Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 12736–12737, November 1997
From the Academy

This paper is a summary of a session presented at the third annual German–American Frontiers of Science symposium, held June 20–22, 1997 at the Kardinal Wendel Haus in Munich, Germany.

Advances in apoptosis research

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ABSTRACT **Apoptosis, also called programmed cell death, has attracted great attention in recent years. After its discovery by Carl Vogt in 1842, apoptosis research was dormant for more than a century. Its rediscovery in the second half of this century, and the coining of the term *apoptosis* in 1972 by Kerr, Wyllie, and Currie, ignited an unparalleled interest in this field of science. The number of publications related to apoptosis has been growing exponentially every year ever since. This is mainly due to three major advances, two of which have been made recently and one that is currently seen. First, studies with the small nematode *Caenorhabditis elegans* have identified a number of apoptosis regulating genes—the first evidence that cell death is an active process under genetic control. Many of these genes have mammalian homologs that, like their worm counterparts, seem to regulate mammalian apoptosis. Second, elucidation of the signal transduction pathways of apoptosis has lead especially to the identification of specific death signaling molecules such as a new family of cysteine proteases, the caspases. Third, it has now become clear that many diseases are characterized by dysregulation of apoptotic programs. Many of these programs involve a family of receptors and their ligands, the death receptor/ligand family. The hope now is to interfere with apoptosis regulation in these systems and to develop new therapeutic concepts.**

The first of three recent breakthroughs in apoptosis research comes from the genetic study of genes that control apoptotic cell death in the nematode *Caenorhabditis elegans*. In the worm three genes—*ced-3*, *ced-4*, and *ced-9*—are directly involved in controlling the execution of apoptosis during development (1). Both *ced-3* and *ced-4* are killer genes, required for the cell to die, whereas *ced-9* is a survival gene required to protect *C. elegans* cells that should survive from inappropriately activating the death program. Interestingly, homologs of these genes also function to control apoptosis in mammals: *ced-3* encodes a protease homologous to the mammalian caspase family. CED-3 activity is negatively regulated by CED-9, which is structurally and functionally homologous to Bcl-2, a mammalian protein that has been shown to inhibit many forms of apoptosis, and other Bcl-2 family members such as Bcl-x_L. To exert its function, CED-9 requires the presence of CED-4, another apoptosis-promoting factor, which acts as a physical bridge between CED-9 and CED-3 and is essential for the processing of proCED-3 (an inactive zymogen) into the catalytically active death enzyme. Apaf-1, a recently identified CED-4 homolog, is similarly required for the processing of caspase-3 in mammalian cells (2). The conservation in both sequence and function between nematode and mammalian cell death genes indicates that apoptosis is of ancient evolutionary origin, and it suggests that worms and humans use similar conserved mechanisms to get rid of cells.

A large number of additional genes have also been identified in *C. elegans* that affect other aspects of apoptotic death. Many of these genes, such as *ced-1, -2, -5, -6, -7,* and *-10*, act distally of the central apoptotic machinery in the cell. These genes are required for efficient recognition and phagocytosis of apoptotic cells by their neighbors. Some genes, such as *ces-1* and *ces-2*, function only in a small number of cells and affect the decision of individual cells to activate the death program. These genes might be involved in cell type-specific "private" signal transduction pathways or regulatory cascades that affect the cell death pathway. The *ces-2* gene has recently been shown to encode a homolog of the human oncogene *Hlf* (hepatic leukemia factor) (3). Interestingly, both CES-2 and Hlf function as cell type-specific negative regulators of cell survival, suggesting that not only the central killing machinery but also the inputs into this machinery have remained conserved through evolution.

The second breakthrough is related to the discovery of a family of receptors that can specifically trigger apoptosis. These receptors belong to the tumor necrosis factor (TNF) receptor superfamily and are characterized by the presence of extracellular cysteine-rich domains. Intracellularly, however, these members contain an area of weak homology required to transduce a cell death signal which was termed the death domain (DD). With the discovery of genuine death receptors the importance of apoptosis induction became most obvious. Members of this new family include CD95 (APO-1/Fas), TNF-R1, DR3 (TRAMP/wsl-1/APO-3/LARD/AIR), and DR4/TRAIL-R1/APO-2 (for review see ref. 4). The DD couples the death receptors to the apoptosis-inducing machinery. A number of signaling molecules carry a DD that directly binds to these receptors. These molecules include FADD/MORT1, TRADD, RIP, RAIDD/CRADD, and MADD. Only some of them, however, have been detected as part of an *in vivo* complex with their receptors formed in a ligand-dependent fashion. FADD was found to be part of the CD95 death-inducing signaling complex (DISC), and TRADD was found associated with TNF-R1 upon binding of TNFα. DD-containing molecules are specialized adapter molecules coupling to the apoptosis executioners, which are in many instances members of the caspase family of proteases. The best-characterized member of these receptor-binding caspases is caspase-8/FLICE/MACH/Mch5. Caspase-8 is recruited to the CD95 DISC by the adaptor FADD. At its N terminus, FADD contains a death effector domain (DED), a motif that was also found in two copies at the N terminus of caspase-8. Caspase-8 is activated by binding to the DISC (5). The active subunits p10/p18 are released into the cytoplasm, likely

