Caspases – Their Role in Apoptosis and Other Physiological Processes as Revealed by Knock-Out Studies

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Abstract. Caspases are crucial mediators of apoptosis, a form of physiological cell death. Their activation is carefully controlled by a philogenetically conserved death program, which is indispensable for the homeostasis and development of higher organisms. Dysregulation of apoptosis contributes to the pathogenesis of many human diseases. As effectors of the apoptotic machinery, caspases are considered potential therapeutic targets. In vitro studies have demonstrated the requirement of caspase activity for both the triggering phase as well as the execution of apoptosis, thus providing a molecular base for the fine-tuning of this process by pharmacological agents. The precise roles of the individual caspases in vivo and their functional relation to each other have been best demonstrated in genetically modified animals. The generation of single caspase-deficient mice have confirmed most of the data obtained in vitro and exposed some new aspects previously undetected in the cell culture system. Interestingly, inactivation of many caspases revealed not only their expected participation in apoptotic events as well as in the maturation of cytokines, but also provided hints about the role of at least some caspases in cell differentiation and stimulatory responses. In this review we will discuss what these studies have unveiled about the role of individual caspases in development, apoptosis, and inflammation, with particular focus on their role beyond the apoptotic process.

Key words: caspase; cell death; development; cell cycle; inflammation.

Introduction

Apoptosis is the main mechanism by which multicellular organisms eliminate unwanted cells to ensure proper development and maintain cellular homeostasis. Several human pathologies are associated with dysregulation of programmed cell death. This includes degenerative and autoimmune diseases, neoplasia, AIDS, and other viral or bacterial infections21, 54, 92. Pioneering studies of developmental cell death, which significantly broadened our understanding of apoptosis, have been done on the nematode Caenorhabditis elegans, in which a set of genes, termed Ced for cell death-defective, regulates the apoptotic machinery16, 29. Two of these genes, ced-3 and ced-4, are required for the execution of cell death. Another gene, ced-9, protects

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cells from undergoing apoptosis. The conservation of a death pathway through evolution became evident upon the discovery that *ced-3* and *ced-9* encode proteins homologous to the known mammalian proteins interleukin 1 β converting enzyme (ICE) and Bcl-2, respectively. With the expected greater complexity in mammals, other mammalian homologues of *ced-3* and *ced-9* were subsequently discovered, forming the *ced-3* caspase and Bcl-2 protein family. 3, 30, 75, 104 Ced-4 is closely related to an apoptosis regulator in mammals, designated Apaf-1 for *apoptotic protease-activating factor* 1.122

Mammalian caspases (cysteiny1 aspartases), Casp, compose a group of at least 14 members.57, 71, 90 All caspases show remarkable specificity for aspartate residue at the (P1) cleavage site of their substrates. Although they exhibit at least partial specificity for other aminocoids surrounding the cleavage site, with the second-most important residue the P4 position, the overall secondary structure of the target protein appears to be important, too. Most caspases participate in apoptosis by proteolytic activation of other caspases and by chopping cellular proteins, thus contributing to the advancement of cell death. Some caspases generate mature proinflammatory cytokines and, thereby, regulate immune responses. Yet, it becomes increasingly evident that caspases and apoptosis-regulatory molecules exert important functions beyond that of cell death, including the control of T cell proliferation and cell-cycle progression (reviewed in59).

Caspases are synthesized aszymogens and are built according to the same scheme. An N-terminal regulatory prodomain of various length is followed by two subunits, p20 and p10, with the catalytic domain localized in p20. The core of the catalytic center is formed by the conserved pentapeptide sequence QACXG. The proforms of caspases are activated by proteolytic cleavage at specific aspartate residues. Generally, an initial cleavage event separates the C-terminal short subunit from the rest of the molecule, allowing assembly of an active protease that autocatalytically cleaves off its prodomain. As revealed by the crystal structures of Casp-1, -3, -7 and -9, the mature enzyme exists as a heterotetrameric complex composed of two large (p20) and two small (p10) subunits, which form a pair of independent active centres.5, 62, 78, 98, 100 Once activated, some caspases can propagate the activation of other family members and, thus, initiate and form a proteolytic apoptotic cascade (reviewed in14). Proteolysis as a signal transduction event assures the irreversibility of the process if it occurs on a large scale, since the cleaved molecules can be restored only by *de novo* synthesis.

According to their structure and function, caspases can be divided into initiator and effector subfamilies. Effector caspases contain only a small prodomain and cleave diverse cellular substrates, whereas initiator caspases have a long prodomain and exert a regulatory role by activating downstream effector caspases. However, Casp-14 cannot be clearly classified into any of these subfamilies15, 53. Although its role may be assigned to “maturating cell the death” of keratinocytes, an apoptosis-unrelated physiological form of cell death which occurs when the upper, cornified layers of cutaneous epithelium are formed, this protease has failed to become activated by a number of typical apoptotic stimuli, which puts its role in classical apoptosis in question.

