differential interference contrast optics (Fig. 2A). When viewed with an electron microscope, these dying cells display features characteristic of the apoptotic cell deaths seen in mammals, including cell shrinkage, nuclear condensation, and phagocytosis of cell corpses (Fig. 2B; Refs. 1 and 12).

To analyze the molecular genetic control of programmed cell death in C. elegans, we have used the approach of classical genetics. Specifically, we have identified and characterized mutations affecting either the pattern of programmed cell death (certain cells that should survive instead die or certain cells that should die instead survive) or the process of programmed cell death (cell deaths do not occur or occur but are abnormal in appearance). These mutations have defined 13 genes involved in programmed cell death in C. elegans. We have analyzed these genes using methods of genetics, developmental biology, molecular biology, and biochemistry (reviewed in Ref. 13).

The ces-2 Cell Death Specification Gene Is Similar to the Human HLF Proto-Oncoogene

Two of the 13 characterized cell death genes appear to regulate the process of programmed cell death in a cell-specific fashion (14). Mutations in these genes, ces-1 (ces, cell death specification abnormal) and ces-2, result in the survival of the sister cells of the two serotonergic NSM (neurosecretory motor) neurons, causing these cells, which normally die, to instead survive. Genetic analyses have suggested that ces-1 can act to prevent NSM sister cell deaths and that ces-2 acts as a negative regulator of ces-1.

The ces-2 gene encodes a bZIP (basic leucine zipper) transcription factor of the PAR (proline- and acid-rich) family; the CES-2 protein is particularly similar to the human proto-oncoprotein HLF (hepatic
HLF has been implicated in acute lymphoblastic leukemia, as a t(17;19) translocation associated with this cancer results in a fusion protein that includes the dimerization and DNA-binding domains of HLF (16). On the basis of our observation that ces-2 regulates programmed cell death in C. elegans, the oncogenic form of HLF was shown to inhibit programmed cell death in mammalian cells, suggesting that the regulation of programmed cell death may be conserved between nematodes and mammals (17).

These findings suggest that the misregulation of different human cell death specification genes could lead to the cancerous proliferation of different cell types.

The *ced-9* Cell Death Protection Gene Is Similar to the Human *Bcl-2* Proto-Oncogene

The *ced-9* (*ced*, cell death abnormal) gene protects cells from programmed cell death. Mutations that decrease or eliminate *ced-9* function cause cells that would normally survive to instead undergo programmed cell death (18). In embryos with diminished *ced-9* function, most cells die, resulting in the death of the embryo. Conversely, a *ced-9* mutation that abnormally activates *ced-9* causes cells that would normally die instead to survive and no programmed cell death seems to occur in such *ced-9* mutant animals. Similarly, overexpression in *C. elegans* of a wild-type *ced-9* transgene prevents programmed cell death (19). These observations indicate that *ced-9* negatively regulates programmed cell death and that many, possibly all, of the cells that survive during *C. elegans* development do so because they are protected by *ced-9* from undergoing programmed cell death.

The *ced-9* gene encodes a 280-amino acid protein that is similar in sequence to the protein product of the human proto-oncogene *Bcl-2* (*Bcl, B cell lymphoma; Ref. 19). *Bcl-2*, like *ced-9*, protects cells against programmed cell death, and a t(14;18) translocation that results in *Bcl-2* overexpression in B cells causes B-cell follicular lymphoma by allowing excessive B-cell survival and subsequent proliferation (e.g., see Refs. 5 and 6). Human *Bcl-2* expressed as a transgene in *C. elegans* both prevents the deaths of cells that normally undergo programmed cell death and can substitute for *ced-9* in a *ced-9*-deficient mutant (19, 20). Together, these results suggest that *ced-9* and *Bcl-2* are functional homologues.

The *egl-1, ced-3*, and *ced-4* Genes All Have Human Counterparts That Act in Apoptosis

The functions of three genes, *egl-1* (*egl, egg-laying abnormal*), *ced-3*, and *ced-4*, are required for programmed cell death to occur (21, 22). Hence, these genes act as killer genes.

The *egl-1* gene was discovered because gain-of-function mutations in this gene cause the two hermaphrodite-specific neurons (HSNs), which are required for egg laying, to undergo programmed cell death (23). Genetic analysis of *egl-1* led to the isolation of a loss-of-function allele, and in animals homozygous for this allele, it appears that essentially all 131 cells that normally undergo programmed cell death instead survive (22). Thus, *egl-1* function is required for programmed cell death. The *egl-1* gene encodes a 91-amino acid protein that contains a BH3-like region (*Bcl* Homology region 3; Ref. 22). BH3 is a protein domain that is found in the CED-9 and Bcl-2 proteins, as well as in other members of this protein family and that has been implicated in mediating heterodimerization between Bcl-2 family members (e.g., Ref. 24; also see reviews in Refs. 25 and 26). The *EGL-1* protein lacks three other Bcl-2 homology regions (BH1, BH2, and BH4) found in many Bcl-2 family members. A number of such BH3-only proteins have been identified in mammals (reviewed in Refs. 25 and 26). These mammalian proteins, like the EGL-1 protein, are thought to function as activators of programmed cell death.

