

Apoptosis—an introduction

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Summary

Apoptosis has become a major research area in the biomedical sciences. As there are more than 13,000 papers published annually on the topic, it is impossible to keep track on all developments in the area. The individual aspects of molecular control of apoptosis are well reviewed, but more general, introductory recent reviews into the field are lacking. This review aims to give a brief overview of the field, providing an introduction into the literature for students and newcomers; as it is written for the un-initiated, wherever possible, review articles will be cited rather than original papers. *BioEssays* 25:888–896, 2003. © 2003 Wiley Periodicals, Inc.

Introduction into cell death

The word “apoptosis” comes from the ancient Greek ἀπόπτωσις, meaning the “falling off of petals from a flower” or “of leaves from a tree in autumn”. The name was first introduced by John Kerr⁽¹⁾ in 1972 and refers to the morphological feature of formation of “apoptotic bodies” from a cell. Carl Vogt, however, first described the phenomenon more than 100 years earlier in 1842. Over the last 10 years, the number of publications related to apoptosis has increased exponentially to now over 2% of the papers published in the life sciences. A timeline of cell death publication chronology can

be found in Ref. 2. This great interest in apoptosis arose due to the recognition that many diseases involve too much apoptosis (e.g., [neuro]degenerative diseases, Parkinson’s, Alzheimer’s, spinal muscular atrophy, AIDS) or too little apoptosis (e.g., cancer [either by virus infection or by DNA mutations such as p53 and Bcl-2] or autoimmune diseases [diabetes type I, encephalomyelitis]). Many toxins and other cellular stresses can also trigger apoptosis (e.g., oxidative stress, alcohol).

Apoptosis is associated with a distinct set of biochemical and physical changes involving the cytoplasm, nucleus and plasma membrane. Early in apoptosis, the cells round up, losing contact with their neighbors, and shrink. In the cytoplasm, the endoplasmic reticulum dilates and the cisternae swell to form vesicles and vacuoles. In the nucleus, chromatin condenses and aggregates into dense compact masses, and is fragmented internucleosomally by endonucleases, which can be analysed by the typical “DNA ladder” formation in apoptosis, for which DNA (either total or cytosolic) is extracted from the cells and separated in an agarose gel.⁽³⁾ The nucleus becomes convoluted and buds off into several fragments, which are encapsulated within the forming apoptotic bodies. In the plasma membrane, cell junctions are disintegrated, whereby the plasma membrane becomes active and convoluted, eventually blebbing. The cell breaks up in a florid manner leading to the “falling away” of several membrane spheres containing the “packaged” cellular contents identified as apoptotic bodies of various sizes.⁽⁴⁾ Under physiological conditions, certain modifications in the plasma membrane occur, which enable the recognition of apoptotic bodies by phagocytic cells. Since the apoptotic bodies are surrounded by an intact plasma membrane, apoptosis usually occurs without leakage of cell content and usually without inflammation. This form of physiological cell death is morphologically quite different from oncosis, in which the cell swells and disintegrates in an unordered manner, eventually leading to the destruction of the cellular organelles and finally rupture of the plasma membrane and leakage of the cell content (necrosis). Necrosis can also be the final result in situations where there is too much apoptosis occurring for phagocytotic cells to cope with, especially in cell culture,⁽⁵⁾ where professional phagocytotic cells are usually lacking.

As apoptosis was introduced as a term describing a specific morphology of cell death, it should not be used synonymously with the term “programmed cell death (PCD)”, which usually occurs via apoptosis. The term PCD refers to time- and position-programmed cell death during development of an

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Abbreviations: AICD, activation-induced cell death; Apaf-1, apoptotic protease activating factor-1; ARF, alternative reading frame; BH, Bcl-2 homology; CAD, caspase-activated DNase; CARD, caspase recruitment domain; DD, death domain; DED, death effector domain; DISC, death inducing signaling complex; DR, death receptor; FACS, fluorescence-activated cell sorting; FADD, Fas-associated death domain protein; GrB, granzyme B; IAP, inhibitor of apoptosis protein; ICAD, inhibitor of caspase-activated DNase; MDM2, murine double minute 2; NAIP, neuronal apoptosis inhibitory protein; PARP, poly-(ADP-ribose)polymerase; PCD, programmed cell death; PM, plasma membrane; PS, phosphatidylserine; ROCK1, Rho-associated coiled-coil forming kinase I; SMase, sphingomylinase; tBid, truncated Bid; TNF, tumor-necrosis factor; TNF-R, tumor-necrosis factor receptor; TRADD, tumor-necrosis factor receptor-associated death domain protein TRAIL, TNF-related apoptosis-inducing ligand; VDAC, voltage-dependent anion-selective channel.

organism (see next section). As apoptosis usually does not lead to inflammation, it can be considered as a physiological form of cell death. PCD, in most instances, follows the apoptotic morphology. However, apoptosis can be induced, for example by anti-cancer drugs. In these instances, the cell death program is initiated, but without treatment the cells would not die; i.e., we are not dealing with programmed cell death.