Activation of initiator caspases is mediated by the binding of adapter molecules to protein interaction motifs in their prodomains. Two general types of interaction have been identified.58, 60, 66, 80. Pro-Casp-8 and -10 each contains two tandem death effector domains (DED), while pro-Casp-1, -2, -4, and -9 contain a caspase-recruitment domain (CARD). Ligation of death receptors such as CD95 and tumor necrosis factor receptor 1 (TNF-R1) facilitates the oligomerization of adapter proteins which provide a spatial framework for the binding and reciprocal proteolytic activation of pro-caspases (Fig. 1). For example, Fas-associated death domain (FADD) protein, which contains a CARD, is recruited directly to CD95, TRAIL-R1, -R2 and indirectly to TNF-R1, resulting in the autoactivation of Casp-8, reviewed in 59, 85. Analogously, the adapter protein RAIDD/CRADD, which contains a CARD, can associate with TNF-R1 and promote activation of pro-Casp-2, 14, 46. Besides death receptor-mediated apoptosis, a related but different way of caspase activation exists which is triggered by cytotoxic drugs, by p53, or by some other stimuli inducing cellular stress, and it is essentially controlled by mitochondria. In the early phases of apoptosis, mitochondria release cytochrome c, which, together with dATP, binds to and activates Apaf-1.31 This event facilitates CARD-CARD interaction between Apaf-1 and pro-Casp-9, while a different region of Apaf-1 self-associates, thus resulting in Casp-9 activation (Fig. 1). Apaf-1, (pro)Casp-9, and dATP form the core of a large protein complex known as the apoposome. But a concurrent, Apaf-1-independent, mitochondrial death pathway exists which probably does not rely on caspases and which may cooperate with the apoposome in cell-death propagation. It is triggered by the release of *apoptosis-inducing factor* (AIF) a mitochondrial flavoprotein localized in the intermembrane space. Recombinant AIF is capable of
evoking chromatin condensation and large-scale fragmentation of DNA in isolated HeLa cell nuclei and of inducing the release of cytochrome c and pro-Casp-9 from purified mitochondria. When microinjected cytoplasmically, it causes externalisation of phosphatidylserine, one of the hallmarks of apoptosis[1].

Although caspases are the essential components of major physiological death pathways, the precise roles of the individual family members remain controversial. Their overlapping tissue expression pattern and similar cleavage sequence specificity raises questions about the compensation mechanisms and/or unique functions of individual caspases in vivo. Some family members, such as Casp-14 and, to a certain extent, Casp-1 and -11, seem to play a negligible role in apoptosis, if at all.

Recent studies using gene targeting and transgene technologies have helped to solve some questions, thus shedding new light on the distinct roles of individual caspases in cell death as well as in other biological processes. The “knock-out technology”, despite its value in defining the biological importance of targeted molecules, tends to underestimate the significance of the deleted genes, especially in a redundant biological context. Indeed, it has been shown recently[108] that cells can well counterbalance the loss of one caspase by compensatory activation of other family members, at least in some experimental systems. Thus, knocking out more than one caspase at a time will be of advantage in defining the roles of these proteases in various biological processes, while pointing out redundancies.

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**Fig. 1.** The principal cell death signaling pathways. The two main apoptotic pathways are indicated. Ligation of a death receptor results in the recruitment of the adapter protein FADD and pro-Casp-8, which becomes cleaved and activated at the receptor complex, initiating caspase cascade. The mitochondria/apoptosome pathway (right) is triggered by a number of apoptotic stimuli. An early, not well understood step is the mitochondrial release of apoptosis-inducing molecules (incl. cytochrome c and AIF) into the cytosol. Initially, cytochrome c, together with dATP, associates with Apaf-1. This event unmask the CARD motif in Apaf-1 and allows binding and activation of pro-Casp-9. Once activated, Casp-9 propagates the apoptotic signal. Please note the positive feedback loops marked A, B, and C. Loop A involves Bid, mitochondria, apoptosome, and Casp-9, -3, -6, and -8. Loop B consists of Casp-8, -3, and -6; and, finally, loop C is composed of Casp-9, -3, and -7. These positive feedback mechanisms assure amplification of apoptotic signals. The apoptosome pathway is further potentiated by AIF through augmentation of the mitochondrial release of cytochrome c and pro-Casp-9.
Casp-1 and Casp-11 – Their Role in the Immune Response as Unveiled by Knock-Out Mice

**Casp-1 knock-out**

In the beginning of the 90’s, Casp-1 was originally identified as ICE, the protease which proteolytically activates pro-IL-1β\(^8\). More recently, Casp-1 has also been shown to process the cytokine precursors of IL-6 and IL-18 and to be involved in the secretion of IL-1α, IL-4, and TNF-α\(^9,25,26,41\). Since the demonstration that Ced-3 of *C. elegans* encodes a protein similar to mammalian Casp-1\(^3\) and that overexpression of both genes promotes apoptosis\(^56,63\), Casp-1 has been an object of intensive research.

The Casp-1 gene was the first of the caspase family to be genetically inactivated. Contrary to most other caspase-targeted mice, the Casp-1\(^–/–\) animals developed normally. The knock-out experiments revealed that Casp-1 plays an important role in the regulation of the immune response, but is presumably dispensable for most apoptotic pathways\(^41,49\). Casp-1\(^–/–\) mice had a major defect in the production of mature IL-1β and impaired IL-1α synthesis (Table 1). Secretion of TNF-α and IL-6 in response to lipopolysaccharide (LPS) stimulation was also diminished. In addition, macrophages from Casp-1\(^–/–\) mice were defective in LPS-induced interferon γ (IFN-γ) production\(^19\) and they were highly resistant to the lethal effects of endotoxin\(^50\). The pro-inflammatory role of Casp-1 was supported by the finding that pharmacological blockade or genetic deletion of Casp-1 decreased necrosis, edema formation, and serum levels of amylase and lipase during experimentally induced pancreatitis\(^22\), which was associated with dramatic survival benefits.

