Mechanisms and Genes of Cellular Suicide

Hermann Steller

Apoptosis is a morphologically distinct form of programmed cell death that plays a major role during development, homeostasis, and in many diseases including cancer, acquired immunodeficiency syndrome, and neurodegenerative disorders. Apoptosis occurs through the activation of a cell-intrinsic suicide program. The basic machinery to carry out apoptosis appears to be present in essentially all mammalian cells at all times, but the activation of the suicide program is regulated by many different signals that originate from both the intracellular and the extracellular milieu. Genetic studies in the nematode *Caenorhabditis elegans* and in the fruit fly *Drosophila melanogaster* have led to the isolation of genes that are specifically required for the induction of programmed cell death. At least some components of the apoptotic program have been conserved among worms, insects, and vertebrates.

Most, if not all animal cells have the ability to self-destruct by activation of an intrinsic cell suicide program when they are no longer needed or have become seriously damaged. The execution of this death program is often associated with characteristic morphological and biochemical changes, and this form of cell death has been termed apoptosis (*1*). During apoptosis, the nucleus and the cytoplasm condense, and the dying cell often fragments into membrane-bound apoptotic bodies that are rapidly phagocytosed and digested by macrophages or by neighboring cells. In this way, dead cells are rapidly removed, and any leakage of their noxious and possibly dangerous contents is avoided. In contrast, during necrosis, a pathological form of cell death that results from overwhelming cellular injury, cells swell and lyse, thereby releasing cytoplasmic material which often triggers an inflammatory response. Apoptosis is usually associated with the activation of nucleases that degrade the chromosomal DNA first into large (50 to 300 kilobases) and subsequently into very small oligonucleosomal fragments (*2*).

It is now widely accepted that apoptosis is of central importance for the development and homeostasis of metazoan animals. For example, apoptosis serves as a prominent force in sculpting the developing organism (*3*), as a major mechanism for the precise regulation of cell numbers (*4, 5*), and as a defense mechanism to remove unwanted and potentially dangerous cells, such as self-reactive lymphocytes (*6*), cells that have been infected by viruses (*7, 8*), and tumor cells (*9*). Not surprisingly, the initiation of apoptosis is carefully regulated. Many different signals that may originate either from within or outside a cell have been shown to influence the decision between life and death. These include lineage information, cellular damage inflicted by ionizing radiation or viral infection, extracellular survival factors, cell interactions, and hormones (*4, 5, 10*). These diverse signals may act to either suppress or promote the activation of the death program, and the same signal may actually have opposing effects on different cell types (*11*).

In addition to the beneficial effects of programmed cell death, the inappropriate activation of apoptosis may cause or contribute to a variety of diseases, including acquired immunodeficiency syndrome (AIDS) (*12*), neurodegenerative diseases, and ischemic stroke (*5, 13*). The realization that apoptosis represents an active, gene-directed mechanism has fostered optimism that it may be possible to control apoptosis with the development of drugs that act against the molecular components of the death machinery. However, despite considerable progress, it has proven difficult to identify the molecules responsible for apoptosis by conventional biochemical and molecular approaches in mammalian systems. Fortunately, there is increasing evidence that apoptosis occurs by a mechanism that has been at least partially conserved throughout animal evolution. Therefore, results obtained from the study of experimentally more accessible invertebrate model systems may be directly relevant for understanding the mechanism of apoptosis in vertebrates. Indeed, much of our current knowledge about specific cell death genes has been derived from genetic studies in the nematode *Caenorhabditis elegans* (*14*). A large number of mutations that affect specific stages of programmed cell death have been isolated in this organism, and the corresponding genes have been ordered into a genetic pathway (Fig. 1).