Loss-of-function mutations in the genes *ced-3* and *ced-4*, like the loss-of-function *egl-1* mutation described above, cause essentially all 131 cells that normally die instead to live (21). Genetic mosaic experiments indicate that *ced-3* and *ced-4* are expressed within cells that die and act within those cells to cause them to undergo programmed cell death (27). This finding revealed that programmed cell death specification genes would be conserved during evolution between nematodes and mammals (17).

Leukemia factor; Ref. 15).

The same cell is shown in A and B. A, light micrograph, Nomarski interference contrast optics. Cell corpse is seen as a dark, electron-dense object and is being engulfed by a neighboring cell. Photographs courtesy of Gillian Stanfield and Erika Hartweg (MIT).

**Fig. 2.** A *C. elegans* cell corpse derived from the ventral ectoblast P12 (see Ref. 9): L1 (first larval) stage hermaphrodite. The ventral side of the animal is at the bottom, and anterior is to the left. The same cell is shown in A and B. A, light micrograph, Nomarski differential interference contrast optics. Cell corpse is seen as flat, disc-like object. B, electron micrograph. Cell corpse is seen as a dark, electron-dense object and is being engulfed by a neighboring cell. Photographs courtesy of Gillian Stanfield and Erika Hartweg (MIT).
death is an active process on the part of dying cells. The ced-3 gene encodes a protein similar to the human cysteine protease ICE\(^3\) (28). ICE was identified and purified on the basis of its ability to cleave the M\(_\text{r}\) 31,000 precursor of interleukin 1\(\beta\) to generate the active M\(_\text{r}\) 17,500 cytokine (29, 30). The similarity between the CED-3 protein and ICE suggested that CED-3 acts as an ICE-like cysteine protease, which indeed is the case (31). Both CED-3 and ICE are generated as pro-proteins, which must be proteolytically cleaved to form active enzymes; this cleavage can be catalyzed by the active CED-3 and ICE proteases. The discovery that CED-3 causes programmed cell death in \textit{C. elegans} by functioning as an ICE-like protease led to the identification of a family of such proteins and the demonstration that many of these proteases act in mammalian apoptosis (reviewed in Ref. 32). These proteases are called caspases, because they are cysteine proteases that cleave after specific aspartate residues.

Like ces-2, ces-9, egl-1, and ced-3, the ced-4 gene also encodes a protein that has a human counterpart. Although when cloned the ced-4 gene was not similar to any other known protein (33), the subsequent characterization of \textit{an in vitro} system for apoptosis led to the purification of a protein called Apaf-1 (apoptotic protease-activating factor), which has a region of similarity to the CED-4 protein (34). Both CED-4 and Apaf-1 have been suggested to require nucleotide binding for their functions (35-37). The recent generation of Apaf-1-deficient mice established that, like CED-4, Apaf-1 functions in apoptosis \textit{in vivo} (38, 39).

**Seven Genes Act in the Phagocytosis and Degradation of Cell Corpses**

Six \textit{C. elegans} genes, ced-1, ced-2, ced-5, ced-6, ced-7, and ced-10, function in the process of phagocytosis during which a dying cell is engulfed by a neighboring cell (40, 41). The ced-6 gene encodes a protein with an apparent phosphotyrosine-binding domain and may act as an adaptor in a tyrosine kinase signaling pathway (42). The ced-7 gene encodes a protein similar to ABC (ATP-binding cassette) transporters (43). ABC transporters mediate the transmembrane movement of diverse substrates, including chemothapeutic agents (44, 45). We proposed that CED-7 functions to transport molecules that mediate the interaction between dying cells and the cells that engulf them (43). The CED-7 protein is most similar to the mammalian ABC1 subfamily of ABC transporters. ABC1 has been suggested to act in macrophages during the phagocytosis of apoptotic cells (46), suggesting that the process of cell corpse engulfment, like those of cell death specification, cell protection, and cell killing, have been conserved through evolution.

Of the six genes that function in the engulfment of cell corpses, three, ced-2, ced-5, and ced-10, also act in the migrations of two specific gonadal cells, the distal tip cells (47), suggesting that these genes function in a process common to engulfment and migration. The ced-5 gene appears to act within the engulfing cells during the process of cell-corpse engulfment, and ced-5 encodes a protein similar to the human protein DOCK180 (downstream of CRK, a proto-oncoprotein; Refs. 48 and 49) and the Myoblast City protein of \textit{Drosophila melanogaster} (47). We proposed that CED-5, DOCK180, and Myoblast City all function in the extension of cell surfaces and in particular that CED-5 functions in programmed cell death in the extension of the surface of the engulfing cell around the dying cell (47).