In contrast to inflammatory responses, Casp-1-null mice did not show any obvious abnormalities in development or grave defects in apoptosis. The thymocytes from young Casp-1\(^–/–\) mice equally underwent apoptosis when triggered by dexamethasone or γ-irradiation. However, these thymocytes revealed a partial resistance towards CD95-induced apoptosis\(^41\). The knock-out studies also indicated that Casp-1 partially mediates apoptosis induced by IFN-γ. In this scenario, a positive amplification loop between IFN-γ and Casp-1 seems to exist. IFN-γ is able to induce Casp-1 expression through activation of the signal transducer and activator of transcription (STAT) signaling pathway\(^10\). IFN-γ did not induce Casp-1 expression and apoptosis in STAT1\(^–/–\)-deficient cells. Therefore, activation of the STAT pathway may trigger apoptosis in some systems through the induction of Casp-1 expression. Casp-1 seems to play a role in some forms of neuronal cell death. The mutation of Casp-1 inhibited apoptosis caused by the withdrawal of trophic factor in dorsal root ganglial cells\(^23\). Also, brain injury caused by middle cerebral artery occlusion, a mouse model of stroke, was significantly reduced in Casp-1-mutant and -deficient mice\(^23,82\). In addition, expression of mutant Casp-1 in neurons of superoxide dismutase-deficient mice, a model of amyotrophic lateral sclerosis, delayed disease progression\(^22\).

**Casp-11 knock-out**

Murine Casp-11 (Ich-3) is the homologue of human Casp-5. Casp-11 transcription and translation is strongly induced upon stimulation with LPS. Overexpression of Casp-11 induced apoptosis, which could be inhibited by CrmA and Bel-2\(^95\). Casp-11-deficient animals developed normally and were resistant to endotoxic shock, as were Casp-1\(^–/–\) mice. Moreover, after middle cerebral artery occlusion, a mouse model of stroke, fewer apoptotic cells and a defect in Casp-3 activation was observed in Casp-11\(^–/–\) mice. Recombinant pro-Casp-11 can autoactivate itself *in vitro*. Furthermore, it is a very efficient activator of pro-Casp-3. These features suggest that Casp-11 may act as an apical/initiator caspase like Casp-8, or -9, at least under certain conditions\(^34\). Following LPS stimulation, production of both IL-1α and IL-1β was blocked in Casp-11\(^–/–\) mice. Casp-11-deficient cells were resistant to apoptosis induced by Casp-1 overexpression, suggesting that activation of Casp-1 requires Casp-11. Based on the striking phenotype similarity between Casp-1\(^–/–\) and Casp-11\(^–/–\), it has been proposed that Casp-1 is activated by a direct physical interaction with murine Casp-11\(^95\). However, as Casp-11 does not directly cleave either pro-Casp-1 or pro-IL-1β *in vitro*, it is likely that a yet to be discovered adapter/chaperon molecule may be required to assist this process in the cell. Thus, Casp-1 and Casp-11 seem to play an important role in inflammation by activating cytokines, but may not mediate apoptosis in development and in most other circumstances.

**Casp-2 knock-out**

Casp-2 (Ich-1, Nedd2) was first identified as a gene highly expressed in embryonic brain, but reduced in the adult nervous system\(^44\). It has been shown to interact with RAIDD, a component of the TNF-receptor signalling machinery\(^1,14\), presumably participating in TNF-signalling. Overexpression of Casp-2 in mammalian cells induced apoptosis that could be blocked by
### Table 1. Phenotypes of caspase-, FADD-, cytochrome c-, AIF- and Apaf-1-deficient mice