Programmed cell death in *C. elegans* can be divided into four distinct stages. These include the decision of whether a given cell will die or adopt another fate, the death of the cell, the engulfment of the dead cell by phagocytes, and the degradation of the engulfed corpse. Mutations that affect each of these stages have been isolated, and these mutations define 14 genes that function in programmed cell death in the nematode. Mutations that interfere with the execution of death, engulfment, or degradation affect all somatic cell deaths, whereas genes implicated in the decision step influence only very few cells. Three genes have been shown to affect the execution of all somatic cell deaths, *ced-3*, *ced-4*, and *ced-9* (*ced* stands for cell death defective). The activity of two of these genes, *ced-3* and *ced-4*, is

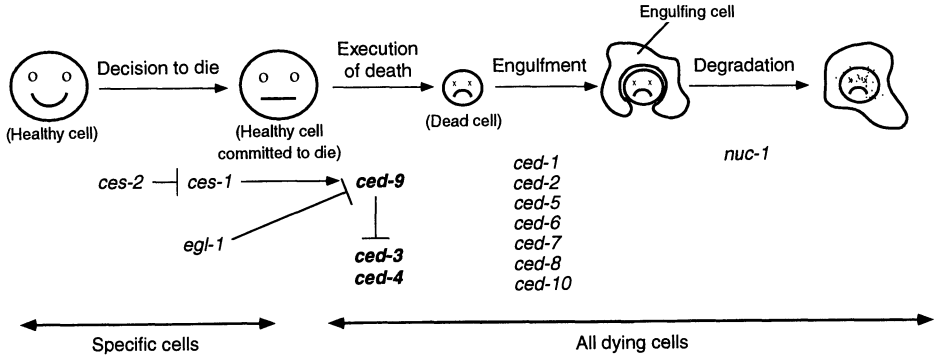

Fig. 1. Genetic pathway for programmed cell death in *C. elegans*. Mutations in 14 different genes have been isolated that affect specific stages of programmed cell death in *C. elegans*. Mutations that influence the decision to die affect only a small number of cells. In contrast, genes involved in all subsequent stages of cell death are common to all somatic cell deaths in this organism. Three genes, *ced-3*, *ced-4*, and *ced-9*, affect the execution of the death program. The activity of *ced-3* and *ced-4* promotes cell death, and *ced-9* activity prevents this process. The epistatic relationship among these genes has been deduced from double-mutant combinations. Symbols: → (positive regulation); ——| (negative regulation). [Adapted from (*14*)]

The author is at the Howard Hughes Medical Institute, Department of Brain and Cognitive Sciences and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

required in dying cells for cell death to occur, and the properties of these genes are summarized below. The third gene, *ced-9*, is required to protect cells that should survive from undergoing programmed cell death (*15*). This gene encodes a protein that is homologous to the Bcl-2 family of cell death regulators (*16, 17*). Furthermore, expression of human Bcl-2 can inhibit cell death in nematodes and can even partially substitute for the loss of *ced-9* function (*16, 18*). These results indicate that at least some components of the apoptotic program have been conserved throughout most of animal evolution. This notion has received further support from reports that expression of the anti-apoptotic p35 protein from baculovirus can protect against programmed cell death in insects, nematodes, and mammalian neurons (*19*). The relevant proteins that interact with Bcl-2 and p35 have not yet been identified, but it is likely that they have an important and conserved function in the execution of the cell death program.

Here I focus on recent advances in characterizing the properties of the cell death program and of the genes implicated in the process of apoptosis. Although the exact mechanism of death is unknown, progress has been made in identifying key components of the apoptotic machinery. First, I will summarize work indicating that the cell death effector molecules, that is, the components that are directly responsible for the morphological changes during apoptosis, are always present but are inhibited in most mammalian cells. Then I will discuss various biochemical mechanisms that have been proposed as the possible cause of apoptotic death. Next, I will review the evidence that implicates proteases of the *ced-3*/interleukin-1β–converting enzyme (ICE) family in causing the onset of apoptosis. Finally, I will present a model for how distinct signaling pathways may converge in order to activate a common apoptotic program.

Properties of the Cell Death Program

Some of the initial evidence for the idea that apoptosis is caused by an active cell suicide program was derived from experiments in which cell death could be suppressed by inhibitors of RNA or protein synthesis (*20*). However, it was subsequently shown that these inhibitors fail to block and may even induce apoptosis in many other situations, which suggests that the apoptotic effector molecules are always present in most mammalian cells (*5*). The most compelling evidence for the idea that all the proteins required for apoptosis are constitutively expressed in mammalian cells has come from experiments with cells whose nuclei have been removed (*21*). If such cytoplasts are deprived of survival factors or are treated with high concentrations of the protein kinase inhibitor staurosporine, they will undergo all of the cytoplasmic changes that are characteristic of apoptosis. Cytoplasts prepared from cells that express high levels of the Bcl-2 protein are protected against these apoptotic changes. Thus, major aspects of apoptosis do not require the transcription of new genes, and Bcl-2 can protect against this process in the absence of a cell nucleus. As discussed below, the requirement of RNA and protein synthesis for the induction of cell death in certain situations may reflect the need to synthesize molecules that activate or derepress the existing cell death machinery, rather than making any components required for the basic cell death program itself.