Apoptosis is over 20 times faster than mitosis. Sightings of dying cells *in vivo* are therefore rare. Apoptotic cells are engulfed and degraded by neighboring cells without a trace. For cell homeostasis to be maintained, a balance between the increase (by differentiation from precursors and by proliferation) and decrease (by further differentiation and cell death) in the number of a cell population has to be neatly balanced. If mitosis proceeded without cell death, an 80-year-old person would have 2 tons of bone marrow and lymph nodes, and a gut 16 km long.⁽⁶⁾

Genetic regulation of apoptosis

For the nematode *Caenorhabditis elegans*, the complete genome has been sequenced, a complete cellular fate map has been established and genetic mutants are easily obtained. *C. elegans* hermaphrodites have 1090 somatic cells, 131 of which commit suicide by apoptosis. 959 cells live and develop into defined tissues. 116 of the 131 dying cells are cells of the nervous system and other ectoderm.

Two “cell death abnormal” genes, *ced-3* and *ced-4* are required for PCD of all 131 somatic cells.⁽⁷⁾ The product of the *ced-9* gene inhibits the products of *ced-3* and *ced-4*. The action of *ces-1*, *ces-3*, *ces-4* and *egl-1* determines whether a cell will die or survive. A range of genes is involved in engulfment of dead cells (e.g., *ced-1* and *ced-6*). Finally, *nuc-1* (a nuclease) is

needed for DNA degradation to occur.⁽⁷⁾ The importance of these discoveries is highlighted by the award of the 2002's Nobel Prize for Physiology or Medicine to Sydney Brenner, Robert Horvitz and John Sulston, for their discoveries concerning the “genetic regulation of organ development and programmed cell death” in *C. elegans*. This lies in the fact that apoptosis is evolutionary conserved (Table 1)—albeit with an increase in complexity with continuing development—and that mammalian homologues of the *C. elegans* death genes have been identified. Examples of the protein homologues are: (1) CED-9—anti-apoptotic Bcl-2 family proteins, e.g., Bcl-2/Bcl-x_L, (2) CED-4—Apaf-1 and related proteins, (3) CED-3—the caspases, and (4) EGL-1—BH₃-only proteins, e.g., Bik/Bad/Bim.

During development, PCD has many functions, including sculpting of structures (i.e., digits, lumina), deleting structures (i.e., the tadpole's tail) and adjusting cell numbers.⁽⁸⁾ The latter, together with the deletion of damaged (and therefore potentially dangerous) cells, is important throughout life.

The family of Bcl-2 proteins

During lymphocyte development, these cells change their apoptotic propensity (sometimes referred to as “apoptotic phenotype”). The major determinants of the “apoptotic phenotype” in lymphocytes are the levels of expression of Bcl-2, Bcl-x_L and of Fas and/or Fas ligand (FasL).⁽⁹⁾ In general, developmental stages at which selection occurs are characterized by expression of low levels of Bcl-2 and/or Bcl-x_L, whereas stages of proliferation are characterized by high expression levels of Bcl-2 and/or Bcl-x_L.⁽¹⁰⁾

Bcl-2 and Bcl-x_L are the two most important anti-apoptotic members of the Bcl-2 family of proteins. In some B-cell lymphoma, the Bcl-2 gene is placed under the control of the

Table 1. Evolutionary conservation of apoptotic pathways

	<i>C. elegans</i>	<i>D. melanogaster</i>		Mammals	
		Intrinsic	Extrinsic	Intrinsic	Extrinsic
Apoptosis promoter	EGL-1	Debcl	Wengen/Eiger	Bax BH3-only proteins	Fas/FasL TNFR1/TNF- α DR4,5/TRAIL
Inhibitor	CED-9	Buffy (?)		Bcl-2, Bcl-x _L	FLIP
Adaptor	CED-4	Dark	dFADD	Apaf-1	FADD TRADD
Initiator caspase	CED-3	Dronc	Dredd	Caspase-9	Caspase-8
Caspase inhibitor		Diap-1	Diap-1	IAP	IAP
Inhibitor of IAP		Reaper, Grim, Hid	Reaper, Grim, Hid	Smac/Diablo Omi/HtrA2	Smac/Diablo
Effector caspase	CED-3	Dcp-1, Drice	Dcp-1, Drice	Caspase-3, caspase-7	Caspase-3, caspase-7