<table>
<thead>
<tr>
<th>Caspase</th>
<th>Development and cytokine expression</th>
<th>Apoptosis</th>
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<tbody>
<tr>
<td>Casp-1</td>
<td>No developmental defects;</td>
<td>Sensitive to most apoptotic inducers;</td>
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<td></td>
<td>Resistant to LPS-induced septic shock;</td>
<td>Reduced ischemic brain injury;</td>
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<td></td>
<td>Increased survival in experimental pancreatitis;</td>
<td>CD95-induced apoptosis attenuated in thymocytes;</td>
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<td></td>
<td>No IL-1β and IL-18 processing, impaired production of IL-1α, IL-6, TNF-α and IFN-γ</td>
<td>Neurons resistant to trophic factor withdrawal</td>
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<tr>
<td>Casp-2</td>
<td>Viable, no marked abnormalities;</td>
<td>Oocytes resistant to drug-induced death;</td>
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<td></td>
<td>Excess numbers of female germ cells</td>
<td>Defective B cell death in response to granzyme B;</td>
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<td></td>
<td></td>
<td>Lymphocytes sensitive to drugs and anti-CD95;</td>
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<td></td>
<td></td>
<td>Increased susceptibility of sympathetic neurons to trophic factor withdrawal</td>
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<td>Casp-3</td>
<td>Mice born at lower frequency and with smaller size;</td>
<td>No membrane blebbing and nuclear fragmentation in hepatocytes and thymocytes;</td>
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<td></td>
<td>Death at 1–3 weeks of age;</td>
<td>Cleavage of caspase substrates delayed or absent;</td>
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<td></td>
<td>Disturbed brain development with excessive numbers of postmitotic cells</td>
<td>Reduced apoptosis in diverse settings including activation-induced T cell death and drug-induced apoptosis of fibroblasts</td>
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<tr>
<td>*Casp-6</td>
<td>Normal development, possible defects in B cell development</td>
<td>No data</td>
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<tr>
<td>*Casp-7</td>
<td>Lethality during early development</td>
<td>No data</td>
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<tr>
<td>Casp-8</td>
<td>Lethal in utero; embryos of smaller size;</td>
<td>Fibroblasts resistant to TNF-RI, CD95, and DR3 but sensitive to drug-induced apoptosis;</td>
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<td></td>
<td>Impaired heart muscle development;</td>
<td>Normal JNK and NF-xB activation</td>
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<td></td>
<td>Congested accumulation of erythrocytes and massive hemorrhage;</td>
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<td></td>
<td>Decreased number of hematopoietic stem cells</td>
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<tr>
<td>Casp-9</td>
<td>Perinatal lethality;</td>
<td>Embryonic stem cells and fibroblasts resistant to several apoptotic stimuli;</td>
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<td>Enlarged and malformed cerebral due to reduced apoptosis during brain development;</td>
<td>Thymocytes resistant to dexamethasone- and γ-irradiation-induced apoptosis, but sensitive to UV-irradiation and CD95;</td>
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<td></td>
<td>Lack of Casp-3 activation in embryonic brains</td>
<td>Splenocytes not protected against drug-induced apoptosis</td>
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<td>Casp-11</td>
<td>No developmental defects; similar to Casp-1 deficiency;</td>
<td>Cells resistant to apoptosis induced by overexpression of Casp-1</td>
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<td></td>
<td>Resistant to endotoxic shock;</td>
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<td></td>
<td>Lack of IL-1α and IL-1β production due to blocked Casp-1 activation</td>
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<tr>
<td>Casp-12</td>
<td>No developmental abnormalities</td>
<td>Resistance to ER/Golgi-stress (tunicamycin, thapsigargin, brefeldin A)-induced apoptosis;</td>
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<td>Normal response to death receptor-, or apoptosome-dependent death stimuli;</td>
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<td>Reduced renal toxicity upon intraperitoneal injection of tunicamycin;</td>
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<td></td>
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<td>Partial resistance of cortical neurons to amyloid-β-, or excitotoxicity-induced death, normal response to other apoptotic stimuli such as staurosporine or trophic factor deprivation</td>
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<tr>
<td>FADD</td>
<td>Cardiac failure and massive hemorrhage;</td>
<td>Fibroblasts resistant to death receptor- but sensitive to drug, E1A- and c-Myc-induced apoptosis;</td>
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<td></td>
<td>Phenotype similar to Casp-8-/- mice;</td>
<td>Thymocytes of RAG-1 chimeras show impaired survival and proliferation</td>
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<td>IL-2 expression of thymocytes intact</td>
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<td>Apaf-1</td>
<td>Lethal at E16.5;</td>
<td>Embryonic fibroblasts exhibit reduced response to various apoptotic stimuli;</td>
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<td></td>
<td>Brain overgrowth, exencephaly;</td>
<td>Thymocytes sensitive to CD95-, but resistant to drug- and irradiation-induced apoptosis</td>
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<td>Severe craniofacial and ossification defects;</td>
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<td></td>
<td>Strong alterations of the lens and retina;</td>
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<td></td>
<td>Persistence of interdigital webs</td>
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<tr>
<td>Cytochrome c</td>
<td>Death in utero during mid-gestation – no obvious developmental abnormalities</td>
<td>Resistance to death induced by UV-irradiation or proapoptotic drugs such as staurosporine, partial resistance to serum withdrawal-induced apoptosis</td>
</tr>
<tr>
<td>AIF</td>
<td>Very early developmental cell death (during blastulation)</td>
<td>Embryonic stem cells resistant to cell death after serum deprivation, essential role in programmed cell death during cavitation of embryoid bodies, the first wave of cell death crucial for early morphogenesis</td>
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* The phenotypes of Casp-6 and -7 knock-out mice still await publication; the preliminary data was obtained from Zheng and Flavell.107
M. Bcl-2. Antisense inhibition of Casp-2 expression delayed cell death triggered by trophic factor deprivation in hematopoietic and neuronal cell lines. Casp-2−/− mice developed and grew normally. The phenotype has become obvious upon careful histological examination. Casp-2 appears to be required for female germ-cell death and it seems to play a protective role (!) in the brain. Over one-half of ovarian germ cells undergo apoptosis at the later stages of fetal life, the remaining ones persisting through day 3 postpartum. At that time the “surviving” oocytes are enclosed by granulosa cells to form primordial follicles. Casp-2-deficient mice contained significantly higher numbers of newly formed primordial follicles than wild-type littermates. Oocytes of Casp-2−/− mice were also resistant to cell death following exposure to some chemotherapeutic drugs.

In contrast, cell death of facial motor neurons was not suppressed, but was even accelerated in Casp-2-deficient mice. Similarly, Casp-2-deficient sympathetic neurons were more sensitive to nerve growth-factor deprivation. However, in other apoptotic models no significant role for Casp-2 in neuronal cell death could be observed. The pro-versus anti-apoptotic effect of Casp-2 may be explained by the existence of splice variants, which are differentially expressed in a tissue- and developmental stage-dependent manner. Alternative splicing generates two Casp-2 mRNA, encoding either proapoptotic Casp-2α or a truncated protein Casp-2β, which antagonizes cell death.

B lymphoblasts from Casp-2-deficient mice failed to undergo apoptosis upon death induction by granzyme B, but not CD95. In contrast, thymocytes and T lymphocytes were equally sensitive to these stimuli and also to apoptosis induction by doxorubicin, etoposide, staurosporine, or γ-irradiation. Unexpectedly, no differences in TNF-induced apoptosis were observed in Casp-2-deficient embryonic fibroblasts, despite Casp-2 having previously been shown to physically interact with RAIDD, a component of the TNF-receptor signaling machinery. The death domain (DD) of RAIDD interacts with receptor-interacting protein (RIP), a DD-containing serine/threonine kinase that is part of the TNF death pathway. Thus, the results obtained with Casp-2-mutant mice suggest that Casp-2 and RAIDD may be redundant in TNF signaling. In conclusion, Casp-2 may be essential for apoptosis in female germ cells, whereas in some other systems it may function to delay cell death. The ultimate action of Casp-2 appears to be dependent on the expression level of its isoforms and the abundance of other caspases. So, for instance, genetic inactivation of Casp-9 leads to strong compensatory activation of Casp-2 following death receptor triggering.