If the apoptotic effector proteins are present in living cells, their potentially lethal activities must be suppressed in cells that normally survive. Raff and colleagues (*4, 5*) have proposed that cells are programmed to commit suicide and require signals from other cells continuously in order to survive. This model is an extension of the well-accepted neurotrophic theory (*22*), and there is indeed growing support for this view. Many different cells in mammals (*4, 5*) and also in *Drosophila* (*10*) require extracellular factors that are produced by other cells in order to stay alive. This social control of cell survival appears to guarantee that appropriately matching numbers of different cell types in a particular tissue are maintained. Several survival factors and their receptors have been molecularly characterized (*23*), but it is unclear how the activated receptors regulate the apoptotic program. Survival factors might prevent the induction of apoptosis by reducing the amount or activity of crucial cell death effector proteins to harmless levels. Alternatively, they may inhibit cell death by boosting the activity of protective, anti-apoptotic proteins, such as Bcl-2 family members. In the absence of *ced-9* activity, nematodes display widespread ectopic cell death. Therefore, at least in this organism, cell death occurs by default in the absence of protective functions (*16*).

The actual cause and mechanism of apoptotic death is still unknown. Owing to the prominence of nuclear changes at an early stage of apoptosis, it has been suggested that cells are killed by the endonucleolytic cleavage of their DNA (*2, 24*). However, cells without a nucleus are physiologically active for a considerable time and can be induced to undergo the characteristic cytoplasmic changes of apoptosis. In contrast, isolated nuclei can exhibit condensation and oligonucleosomal DNA degradation in a cell-free system (*25*). These observations imply that multiple factors may contribute to death, and that different cellular compartments have considerable autonomy in undergoing structural changes during apoptosis. It has been suggested that apoptosis may be caused by the accumulation of reactive oxygen species in a process that might be controlled by levels of Bcl-2 (*26*). This idea resulted from experiments showing that Bcl-2 protects against peroxide-induced death, and that certain antioxidants protect against death in response to cytokine deprivation. However, it is questionable whether reactive oxygen species are widely used by cells to commit suicide. Apoptosis and Bcl-2 protection have been observed in the absence of mitochondrial respiration and in cells grown essentially without oxygen, conditions that should greatly reduce the amount of free radicals (*27*).

Finally, numerous overall similarities between apoptosis and the cell cycle have been noted, and it has been suggested that apoptosis and mitosis may be mechanistically related or even coupled. An extreme view of this idea is that apoptosis may be an aberrant mitosis (*28*). Some support for a connection between apoptosis and mitosis has come from implicating genes that play a role in the regulation of cell proliferation, such as p53, c-myc, Rb-1, E1A, cyclin D1, c-fos, and $p34^{cdc2}$ kinase in the control of apoptosis (*8, 29, 30*). Some of these genes affect apoptosis in specific situations. For example, p53 appears to be predominantly required for mediating the apoptotic response to chromosomal damage (*31*). In contrast, developmental cell death is not significantly affected in p53 null mice, which develop normally into adult animals. These results place p53 in an activation pathway that is upstream of the basic cell death program. Similar conclusions apply to several other genes on this list. Other genes associated with cell proliferation, such as c-*myc*, are capable of inducing apoptosis when they are aberrantly expressed (*30*), and it has been suggested that *myc* functions normally to induce both proliferation and apoptosis. However, it has been difficult to rule out the alternative interpretation that apoptosis under these conditions is simply the result of a conflict of incompatible growth signals (*32*). The observation that p53 is required for *myc*-induced death (*33*) also suggests that *myc* does not regulate developmental cell death, which can occur in the absence of p53 function (*31*). Likewise, a clear demonstration that any of the other growth control or cell cycle genes function in apoptosis in vivo is still outstanding. In any event, it is likely that some components of the apoptotic program are shared with other cellular processes, including mitosis. However, it appears that the terminal nature and specialized features of apoptosis require at least some components that are specifically devoted to this process.