The basic pathways of apoptosis have been conserved throughout animal evolution, but more players have evolved with progress in evolution. Thus, in the worm, CED-3 acts as initiator and executioner caspase, whereas these functions are performed by different caspases in mammals.

immunoglobulin heavy chain enhancer, resulting in high expression levels and a transformed phenotype. Bcl-2 was the first proto-oncogene to be discovered that regulates progression through the cell cycle by delaying entry into the S-phase. Its oncogene characteristics are due to its ability to prevent apoptosis (rather than stimulate proliferation), highlighting the importance of impediment of apoptotic signaling for cancer development. It was reported to act in an antioxidant manner.⁽¹¹⁾ Like CED-9, Bcl-2 contains four so-called Bcl-2 homology domains (BH1–BH4), which are absolutely required for its survival functions. At present, three groups of the Bcl-2 family proteins can be distinguished: (1) the anti-apoptotic proteins (most of which contain a C-terminal membrane anchor and the four BH domains), like Bcl-2 and Bcl-x_L, (2) the pro-apoptotic members (which lack some of the four Bcl-2 homology [BH] domains; e.g., Bax, Bak) and (3) the BH3-only proteins (that, as the name suggests, only contain the 3rd BH domain, an amphipathic helical structure, and are all pro-apoptotic; e.g., Bad, Bik, Bid, Bim). The relative levels of pro- and anti-apoptotic proteins determine a cell's susceptibility to apoptosis (rheostat hypothesis, Ref. 12). Several members of this protein family are capable of forming death-promoting or -inhibiting homo- and/or heterodimers. Many death signals converge through BH3-only proteins at the mitochondria. We still do not fully understand the mechanism of action of the Bcl-2 family proteins; however, there is evidence for their involvement in the regulation of the formation of pores in (mitochondrial) membranes. For a recent review on Bcl-2 family proteins see, for example, Ref. 13.

Receptor-mediated apoptosis

One major pathway for the induction of apoptosis is the receptor-mediated or extrinsic pathway. The receptors triggering this pathway are located in the plasma membrane of the cell that is to undergo apoptosis and they are activated by extracellular ligands. Typical death receptors are Fas (also called Apo-1 or CD95) and tumor-necrosis factor receptor (TNF-R) 1; they belong to TNF-R family and contain a cytosolic death domain (DD). The key enzymes orchestrating and executing apoptosis are the cysteine aspartic acid proteases (caspases, see next section). The receptor-induced pathway leads to the recruitment of caspase-8 or -10 (initiator caspases, the caspases at the beginning of the cascade) to the DISC (death inducing signaling complex). The activated caspase is capable of directly activating effector caspases (also called executioner caspases), the caspases that are activated by initiator caspases and that degrade cellular targets).

T-cell activation results in expression of FasL on the activated T cell. By binding to Fas expressed on target cells, these activated T cells can kill these target cells. Furthermore, in an autocrine loop, they can activate their own Fas and commit suicide (activation-induced cell death, AICD).