The Effect of Apoptosis as revealed by Casp-3, -6, and -7-Deficient Mice

Casp-3 knock-out

Casp-3, (CPP32, YAMA, Mch2, apopain), a prototype effector caspase able to degrade a number of “death substrates”, is probably the most extensively studied member of the family of proteases. Its three-dimensional structure resembles Casp-1 in general, but its S4 substrate residue (accommodating the fourth amino acid’s N-terminal of the cleavage site) is different. This variance evokes the distinct substrate specificity of the two proteases and may enable the design of selective inhibitors capable of separately targeting either cytokine maturation or apoptosis.

Casp-3−/− mice were historically the first to harbour significant defects in apoptosis. Interestingly, the mice were born at a lower than expected frequency, they were smaller than their littermates and died within the first weeks after birth. The defects were seen exclusively in the central nervous system, while no discernible abnormalities could be found in other organs, such as heart, lung, liver, or kidney. Casp-3-deficient animals showed massive hyperplasia and ectopic tissue masses in the brain, with a variety of disorganised histologic structures. For example, compression of the eye lens, caused by neuroepithelial tissue protruding into the retina, could be frequently observed. In general, the defects in the brain were similar, but less pronounced than in Casp-9−/− mice. This indicates that Casp-3 plays a critical role during morphogenetic cell death in the mammalian brain, where it depends on Casp-9 for activation.

Careful examination of other tissues revealed more discrete, but still expected apoptotic defects. Casp-3−/− hepatocytes or thymocytes were killed at a similar rate as control cells upon death induction by CD95, but neither membrane blebbing nor DNA fragmentation were detected even after 6 h of observation. The cleavage of the majority of caspase substrates, including gelatin, fodrin, lamin B, and DFF/ICAD, an endonuclease inhibitor, was delayed or absent in Casp-3−/− cells. These findings may explain the lack of typical apoptotic morphology, frequently observed in Casp-3−/− cells. Unexpectedly, thymocytes from Casp-3−/− and wild-type mice were equally sensitive to induction of apoptosis by anti-CD95, dexamethasone, ceramide, staurosporine, and γ-irradiation. In contrast, peripheral T cells were resistant to activation-induced cell death and apoptosis triggered by anti-CD3 and anti-CD95. In embryonic stem cells, Casp-3 was necess-
ary for efficient apoptosis following UV-irradiation, but not γ-irradiation or cytotoxic killing of target cells. TNF-α treatment induced normal levels of apoptosis in Casp-3−/− thymocytes, but defective apoptosis in oncogene-immortalised fibroblasts101. It is likely, that Casp-3 deficiency influences apoptosis in both a cell type- and stimulus-specific manner. Other members of the effector caspase subfamily, such as Casp-6 and -7, are compensatorily activated by certain stimuli in Casp-3-deleted cells108.

Casp-6 and -7 knock-outs

Although papers providing detailed analyses of mice lacking the other effector caspases, Casp-6 and -7, still await publication, the preliminary data revealed so far are rather unexpected and exciting. Casp-6−/− mice develop normally and, apart from possible discrete defects in the B cell development, no obvious abnormalities can be detected. In contrast, disruption of Casp-7 is associated with early embryonal death107. Obviously, the lack of Casp-6 expression can be compensated by other subfamily members, but this does not seem to be true for Casp-7. One explanation would be a possible spatial and temporal difference in developmental expression of both proteases. The fact that the developmental defect seen in Casp-7−/− mice appears earlier than in any of the initiator caspase knock-outs suggests that either a redundancy exists among initiator caspases or that Casp-7 can serve both as initiator and effector. However, this hypothesis finds little support in the primary structure of pro-Casp-7. Like other effector caspases, it possesses a very short prodomain, probably inadequate to participate in the formation of multiprotein-complexes typical for the initiation of the caspase cascade. Still, it cannot be excluded that this caspase may initiate the apoptotic program upon activation by a novel mechanism yet to be discovered. Since the previously published in vitro data do not suggest an obvious distinct role for Casp-7 in any of the known death pathways or other physiological cellular processes, careful examination of the Casp-7−/− phenotype will definitively contribute significantly to our knowledge on the role of caspases in apoptosis and development.

The Functional Characterization of Receptor Associated Caspases and FADD

CD95/Apo-1/Fas- and TNF-induced apoptosis dominated programmed cell death research in the last decade. The important players in these pathways are Casp-8 and the FADD adaptor molecule. Casp-8 (FLICE, Mach, Mch5) was initially identified as the proximal caspase in the death-inducing signaling complex (DISC), a signal transduction machinery associated with CD956, 65, 89. The protodomain of Casp-8, as well as that of Casp-10, contains duplicates of a DED which enables Casp-8 to interact with FADD, an adapter molecule associating with the CD95 receptor (Fig. 1). Casp-8 can autoproteolytically activate itself upon recruitment and oligomerization in the vicinity of death receptors60, 66. A similar principle of apoptotic signal transduction is utilized by other death receptors, including the TNF-R1, DR3, and TRAIL receptors, although Casp-2 and Casp-10 may also play a role here.