One gene, nuc-1 (nuc, nuclease abnormal), has been identified that is essential for the normal degradation of DNA in dying cells (40). The nuc-1 gene has not been cloned, so it is unknown whether this gene is similar to the gene that encodes the mammalian nuclease CAD (caspase-activated DNase), which acts in apoptosis (50).

**The Genetic Pathway for Programmed Cell Death in \textit{C. elegans}**

Genetic experiments have ordered the functions of the 13 \textit{C. elegans} genes that act in programmed cell death and have defined a genetic pathway (Fig. 3). As described above, loss-of-function mutations in the cell death protector gene ced-9 lead to lethality because cells that should survive instead undergo programmed cell death. This ectopic programmed cell death can be suppressed by loss-of-function mutations in ced-3 or ced-4 (18) but not by the loss-of-function mutation in egl-1 (22). A simple genetic pathway consistent with these observations places egl-1 upstream of ced-9 and ced-9 upstream of ced-3 and ced-4. The order of action of ced-3 and ced-4 has been inferred from experiments in which these genes have been overex-

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\(^3\)The abbreviation used is: ICE, interleukin 1\(\beta\)-converting enzyme.
pressed from transgenes in specific developing C. elegans neurons (51). Overexpression of either ced-3 or ced-4 can cause those neurons to undergo programmed cell death, but whereas the killing activity of ced-4 depends upon ced-3 function, the killing activity of ced-3 does not depend upon ced-4 function. A linear pathway consistent with these observations places ced-4 upstream of ced-3. A physical basis for the results of these genetic studies is suggested by the findings that the EGL-1 and CED-9 proteins interact directly (22), that the CED-9 and CED-4 proteins interact directly (reviewed in Ref. 52), and that the EGL-1 and CED-9 proteins interact directly (22), that the CED-9 proteins interact directly (22), and that the engulfment genes and apopstatin of HeLa cells (53). Other genetic experiments have indicated that the cell death specification genes ces-2 and ces-1 act upstream of egl-1 (22) and that the engulfment genes and nue-1 act downstream of ced-3 (e.g., Ref. 21).

On the basis of the genetic and molecular data, we proposed a model for the activation of programmed cell death in C. elegans (Fig. 4; Refs. 13 and 22). After activation by upstream signals (e.g., from ces genes), the EGL-1 protein physically interacts with the CED-9 protein, thereby releasing the CED-4 protein from a complex that consists of at least CED-9 and CED-4. Free CED-4 then facilitates the proteolytic processing of inactive pro-CED-3 to the active caspase. CED-3 protease activity then causes programmed cell death by: (a) activating a protein or set of proteins that function to kill cells, (b) inactivating a protein or set of proteins that function to protect cells from programmed cell death, and/or (c) inactivating a protein or set of proteins involved in cellular homeostasis and for this reason essential for cell viability.

Cell Death Genes May Define New Classes of Proto-Oncogenes and Tumor Suppressor Genes

As discussed above, the human Bcl-2 gene, which encodes a protein similar to the C. elegans CED-9 protein, and the gene for human hepatic leukemia factor (HLF), which encodes a protein similar to the C. elegans CES-2 protein, are implicated in follicular lymphoma and acute lymphoblastic leukemia, respectively. Both of these genes appear to act as oncogenes by suppressing programmed cell death. These observations and the striking conservation between C. elegans and mammals of the overall pathway for programmed cell death together suggest that other genes that function in the process of programmed cell death in the nematode could have counterparts responsible for cancer in humans. For example, mutations in other human genes that act like mutations in ces-1 and ces-2 to prevent the deaths of specific classes of cells could lead to the uncontrolled proliferation of those cell types and predispose or contribute to malignancy. Similarly, mutations that reduce the activities of killer genes of the EGL-1/BH3-only, CED-4/Apaf-1, or CED-3/caspase families could prevent the normal process of programmed cell death and thereby promote malignancy, just as does overexpression of Bcl-2. Such killer genes would define new classes of tumor suppressor genes.

The identification of antagonists of genes that act to prevent programmed cell death or of agonists of genes that act to cause programmed cell death could lead to the development of new anticancer agents. My hope is that studies of the genetics of programmed cell death in C. elegans will help identify both novel drug targets and novel therapeutic agents that will be important in cancer therapy.

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Genetic Control of Programmed Cell Death in the Nematode
*Caenorhabditis elegans*

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