Role of Cysteine Proteases in Apoptosis

Systematic genetic studies in the nematode *C. elegans* have led to the identification of two genes, *ced-3* and *ced-4*, that are required for all somatic cell deaths (*14*). If either gene is inactivated, all the cells that normally die during development survive. Genetic mosaic studies indicate that these genes act autonomously in dying cells, consistent with a function of *ced-3* and *ced-4* in mediating cellular suicide (*34*). Furthermore, mutations in these genes suppress the ectopic cell deaths otherwise seen in animals that have lost *ced-9* function (*15*). Double mutants between *ced-3* and *ced-9* or *ced-4* and *ced-9* lack all programmed cell death and have phenotypes equivalent to either *ced-3* or *ced-4* single mutants. The simplest interpretation of these results is that *ced-9* inhibits the activities of *ced-3* and *ced-4*. If these genes operate in a linear pathway, *ced-9* would be expected to act upstream of *ced-3* and *ced-4* (Fig. 2). Because it is unlikely that Ced-9/Bcl-2 proteins regulate gene expression, these results also indicate that *ced-3*, *ced-4*, and *ced-9* are expressed in many more cells than in those that actually die. There appears to be a delicate balance between the opposing activities of proteins that promote and those that inhibit cell death.

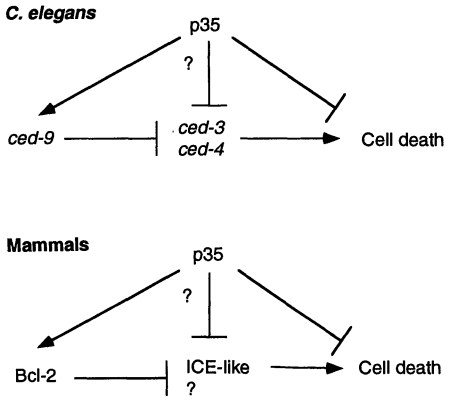

Fig. 2. At least some components of the cell death pathway have been conserved throughout animal evolution. Two of the three genes that control the initiation of programmed cell death in *C. elegans* are homologous to mammalian genes that are thought to play a similar role during apoptosis. In addition, the baculovirus p35 protein has been shown to suppress cell death in insects, nematodes, and mammals, indicating that it interacts with a component of the cell death program that has been conserved in evolution. The identity of this component has not yet been determined. The p35 protein could act by either increasing the activity of protective functions or by blocking the activity of ICE-like proteases or other cell death effector proteins. This regulation could occur by direct protein-protein interactions or could involve other intermediate components. Symbols are as in Fig. 1.

The genes for both Ced-3 and Ced-4 have been cloned and molecularly characterized. The *ced-4* gene encodes a 63-kD protein with no significant similarity to other known polypeptides (*35*). In contrast, the *ced-3* gene encodes a protein that is similar to the family of cysteine proteases, which includes interleukin-1β–converting enzyme (ICE) (*36–38*), *nedd-2/Ich-1* (*39, 40*), and CPP32 (*41*). Furthermore, overexpression of either *ced-3*, ICE, or Nedd-2/Ich-1 in mammalian cells causes apoptosis (*40, 42*). These observations suggest that ICE or a related cysteine protease may function in mammalian cell death. Additional support for this idea comes from the observation that expression of the cowpox virus *crmA* gene, a very potent and specific inhibitor of ICE-like proteases (*43*), can protect cells against apoptosis in response to growth factor withdrawal (*44*). However, it remains to be shown—by targeted gene knockout, for example—whether ICE itself or a similar protease encoded by another gene has a physiological role during apoptosis in vivo that is comparable to that of *ced-3*. Recently, three nuclear proteins, poly(A)DP ribose polymerase, lamins, and U1-70kD have been identified as substrates for proteolytic cleavage by an activity that is very similar to but distinct from that of ICE (*45*). Therefore, although several genes encoding proteins homologous to ICE have already been isolated (*39–41*), it is possible that additional family members, including the true functional homolog or homologs of *ced-3*, remain to be discovered.