Casp-8 knockout

Casp-8 plays a central role in propagating death receptor-mediated signals, though, it also becomes activated in a receptor-independent manner, i.e. during chemotherapeutic drug-induced apoptosis20, 99. Here, it fulfills the role of a “downstream/effector” caspase, activated by Casp-6 which in turn becomes activated by Casp-3, downstream of Apaf-1/Casp-985. A Jurkat T cell line deficient in Casp-8 was not only completely resistant to apoptosis triggered by death receptors, but also partially resistant to cell death induced by UV-irradiation, Adriamycin and etoposide13. Complementation of these cells with Casp-8 restored apoptosis sensitivity. In addition, adenoviral protein E1A instigated pro-Casp-8 processing and apoptosis in cells deleted of FADD, indicating the existence of alternative activation pathways80. A likely candidate in this context could be the death-inducing protein complex localized in the endoplasmic reticulum (ER). It is composed of the integral ER membrane protein p28 Bap31, Casp-8/Casp-12 and, possibly, Apaf-1-related molecule67, 69. Activation of this complex might be induced upon viral infection or by ER/Golgi poisons such as tunicamycin, thapsigargin or brefeldin A.

While Casp-8 can be activated indirectly by the mitochondrial pathway, it has also been found that, conversely, CD95-mediated activation of Casp-8 can deliver a signal to mitochondria80. This event is initiated by Casp-8-mediated cleavage of the Bcl-2 protein Bid. The generated c-terminal fragment triggers cytochrome c release, thus accelerating apoptosis82, 58. Therefore, the death receptor and the mitochondrial pathways of caspase activation may be interconnected, leading to the amplification of an apoptotic signal.

However, for a rather unexpected reason, homozy-
gous targeting of the mouse Casp-8 gene was found to be lethal in utero\textsuperscript{93}. Casp-8\textsuperscript{–/–} embryos presumably died from cardiac failure. The two prominent features of Casp-8-null mice were impaired heart muscle development and abdominal hemorrhage. Extensive erythrocytosis was also present in other organs, such as the liver and lungs. The reason for this is not well understood. Recently published data indicate that caspases may either promote hematopoiesis by facilitating maturation of erythrocytes or play an inhibitory role by cleaving the transcription factor GATA-1\textsuperscript{112,105}. The exact role of Casp-8 in erythropoiesis still awaits discovery. The other prominently affected organ, the heart, was of a similar size as in normal mice; however, the trabecula and the ventricular musculature were thin and resembled early mesenchyme. While little is known about Casp-8 participation in this developmental process, recent studies have demonstrated caspase activation during the remodeling and shortening of the cardiac outflow tract\textsuperscript{79}. That the heart was hypotrophic rather than enlarged may indicate that Casp-8 participates in the transmission of survival rather than death signals. This is supported by the fact that hematopoietic precursor cells from Casp-8-null mice revealed a strongly impaired colony-forming activity. The disruption of Casp-8 appears to result in a primary or secondary depletion of the hematopoietic precursor pool\textsuperscript{93}.

Casp-8\textsuperscript{–/–} embryonic fibroblasts were completely resistant to apoptosis mediated by death receptors, such as CD95, TNF-R1, and DR3, whereas they retained sensitivity to a wide range of apoptotic stimuli, including UV-irradiation, ceramide, chemotherapeutic drugs, and infection with the cytopathic vesicular stomatitis virus. In contrast, embryonic fibroblasts responded normally to non-apoptotic signals emanating from death receptors and activated Jun N-terminal kinases as well as transcription factor NF-κB just as wild-type cells. Hence, these findings indicate that Casp-8 plays a necessary and non-redundant role in apoptosis induction by death receptors.

**FADD knock-out**

Gene targeting of the adapter protein FADD resulted in a lethal phenotype with profound signs of cardiac failure and hemorrhage\textsuperscript{102}. As expected, the phenotype was strikingly similar to Casp-8\textsuperscript{–/–} mice, confirming that both molecules function within the same pathway. Overexpression of CD95, TNF-R1 and DR3 could not induce apoptosis in embryonic fibroblasts from FADD\textsuperscript{–/–} mice, whereas death induction by DR4, chemotherapeutic drugs, as well as by overexpression of oncogenes c-Myc and E1A, was not markedly affected. Since FADD\textsuperscript{–/–} mice die in utero, T cell maturation has been analyzed in chimeras, deficient for the recombination-activating gene product RAG-1, which activates the re-arrangement of immunoglobulin and T cell receptor genes\textsuperscript{106}. T lymphocytes from FADD\textsuperscript{–/–} chimaera were completely resistant to CD95-induced apoptosis. Thymocyte populations were apparently normal in newborn chimeras, but their number strongly decreased with age. Thus, FADD\textsuperscript{–/–} mice were inefficient in maintaining thymic cellularity, possibly due to an intrinsic survival defect. Another surprising result was the absence of lymphoproliferative disease and the lack of autoreactive antibodies, both characteristic of abnormalities of the CD95 system, as observed in the gld/lpr mutant mice. A likely explanation would be that the expected phenotype does not evolve, due to a defect in the proliferation of T cells of FADD\textsuperscript{–/–} mice, “compensating” the decreased CD95-dependent death. Indeed, activation-induced proliferation was impaired in FADD\textsuperscript{–/–} T cells, despite normal production of IL-2. Moreover, T cells lacking FADD frequently arrested at the G0/G1 transition of the cell cycle. Thus, targeted disruption of FADD revealed an unexpected connection between cell proliferation and apoptosis\textsuperscript{106}. The role of FADD in cell proliferation was further supported by the phenotype of transgenic mice expressing a dominant-negative FADD (FADD-DN) mutant under the control of a T cell specific promoter\textsuperscript{88}. Expression of FADD-DN enhanced negative selection of self-reactive thymic lymphocytes. FADD-DN mice displayed increased apoptosis and reduced proliferation and clonogenic growth of mitogen-activated T cells. However, impaired T cell proliferation was not observed in CD95-deficient mice or animals overexpressing the viral caspase inhibitor CrmA\textsuperscript{86}, suggesting FADD involvement in a yet to be identified pathway important for cell proliferation. Indeed, FADD has recently been proved to be phosphorylated by a yet unknown serine protein kinase during the G2/M transition of the cell cycle, while it was not phosphorylated in cells arrested at G1/S transition\textsuperscript{83}. This event provides further evidence for the role of FADD in the control of cell cycle. Since T lymphocytes from CrmA transgenic mice responded normally to mitogens, it can be speculated that, unlike the pathway leading to apoptosis, the FADD-dependent growth-promoting signal in lymphocytes does not require activation of Casp-8. Furthermore, because CD95-deficient mice do not show any cardiovascular or hematopoietic abnormalities, another receptor may exist which employs the FADD/Casp-8 pathway in order to induce proliferative or morphogenetic signals during development.
Mitochondrial Death Pathways as Revealed by the Knock-Out Technology