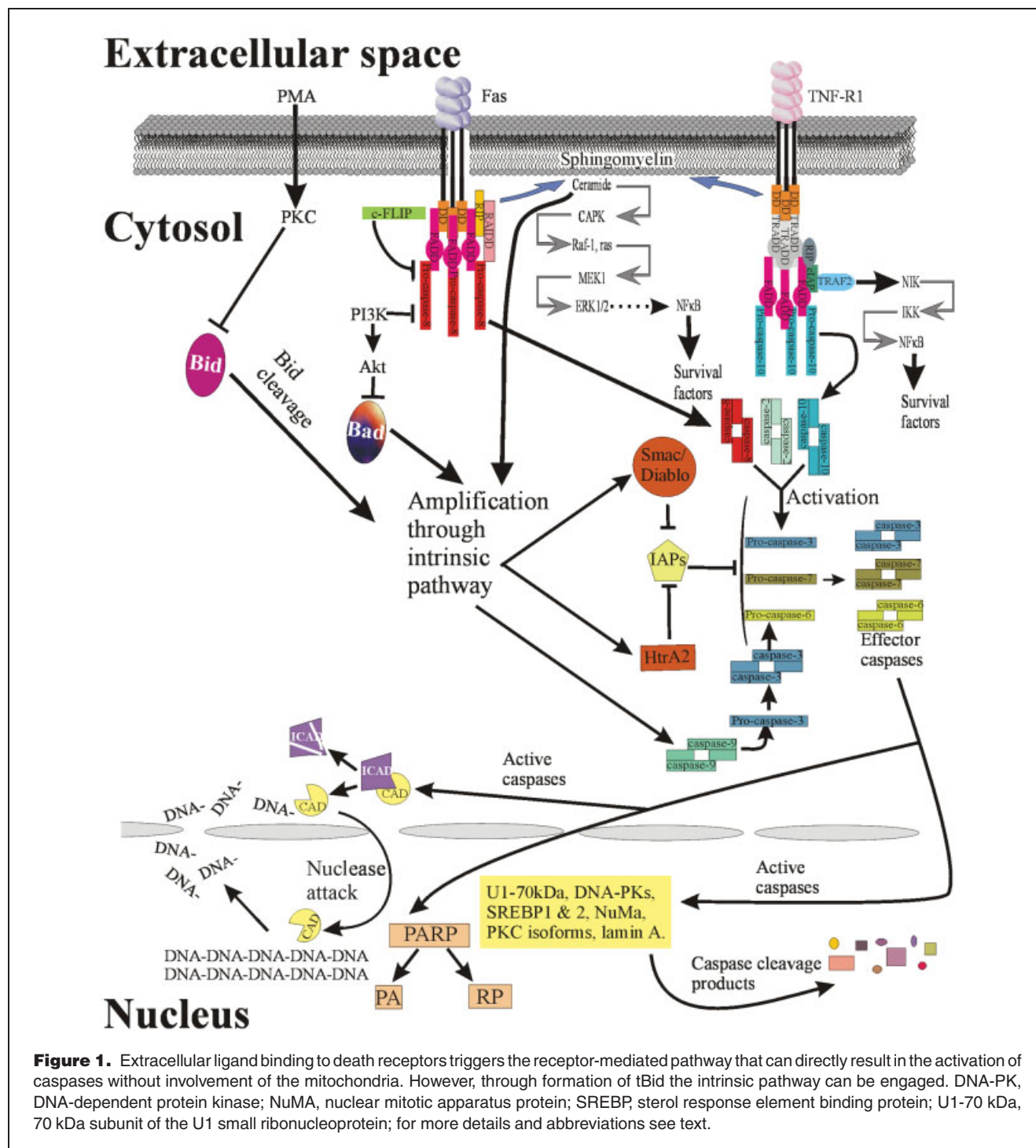
In Fas signaling, the FasL binds to Fas, leading to receptor trimerization. Adaptor proteins via their DDs (Fas-associated death domain protein, FADD) then bind to the cytosolic death domains (DD) of Fas. FADD in addition contains a death effector domain (DED), to which the DED of pro-caspase-8 can bind. The complex of Fas, FasL, FADD and pro-caspase-8 is called the DISC. The procaspase-8 molecules are brought into close proximity in the DISC, so that they can transactivate one another. Active caspase-8 then can directly cleave caspase-3 or other executioner caspases, eventually leading to the apoptotic outcome. Caspase-8 also can cleave the BH3-only protein Bid. The resulting truncated Bid (tBid) then moves to the mitochondria and induces cytochrome *c* release, leading to activation of caspase-9 and caspase-3. DISC signaling can be inhibited by expression of c-FLIP, a dominant negative caspase-8, that leads to the formation of a signaling-inactive DISC. A schematic summary of the pathway is given in Figure 1; for a recent review see, for example, Ref. 14.

Cytotoxic T lymphocytes can use, in addition to Fas signaling, the granzyme B/perforin system to kill target cells. Granzyme B (GrB, a serine protease) and perforin (a molecule capable of forming pores in intracellular membranes) are taken up by the target cell. GrB apparently can directly activate the target cell's caspases and by doing so induce apoptosis.⁽⁹⁾ GrB can also bypass caspases by directly cleaving ICAD (see section on caspases).⁽¹⁵⁾

The importance of the Fas signaling pathway is exemplified by two mutant mice, *lpr/lpr* and *gld/gld*. *lpr/lpr* (lymphoproliferation) mice are a model for lupus, an autoimmune disease in which autoantibodies to a wide range of self DNA and proteins are formed, and they lack a functional Fas. Another autoimmune diseased mouse, *gld/gld* (generalized lymphoproliferative disease) lacks functional FasL. Both mice obviously fail to appropriately remove autoreactive lymphocytes from their immune systems via Fas-induced apoptosis.⁽¹⁶⁾

Immune privileged tissues (e.g., eye, testes, brain) are tissues in which transplants are often accepted without tissue matching or immunosuppressive therapy. These tissues appear to express FasL, so it was proposed that infiltrating Fas-positive killer cells are killed before they can kill themselves. This interpretation, however, was recently challenged by data showing rapid killing by neutrophils of graft islet β -cells and tumor cells that exogenously expressed FasL and further data are needed to delineate the exact role of FasL in immune privilege.⁽¹⁷⁾