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	Death receptors	Adaptors	1. Level caspases	Regulators			2. Level caspases
C. elegans	?	?	?	CED-9		CED-4	CED-3
				Inhibitors	Promotors		
Humans	CD95/APO-1/Fas TNF-R1 DR3/TRAMP/ wsl-1/APO-3/LARD DR4/APO-2/TRAIL-R	FADD/MORT1 TRADD MADD RAIDD/CRADD RIP	caspase-8 caspase-10 caspase-2 (caspase-1-like caspases)	Bcl-2 Bcl-x_L A1 Mcl-1 Bcl-w	Bax Bik/Nbk Bak Bad Bid	Apaf-1	caspase-3-like caspases

FIG. 1. *C. elegans* apoptosis-regulating genes and their human homologs.

cleaving various death substrates that still need to be identified.

The elucidation of the apoptosis pathway in *C. elegans* has been helpful to better understand apoptosis signaling pathways in higher eukaryotes. Key components of the apoptosis machinery seem to be conserved between humans and nematodes. However, neither an apoptosis-inducing receptor nor coupling adaptor molecules have yet been identified in *C. elegans* (Fig. 1). In human cells, at least 10 caspases are expressed, all of which contain regions homologous to the *C. elegans* CED-3. Recent data indicate that some of these caspases, such as caspase-8, act upstream of the human CED-9 homolog Bcl-2/Bcl-x_L, whereas others, such as caspase-3, seem to be located downstream of Bcl-2/Bcl-x_L in the pathway. The latter caspases may require a protein similar to the nematode CED-4 protein for their activation—e.g., the recently identified Apaf-1 (Fig. 1).

The third reason why apoptosis research has experienced such a rapid development relates to its biological relevance. Apoptosis is the physiological way for nucleated cells to die. Apoptosis takes care of unwanted, injured, or virus-infected cells. Autoreactive T and B cells, millions of which are produced by the immune system every day, are also eliminated by apoptosis. Recently, dysregulation ("too much or too little") of apoptosis has emerged as a new concept to explain important features in the development of several as yet poorly understood diseases (Table 1). Unregulated excessive apoptosis may be the cause of various degenerative and autoimmune diseases that are characterized by an excessive loss of normal or protective cells, such as in multiple sclerosis, type-I diabetes mellitus, Hashimoto thyroiditis (6), Sjögren syndrome, and certain cancers such as melanoma (7). Conversely, an inappropriately low rate of apoptosis may promote survival and accumulation of abnormal cells that can give rise to tumor formation and prolonged autoimmune stimulation such as in cancers and Graves disease. Remarkably, even within the same organ such as the thyroid gland, entirely different states of disease may be initiated depending upon the rate at which apoptosis continues to proceed. For instance, in the atrophic variant of immunogenic hypothyroidism (Hashimoto thyroiditis), infiltrating immune effector cells may trigger apoptosis in target cells within thyroid follicles by means of the CD95/CD95L system (6). Moreover, upon exposure to interleukin-1, thyroid follicular cells also appear to use the CD95/CD95L system to transmit the apoptotic signal onto their neighboring cells. By contrast, in the autoimmune hyperthyroidism of Graves disease, intrathyroidal fibroblasts have been identified to act as potent inhibitors of the apoptotic machinery of intrathyroidal B lymphocytes, thereby enhancing their life span, differentiation, and immunoglobulin secretion within the diseased gland. In studies of the mechanisms of fibroblast-mediated suppression of lymphocyte apoptosis, coculture experiments with intrathyroidal fibroblasts and B cells have revealed that both fibroblast-derived soluble factors and direct cell contact most effectively inhibit apoptosis in intrathyroidal B cells (8). It seems likely that pro- and anti-apoptotic factors determine either susceptibility or resistance to apoptosis, and, consequently, play a crucial role in the evolution, propagation, and chronicity of degenerative, cancerous, and autoimmune conditions. Thus, precise identification of the distinct errors in the complex apoptotic machinery holds great promise for elucidating the pathogenesis of various important diseases and for devising more specific and effective treatments.

Table 1. Apoptosis in disease

Too much	Too little
AIDS	Canale–Smith syndrome (CSS; autoimmune lymphoproliferative syndrome)
Cancers (e.g., melanoma, hepatoma colon cancer)	
Liver failure	Lymphoma
Wilson disease	Leukemia
Myelodysplastic syndromes	Solid tumors
Neurodegenerative diseases	Autoimmune diseases (e.g., hypereosinophilia syndrome, lupus erythematosus, rheumatoid arthritis, Graves disease)
Multiple sclerosis	
Aplastic anemia	
Chronic neutropenia	
Type I diabetes mellitus	
Hashimoto thyroiditis	
Ulcerative colitis	

Listed are diseases in which dysregulation of apoptosis has been shown or is currently being discussed as being involved.

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