ICE has a rather unusual substrate specificity, cleavage at Asp-X bonds (where X is any amino acid), that is shared with only one other known eukaryotic protease, granzyme B (also known as cytotoxic cell proteinase–1, fragmentin 2, and RNKP-1) (*46, 47*). In addition, ICE and granzyme B can be inhibited by similar types of reagents (*37*). These observations are intriguing, because granzyme B is a serine protease that is responsible for apoptosis caused by cytotoxic T lymphocytes (CTLs) (*46, 48*). CTLs can kill cells by delivering the contents of enzyme-bearing granules to the target. Even though CTL-mediated cell killing was originally viewed as a "murder," it has become clear that this process involves the induction of apoptosis in the target cells (*46, 49*). The delivery of granzyme B appears to be both necessary and sufficient for the induction of apoptosis (*46, 48*). Thus, granzyme B may induce apoptosis either by mimicking the action of a *ced-3*/ICE-like cell death gene or by converting such an enzyme, through proteolytic processing of its inactive precursor, to its active state (*7*). The appeal of this hypothesis is that it provides a unified view of the mechanisms underlying programmed cell death and CTL-mediated cytotoxicity. Although it has been reported that ICE itself is not a substrate for granzyme B cleavage (*50*), other ICE-family members may be processed by granzyme B. The identification and biochemical characterization of additional *ced-3*/ICE homologs and their substrates will clearly be an important goal for the near future. Despite these uncertainties, there are good reasons to believe that a *ced-3*/ICE-like cysteine protease initiates the irreversible stage of apoptosis in both nematodes and mammals, by acting at or very close to the cell death effector level (Fig. 2). Two of the three genes that are known to control the onset of programmed cell death in nematodes, *ced-3* and *ced-9*, are similar to mammalian genes that are thought to play a similar role in apoptosis (Fig. 2). Because nematodes and vertebrates have origins that date back to very early stages of animal evolution, predating the separation of chordates and arthropods, it is likely that similar genes operate during programmed cell death in all animals. Furthermore, because these genes can at least partially function in heterologous systems, additional components of the cell death pathway must have been conserved as well.

Universal Regulators of Apoptosis

A fundamental unresolved question is how the apoptotic program is regulated so that only certain cells are selected to die. Apoptosis is controlled by many different, distinct signals, and this work has been extensively reviewed in the recent past (*4, 5, 10, 32, 51*). Yet, it appears that different signaling pathways ultimately converge to activate a common apoptotic program. The expression of anti-apoptotic proteins, such as Bcl-2 and baculovirus p35, can inhibit apoptosis in response to many different death-inducing signals (*17, 19*). This indicates that these proteins interact with components that are either shared among different signaling pathways or act downstream from the convergence point of these pathways.

A step toward understanding how different signals may converge to activate a common cell death program has come from genetic studies on the control of apoptosis in the fruit fly, *Drosophila melanogaster*. In *Drosophila*, as in mammals, the onset of apoptosis is influenced by many different intra- and extracellular signals that may either promote or suppress cell death (*10*). Nevertheless, it appears that most, if not all programmed cell deaths in this organism, are mediated by one common mechanism. Genetic analyses have led to the isolation of a gene, *reaper*, that is capable of integrating information from different signaling pathways to activate the apoptotic program (*52*). Deletions that include *reaper* suppress

apoptosis in response to every apoptotic stimulus tested to date. In the *Drosophila* embryo, *reaper* messenger RNA is specifically found in cells that are doomed to die, and the onset of expression precedes the first morphological signs of apoptosis by 1 to 2 hours. This gene is also rapidly induced upon irradiation with x-rays (*53*), and deletions that include *reaper* offer substantial protection against radiation-induced apoptosis. These observations show that multiple signaling pathways for the activation of apoptosis converge onto the *reaper* gene. Furthermore, expression of *reaper* is sufficient to induce apoptosis in cells that would normally survive (*52*, *54*). Taken together, these results implicate *reaper* as a universal activator of apoptosis in *Drosophila*.