Although Fas is the archetype of a death receptor, there are other members of the TNF-receptor family that contain death domains and therefore can signal for apoptosis; many of these are not as exclusive as Fas. In addition to Fas, DR4 and DR5, CAR1, p75 and TNF-R1 all contain death domains. Death receptors (DR) 4 and 5 bind to and are activated by TRAIL (TNF-related apoptosis-inducing ligand). Through their



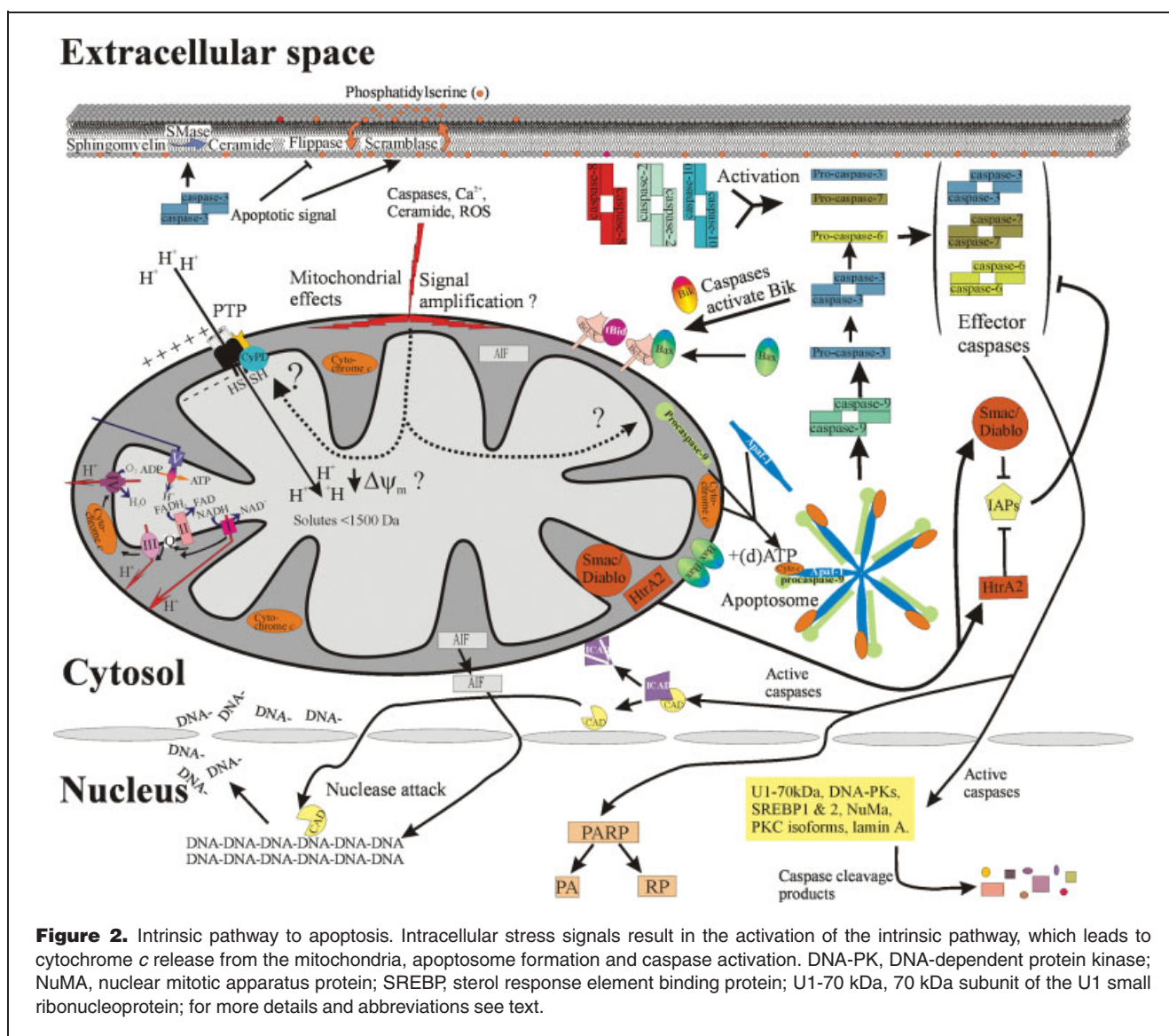
cytosolic DD, they recruit FADD and form a DISC as described for Fas. Most cells appear to express DR4 and/or DR5. Normal cells also express decoy receptors for TRAIL that will lead to the formation of signaling-inactive DISCs. However, many cancer cells appear not to express these decoy receptors for