The induction of cell death in response to a variety of apoptotic stimuli is associated with the early mitochondrial release of cytochrome c, AIF, and some other molecules. This event is blocked by anti-apoptotic members of the Bcl-2 family. The death receptor and the mitochondrial pathways can cross-communicate to amplify apoptotic signaling (Fig. 1). Generation of Casp-9-, Apaf-1-, and AIF-deficient mice as well as cytochrome c−/− cell lines have brought new insight into the roles of different components of the mitochondrial death machinery^8, 27, 32, 40, 48.

Casp-9 and Apaf-1 knock-outs

The phenotypes of these mice resembled Casp-3- and Casp-9-deficient animals, since they primarily developed brain malformations and overgrowth of cells in the central nervous system. The ectopic tissue masses consisted of differentiated post-mitotic cells that had escaped apoptosis. A nearly 10-fold reduction of apoptosis was found in the brain of Casp-9−/− mice at E12.5. Both Casp-9−/− and Apaf-1-deficient mice died at about day 16.5 of development. Unlike the brain, other non-neural organs, such as the heart, lungs, liver, or spinal cord, were not affected and appeared remarkably normal. The phenotypic similarity between Casp-3−/−, Casp-9−/−, and Apaf-1-deficient mice, therefore, indicates that the molecules act in a common and non-redundant death pathway. The most severe morphogenetic distortions were found in Apaf-1-deficient mice^8, 103. They exhibited strong craniofacial abnormalities such as ossification defects, deficient midline fusion of the palatal shelves, protruding cauliflower-like masses in the facial region, as well as dramatic alterations of the lens and retina. An enlarged and malformed cerebrum due to reduced apoptosis was also observed in Casp-9−/− targeted mice, but the malformations were much less pronounced. Another apoptosis-dependent developmental process, the removal of interdigital webs, was slightly delayed in Casp-9−/− mice, but it could not be completed in animals devoid of Apaf-1. This indicates that either Apaf-1 may interact with another caspase or play additional roles beyond that of mitochondria-dependent activation of the caspase cascade. It has been proposed that Casp-8 can physically interact with Apaf-1 in overexpression systems. Detailed analysis, however, showed that Casp-9 is the only Apaf-1-interacting protease in living cells^35. The discussed data on Apaf-1−/− have to be analyzed critically, as another experimental genetic inactivation of Apaf-1 outlined an incomplete in utero lethality of the mutant. Despite prenatal death of the majority of Apaf-1−/− mutants, about 5% of them successfully survived to adulthood. In these survivors, the brain developed normally, but males exhibited a degeneration of the spermatogonia, resulting in the virtual absence of sperm^31. A likely explanation could be that under certain conditions, which are probably genetic background-dependent, developmental cell death in the brain can occur without the involvement of Apaf-1. Strikingly, it has also been observed recently that a small percent (<2%) of Casp-9−/− mutants may develop normally. In these cases, the likely mediator of developmentally indispensable apoptosis could be a yet unknown Apaf-1 homologue, other caspases released from mitochondria, or more likely, the AIF-governed apoptotic program, since Apaf-1/Casp-9-dependent apoptosis, like AIF, also relies on mitochondria-derived signaling.

AIF and cytochrome c knock-outs

Both molecules are the primary mediators of the mitochondria-dependent apoptotic cascade. Although acting independently, they can cooperate and probably potentiate their action in a reciprocal manner. Like cytochrome c, AIF, which is normally present in the mitochondrial intermembrane space, is also released in response to death stimuli^15, 91. Extra-mitochondrial targeting of AIF, micro-injection of recombinant AIF protein into cells, or addition of AIF to isolated nuclei results in apoptotic phenotypes, such as chromatin condensation, large-fragment (>50 kb) DNA degradation, and phosphatidylserine exposure. It can also induce the release of cytochrome c and pro-Casp-9 from purified mitochondria, thus potentiating the apoptosome pathway. Genetic inactivation of AIF rendered embryonic stem cells resistant to cell death after serum deprivation. AIF was also essential for programmed cell death during cavitation of embryoid bodies, the very first wave of cell death indispensable for morphogenesis^32.