The mechanism by which *reaper* induces apoptosis is not yet clear. The *reaper* gene encodes a small polypeptide of 65 amino acids with no significant similarity to other known proteins, so the sequence provides no information about its biochemical function. However, it appears that *reaper* is not part of the actual death program. This conclusion is based on the observation that some apoptosis can be induced in embryos deficient for *reaper* by irradiation with very high doses of x-rays. Even though the induction of radiation-provoked cell death is much more efficient in the presence of *reaper*, the morphology of those cells that do die is unaffected by its absence. This suggests that *reaper* encodes a regulatory molecule, rather than an apoptotic effector. The selective expression of *reaper* in cells that are doomed to die is consistent with this model, because cell death effectors, at least in mammalian cells, are expected to be constitutively expressed. A highly speculative model for the role of *reaper* in apoptosis with respect to other cell death genes is shown in Fig. 3. One prediction of this model is that *reaper* acts upstream of cell death effectors—for example, by activating a *ced-3*/ICE-like protease. Alternatively, *reaper* may inhibit negative regulators of cell death, such as *ced-9*/Bcl-2 family members. In either case, *reaper* would serve as an integrator that links different signaling pathways with the basic cell death program. In the future, it will be important to identify downstream targets for *reaper* and also to investigate whether this gene and its function have been conserved during evolution. Another interesting question is how *reaper* mRNA expression is regulated in response to different death-inducing signals. It is possible that the convergence of different signaling pathways occurs at the level of the *reaper* promoter. In this case, pathway-specific transcription factors may bind to distinct control elements of the *reaper* gene. Alternatively, the convergence of signaling pathways may occur upstream of *reaper*. In this case, a death-specific transcription factor may be activated and induce *reaper* transcription by binding to a single regulatory site. In principle, it should be possible to distinguish between both models by standard promotor analyses.

The universal requirement of *reaper* for the activation of programmed cell death in *Drosophila* poses an apparent paradox in light of the finding that apoptosis can be induced in mammalian tissue culture cells in the absence of de novo protein synthesis. One possible interpretation is that there are fundamental differences in the expression of cell death genes between *Drosophila* and mammals. Yet one does not have to invoke such differences at this point. As discussed above, the inhibition of apoptosis by protective, anti-apoptotic proteins is of fundamental importance for cell survival, and it appears that many cells require the constant production of these proteins in order to survive. The absence of protein synthesis (or a cell nucleus) should lead to a steady decrease in the levels of both protective and death-promoting proteins. Therefore, a block of RNA or protein synthesis will set up a "race" between proteins with opposing functions in cell death. The outcome of this competition will depend on the relative amounts, activities, and stabilities of these proteins. Because different cell types are known to express distinct levels and types of protective functions, it is expected that different results will be obtained for different cell types. According to this model, cells that start out with a relative excess of protective proteins should continue to be protected against apoptosis upon blocking protein synthesis. However, other cells may lose their protection more rapidly than the proteins that mediate apoptosis, and such cells will die upon treatment with inhibitors of RNA or protein synthesis. It is easy to envision that the outcome of this competition can also be affected by a variety of drugs and manipulations that may influence the activity and stability of the relevant components, perhaps often in ways that differ from the mechanisms normally used by cells to control apoptosis. Consequently, whereas *reaper* and related molecules may be used to initiate apoptosis in vivo, it may be possible to bypass the requirement for such positive regulators of cell death in less physiological situations.

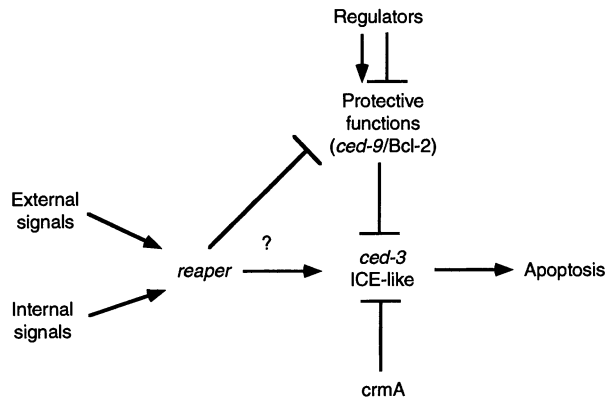

Fig. 3. Model for the convergence of death signals on the apoptotic program. The *Drosophila reaper* gene may serve as a universal activator of apoptosis in response to different death-inducing signals. Expression of *reaper* gene is induced by many different death signals, and deletions that include *reaper* suppress cell death in response to these signals. The *reaper* gene appears to function as a regulator, and not part of the basic cell death effector machinery. Therefore, it should act upstream of cell death effectors, either by activating death-promoting genes, such as *ced-3*/ICE-like proteases, or by inhibiting negative regulators, such as *ced-9*/Bcl-2. This model is highly speculative, because *reaper* homologs have not yet been reported in other systems and interactions between *reaper* and other cell death genes have not yet been experimentally tested. Symbols are as in Fig. 1.