TRAIL, making DR4 and DR5 good potential targets for anti-cancer treatments, especially since TRAIL acts independently of p53,^(14,18–21) a transcription factor and tumor suppressor gene that is mutated in many cancers. As with the mice lacking a functional Fas pathway (discussed above), TRAIL knockout

mice suffer from a much higher incidence of developing autoimmune diseases than their wild-type counterparts,⁽²²⁾ indicating that TRAIL also plays a role in the apoptotic depletion of autoreactive T lymphocytes. TNF-R 1 can signal for proliferation (in inflammation reactions) as well as apoptosis. Whether it signals for death or proliferation appears to depend on the molecules expressed in the cell that can be recruited to the DISC. The first adaptor molecule to be recruited to TNF-R1 is the TNF-R-associated death domain protein (TRADD). This can either recruit FADD, leading into signaling for apoptosis, or RIP1 and TRAF, leading into anti-apoptotic signaling (Fig. 1).^(14,23) The TNF-R family includes members that cover the entire range of signaling—from almost exclusively signaling for apoptosis (e.g., Fas) to signaling almost exclusively for survival/proliferation (e.g., TNF-R2). However, the situation

is complex as there seems to be cross-talk and transactivation.⁽²³⁾

A second messenger initially described to be essential in Fas-induced apoptosis, ceramide,⁽²⁴⁾ later was identified not to be needed for Fas signaling (however, it may constitute an amplification loop).⁽²⁵⁾ Ceramide can be formed, aside from de novo biosynthesis, by the action of sphingomylinase (SMase), which hydrolyses sphingomyelin in the plasma membrane to form ceramide. The SMase can be directly stimulated by caspase-3 (Fig. 2 and Ref. 25). Ceramide (and its soluble analogues) itself can induce apoptosis and many apoptosis-inducing drugs will increase ceramide concentrations. For example, etoposide and dexamethasone stimulate SMase and daunorubicin appears to stimulate both SMase and de novo biosynthesis.^(26,27)



Caspases

Induction of apoptosis can occur by external or internal stimuli. Two major general pathways of induction of apoptosis exist: the receptor or *extrinsic* pathway (described in the previous section) and the mitochondrial or *intrinsic* pathway (next section). Both apoptotic signaling pathways converge at the level of the specific proteases—the caspases (Figs. 1 and 2). There are 14 mammalian caspases identified to date.⁽²⁸⁾ They are synthesized as pro-enzymes, which usually undergo proteolysis and activation by other caspases in a cascade.⁽²⁹⁾ Peptide caspase inhibitors can inhibit downstream caspase activation and subsequently apoptosis.⁽³⁰⁾ Caspases can be grouped into subclasses in various ways. Functionally, we can distinguish three classes of caspases; (i) the initiator caspases that are characterized by long prodomains (>90 amino acids) containing either DED domains (caspase-8 and caspase-10) or a caspase recruitment domain (CARD) (caspase-2 and caspase-9; CED-3); (ii) the executioner or effector caspases containing short prodomains (caspase-3, caspase-6 and caspase-7) and (iii) the remaining caspases whose main role lies in cytokine maturation rather than apoptosis.⁽³¹⁾ Upon activation, the prodomains are cleaved off and the large and small subunits are separated by caspase action (all cleavages occur after Asp residues). The active site is formed by the interface of the two subunits by 1 Arg, 1 His, 1 Cys of the large subunit and 1 Arg of the small subunit. The active caspases form heterotetramers.⁽³¹⁾ Initiator caspases cleave and activate effector caspases. These then cleave cellular substrates, which leads to all phenomena of the apoptotic morphology. One effector caspase, caspase-3, when activated is capable of cleaving many important cellular substrates, including ICAD (inhibitor of caspase-activated DNase), ROCK1 (Rho-associated coiled-coil forming kinase I), poly(ADP-ribose)polymerase (PARP, a DNA repair enzyme), actin, fodrin and lamin. Active caspase-3 can cause membrane blebbing (via ROCK1 cleavage that leaves the kinase constitutively active, permanently phosphorylating myosin light chain, Refs., 32–34), disassembly of the cell structure and DNA fragmentation [via ICAD (inhibitor of caspase-activated DNase) cleavage that sets CAD (caspase-activated DNase) free to move into the nucleus and cleave DNA internucleosomally, Ref. 35], which eventually lead to cell death.

Several cellular and viral proteins act as caspase inhibitors. For example, cells contain inhibitor of apoptosis proteins (IAPs) that can inhibit activated caspases. Neuronal cells typically contain such proteins (neuronal apoptosis inhibitory protein, NAIP) to protect them from premature apoptosis. Thus NAIP expression appears to protect neurons from dying in some cases of Parkinson's disease.⁽³⁶⁾ Many viruses also contain viral IAPs, viral anti-apoptotic Bcl-2 proteins or other inhibitors of apoptosis in order to prevent infected cells from dying.⁽³⁷⁾ A very powerful pan-caspase inhibitor is the

baculoviral p35 protein, nicely exemplified by three movies on-line available as supplementary material to Ref. 38.

Structurally, caspases follow the caspase fold. This structural motif consists of the large (α) and the small (β) subunit. Each α/β heterodimer comprises six β strands (a to f), of which strands a to e are parallel, whereas strand f is antiparallel. This six-stranded β -sheet forms a twisted structure around five α -helices (H1 to H5). The structure of caspase-1, caspase-3, caspase-7, caspase-8 and caspase-9 have been solved.⁽³⁹⁾ Three groups can be distinguished, each of which has different substrate specificities due to three loop regions close to the active site that differ from group to group. The consensus sequences are WEHD for group I (caspase-1, caspase-4, caspase-5 and caspase-14), DEXD for group II (caspase-2, caspase-3 and caspase-7) and (I/V/L)EXD for group III (caspase-6, caspase-8, caspase-9 and caspase-10), where X stands for any amino acid.^(31,40)

The intrinsic pathway

The mitochondrial pathway is activated by a variety of extra- and intracellular stresses, including oxidative stress and treatment with cytotoxic drugs. The apoptotic signal leads to the release of cytochrome *c* from the mitochondrial intermembrane space into the cytosol, where it binds to the Apoptotic Protease Activating Factor-1 (Apaf-1), a mammalian CED-4 homologue. Early data suggested early loss of mitochondrial membrane potential and the opening of the mitochondrial permeability pore to be necessary steps for cytochrome *c* release. However, recent data seem to suggest that both events are not needed for apoptotic cytochrome *c* release in all instances.^(41,42) Binding of cytochrome *c* to Apaf-1 triggers the formation of the apoptosome, an ~ 1 MDa oligomeric, Apaf-1-containing complex that catalyses activation of caspases. It contains seven Apaf-1, seven cytochrome *c*, seven (d)ATP and seven procaspase-9 molecules. Procaspase-9 is the initiator caspase of the apoptosome.⁽⁴¹⁾ The apoptosome-bound procaspase-9 is activated and can then activate an effector caspase (e.g., caspase-3), which then can cleave the cellular substrates needed for the orchestration of apoptosis (see the previous section and Fig. 2). The apoptosome structure has been solved by low-resolution cryo-electron microscopy: it forms a “wheel of death”, with a seven-fold symmetry. As caspase-9 appears to only contain one active site per tetramer and as caspase-9 activity appears to be three orders of magnitude higher when bound to the apoptosome, one hypothesis is that the seven molecules of pro-caspase-9 will recruit and activate seven molecules of caspase-9 to the apoptosome to form the caspase-9 holoenzyme.^(43–45)

Brains of Apaf-1, caspase-9, and caspase-3 mutant mice demonstrate forebrain extrusions, reflecting the loss of the capacity to appropriately delete cells and demonstrating the importance of apoptosis during brain development.⁽⁴⁶⁾ Proteins of the intrinsic pathway can be upregulated or

downregulated by oncogenes,⁽⁴⁷⁾ e.g., many melanoma express very low levels of Apaf-1.⁽⁴⁸⁾

Upon activation of the intrinsic pathway, a range of pro-apoptotic molecules in addition to cytochrome *c* is released from the mitochondria.⁽⁴⁹⁾ One such molecule is Smac/Diablo, an inhibitor of cellular IAPs.^(50–53) Mitochondria can also release an apoptosis-inducing factor, AIF, which appears to induce an apoptosis-like cell death that is independent of caspases.^(54,55)

Two major questions about the intrinsic pathway remain unanswered: are mitochondria *central* to the intrinsic pathway of apoptosis?⁽⁵⁶⁾ and what forms the cytochrome *c* (and other pro-apoptotic proteins)-releasing pore?⁽⁵⁷⁾ There is increasing evidence that the release of cytochrome *c* itself may be (directly or indirectly) stimulated by caspase activation (e.g., for example, Ref. 58 and references discussed therein) and may therefore constitute an amplification loop rather than a trigger for caspase activation—similar to the situation in the extrinsic pathway. There have been many molecules suggested to be involved in forming the pore that releases cytochrome *c*, including Bax, VDAC, the mitochondrial permeability transition pore PTP, Bax/VDAC, lipids or something else as yet unidentified. Perhaps the strongest data are available for the involvement of Bax and/or VDAC in the formation of the pore-releasing cytochrome *c*.⁽⁵⁹⁾