Future Prospects

During the past few years, substantial progress has been made in understanding the control and mechanisms of apoptosis. Nevertheless, major aspects of the apoptotic pathway remain undefined, and it is not even clear what exactly causes cells to die. However, the availability of several important cell death genes should facilitate the isolation of interacting proteins and additional components of the cell death pathway. The development of cell-free model systems for apoptosis (*25*) should help to elucidate the biochemical basis of this process. Finally, one should expect many more contributions to understanding the mechanism of apoptosis from studies of cell death in highly accessible genetic model systems, such as *C. elegans* and *Drosophila*. It appears that *Drosophila* has been underutilized in the past for this purpose. In this organism, apoptosis is under epigenetic control, providing the opportunity to apply an extremely powerful combination of genetics and molecular and cell biology to studying both the signaling pathways and the mechanism of apoptosis. Because the basic cell death program appears to have been remarkably well conserved during evolution, knowledge gained from further studies of programmed cell death in worms and flies should contribute to a better understanding of mammalian apoptosis.

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The Fas Death Factor

Shigekazu Nagata* and Pierre Golstein

Fas ligand (FasL), a cell surface molecule belonging to the tumor necrosis factor family, binds to its receptor Fas, thus inducing apoptosis of Fas-bearing cells. Various cells express Fas, whereas FasL is expressed predominantly in activated T cells. In the immune system, Fas and FasL are involved in down-regulation of immune reactions as well as in T cell–mediated cytotoxicity. Malfunction of the Fas system causes lymphoproliferative disorders and accelerates autoimmune diseases, whereas its exacerbation may cause tissue destruction.

Homeostasis of multicellular organisms is controlled not only by the proliferation and differentiation of cells but also by cell death (*1*). The death of cells during embryogenesis, metamorphosis, endocrine-dependent tissue atrophy, and normal tissue turnover is called programmed cell death. Most of programmed cell death proceeds by apoptosis, a process that includes condensation and segmentation of nuclei, condensation and fragmentation of the cytoplasm, and often extensive fragmentation of chromosomal DNA into nucleosome units.

Apoptosis in vertebrate development often occurs by default when cells fail to receive the extracellular survival signals needed to suppress an intrinsic cell suicide program (*2*); the survival factors can be produced by neighboring cells of a different type (a paracrine mechanism), or of the same type (an autocrine mechanism). In contrast, in the immune system there are situations where cells actively kill other cells; for example, cytotoxic T lymphocytes (CTLs) or natural killer (NK) cells induce apoptosis in their targets such as virus-infected cells or tumor cells (*3*). In these cases, an effector molecule expressed at the surface of CTLs or NK cells or a soluble cytokine produced by these effector cells is thought to be responsible for target cell death.

Molecular and cellular characterization of Fas, a cell surface protein recognized by cytotoxic monoclonal antibodies, revealed its role as a receptor for a Fas ligand (FasL) (*4*). When FasL binds to Fas, the target cell undergoes apoptosis. Spontaneous mutations for Fas and FasL have been identified in mice, and from the phenotypes of these mutants and from studies on mechanisms of cytotoxicity, it was concluded that the Fas-FasL system is involved not only in CTL-mediated cytotoxicity but also is down-regulation of immune responses. In this article, we summarize current knowledge on Fas and FasL and discuss their physiological and pathological roles in the immune system.

Fas and Fas Ligand

In 1989 two groups independently isolated mouse-derived antibodies that were cytolytic for various human cell lines (*5*, *6*). The cell surface proteins recognized by the antibodies were designated Fas and APO-1, respectively. The antibody to Fas (anti-Fas) was an immunoglobulin M (IgM) antibody, whereas the antibody to APO-1 was classified as IgG3. The Fas complementary DNA

S. Nagata is with the Osaka Bioscience Institute, 6-2-4 Furuedai, Suita, Osaka 565, Japan. P. Golstein is with the Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille Cedex 9, France.

*To whom correspondence should be addressed.