Apoptosis and proliferation

Apoptosis can result from perturbations of the cell cycle. Signaling molecules cover a continuum from proliferation to death. Many genes involved in cell cycle regulation are also involved in regulation of apoptosis (e.g., *c-myc*, *c-fos*, *c-jun*, p53, many kinases and phosphatases).⁽⁶⁰⁾ Thus, signals that promote proliferation can also promote apoptosis. If apoptosis is blocked by survival signals, increase in cell numbers occurs, which can manifest in cancer.⁽⁴⁵⁾ However, many neurons undergo PCD as postmitotic neurons—in these, factor deprivation appears to be the signal for apoptosis.⁽⁶¹⁾ T cell receptor signaling leads to proliferation and—with some delay—to AICD.⁽⁶²⁾ Often the meaning of a signal has to be specified by a second signal, for example *c-myc* plus *bcl-2* leads to proliferation, *c-myc* plus p53 leads to apoptosis.⁽⁶³⁾ p53 is a key element in apoptosis induction in cells in response to DNA damage. p53 is inhibited by MDM2 (murine double minute 2), a ubiquitin ligase that targets p53 for destruction by the proteasome. MDM2 is inactivated by binding to ARF (alternative reading frame). Cellular stress, including that induced by chemotherapy or irradiation, activates p53 either directly, by inhibition of MDM2 (including cleavage by caspases), or indirectly by activation of ARF.^(64–66) ARF can also be induced by proliferative oncogenes such as RAS. Active p53 transactivates pro-apoptotic genes—including Bax, Noxa, CD95 and DR4—to promote apoptosis. p53 can also move directly to the mitochondria where it exerts pro-

apoptotic activity.⁽⁶⁶⁾ Akt is a major kinase involved in anti-apoptotic signaling, Akt knockout mice show enhanced of spontaneous apoptosis.⁽⁶⁷⁾ One important substrate of Akt is the BH3-only protein Bad, which upon phosphorylation binds to 14-3-3 proteins. These sequester it to the cytoplasm, preventing it from translocating to the mitochondrion where it exerts its pro-apoptotic action. Furthermore, Akt phosphorylates forkhead transcription factors, which, again by binding to 14-3-3 proteins, become excluded from the nucleus and cannot initiate transcription of proapoptotic genes like FasL and the BH3 only protein Bim. Through phosphorylation of the cAMP-response element binding protein (CREB) and activation of NF- κ B, Akt can also initiate the transcription of anti-apoptotic genes like Bcl-2 and IAP.^(68,69)

Phagocytosis of apoptotic bodies

In vivo, apoptotic bodies are rapidly cleared away by phagocytosis, either by professional phagocytotic cells or simply by cells in the direct vicinity of the apoptotic bodies. A phosphatidylserine (PS) receptor is involved in phagocytosis of apoptotic cells.⁽⁷⁰⁾ Usually, PS is maintained at the inner layer of the plasma membrane (PM) by the action of an ATP-dependent PS flippase. This flippase is inactivated by caspases, and a scramblase is activated, leading to a redistribution of PS to the outer leaflet of the PM (Fig. 2).⁽⁷¹⁾ PS externalisation is widely used to analyse apoptosis in FACS analysis due to PS binding to annexin V.⁽⁷²⁾ A range of cell surface molecules (e.g., thrombospondin 1 and its receptor, CD36, involved in recognition of the apoptotic bodies) and intracellular molecules (e.g., the DOCK180 [180 kDa protein downstream of CRK]/CRKII/RAC complex, involved in signaling in the engulfing cell) has been identified as being involved in the phagocytosis of mammalian apoptotic bodies and many of them are homologues of the *C. elegans* genes involved in dead cell removal in the worm.^(73–76) Phagocytosis of dead cells appears to have a different meaning beyond that of waste disposal depending on the form of death that the cell underwent. Whereas phagocytosis of oncotic corpses by dendritic cells leads to inflammation and immune response, phagocytosis of apoptotic bodies results in the release of anti-inflammatory cytokines and immune tolerance.^(74,77)

Conclusion and outlook

During the past decade research into the mechanisms of apoptosis has made immense progress. Tens of thousands of papers exist on the topic and, as the field has expanded enormously, it is difficult to find recent general overviews. Being aware of the necessary shortfalls of the present attempt to give such an overview (i.e., omissions [e.g., caspase-independent apoptosis and autophagy] and the restriction on mainly reviews on the various subtopics; see also Ref. 78), I tried to give a brief introduction into this exciting field of recent biomedical research. Though many details have been intrically

described during the past years, there is still a plethora of open questions that will allow many researchers exciting times in their laboratories. A summary of most of the apoptotic pathways mentioned in this review is given in Figures 1 and 2.

Acknowledgments

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