Cytochrome c, probably the key mitochondrial activator of apoptosis, is released from the intermembrane space of mitochondria in response to a variety of death-promoting stimuli. The exact mechanism responsible for the release is not well understood, but changes in mitochondrial membrane permeability, membrane potential, and ultrastructure have been considered^29. Once in the cytosol, cytochrome c binds Apaf-1 with high affinity, an event that triggers oligomerization of Apaf-1/cytochrome c and dATP into complexes that activate pro-Casp-9^113. Activated Casp-9 passes the
death signal on to Casp-3, -6, and -7, the downstream effector components of the apoptotic cascade (reviewed in 59). Unlike Apaf-1, Casp-9, or other components of the death machinery, the biological and biochemical function of cytochrome c could not be tested in animals bearing null mutations. The major obstacle is the absolute requirement of cytochrome c for mitochondrial respiration, the primary source of energy in almost every animal cell. Murine embryos devoid of cytochrome c died in utero by midgestation, but cell lines established from early cytochrome c-null embryos were viable under conditions that compensate for defective oxidative phosphorylation 60. In comparison to wild-type cells, lack of cytochrome c caused resistance to death induced by UV-irradiation or proapoptotic drugs such as staurosporine. The cytochrome c-deficient cells were also partially resistant to the induction of apoptosis by serum withdrawal. In contrast, the death response to TNF-α treatment was preserved and even augmented in the absence of cytochrome c 61. In conclusion, cytochrome c is required for apoptosis-dependent activation of Casp-3 and for apoptosis provoked by common forms of cellular stress, but not for apoptosis driven by TNF-α and other death receptor pathways.

**Endoplasmic Reticulum-Activated Death Pathway-Revealed by the Disruption of Casp-12**

**Casp-12 knock-out**

Casp-12 is ubiquitously expressed in murine tissues. It is found at high levels in muscle, liver, kidney and at moderate levels in the brain, where it is present in cortical neurons, Purkinje cells, brainstem neurons, and olfactory neurons. Subcellularly, Casp-12 is primarily localized in the ER. It is activated by stimuli which cause ER stress, including disruption of ER calcium homeostasis and accumulation of excess proteins in ER (upon treatment with brefeldin-A, tunicamycin, thapsigargin), but not by death receptor-mediated or mitochondrial-dependent apoptotic stimuli. Murine Casp-12 is closely related to murine Casp-1 (39% identity), mCasp-11 (38% identity), human Casp-4 (48% identity), Casp-5 (45% identity) and human Casp-13 (also 45% identity at the protein level). Overexpression of Casp-12 induces apoptosis, which is inhibited by the broad-spectrum caspase inhibitor z-VAD-fmk and partially by overexpression of Bcl-XL, but not by CrmA. Mice that are deficient in Casp-12 are resistant to apoptosis occurring upon ER stress, but their cells die in response to other death stimuli which rely on death receptor or mitochondrial activation of the caspase cascade. As investigated at the organ level, renal toxicity induced by intraperitoneal injection with tunicamycin is drastically reduced in Casp-12Δ mice. In addition, cortical neurons devoid of Casp-12 are defective in apoptosis induced by amyloid β, or due to excitotoxicity, but not by staurosporine or trophic factor deprivation 67, as reviewed in 79. This is in agreement with previously published data blaming disturbance of intracellular Ca2+ homeostasis as causing neuronal cell death induced by excitotoxicity 52, as reviewed in 79. Certainly, the physiological significance of this newly discovered ER-dependent pathway of caspase activation upon ER stress or disruption of intracellular Ca2+ homeostasis requires further investigation and experiment. Targeting more than just a single caspase could reveal the true role of Casp-12, probably hidden by compensatory mechanisms.

**Concluding Remarks**

A very remarkable feature of the mice studied is their rather restricted phenotype. For instance, Casp-3-, Casp-9-, and Apaf-1-null mice revealed massive malformations, which were mostly restricted to neural tissues. The phenotypes of the three mutant mice were similar, but did not accurately resemble each other. Although disruption of either Casp-9 or Casp-3 caused brain malformations, the abnormalities were more severe in mice lacking Casp-9. Casp-3-deficient cells rarely exhibited a grave exencephal abnormality. One possibility to account for this difference in severity of the malformation is that the function of Casp-3 may be at least in part compensated by other effector caspases, such as Casp-6 or Casp-7 108. Interesting features were observed in the thymus of the Apaf-1- and Casp-9 Δ mice. The thymic architecture appeared to be normal in both of them. Both mutants contained a comparable number of double-positive thymocytes, indicating that the apoptosome is dispensable for negative selection. Interestingly, thymocytes from Apaf-1 Δ mice were resistant to the depolarisation of mitochondrial membrane upon numerous apoptotic stimuli, an event widely believed to precede cytochrome c release 103. It has been proposed that the opening of the mitochondrial permeability pore and loss of transmembrane potential may be an initial event for cytochrome c release and subsequent caspase activation 38. The results observed in Apaf-1 Δ mice, however, strongly suggest that the decrease of the transmembrane potential is downstream of Apaf-1-mediated caspase...
activation. This assumption is supported by the fact that in several apoptotic systems cytochrome c release preceded mitochondrial membrane depolarisation by many hours.

The exact mechanism of mitochondrial cytochrome c release still awaits discovery. Apart from the “Permeability Transition” theory, several other models, including rupture of the outer mitochondrial membrane and the escape of cytochrome c through megachannels or pores formed by pro-apoptotic Bcl-2 family members, have been proposed. It has been described recently that cytochrome c can escape from apoptotic cells in an early death phase and can be used as an apoptosis-specific marker in vitro and in vivo. The fact that it can be detected in the extracellular space just hours after death induction, i.e. much earlier than typical morphological changes characteristic for apoptosis can be observed, prompts a tempting hypothesis predicting that cytochrome c may cross lipid membranes relatively freely, but its association with other components of the electron transport machinery can be the regulatory event. Since cytochrome c itself is rather a hydrophilic molecule, a suitable carrier/receptor molecule must exists to assist this event.

The Casp-8/FADD-deficient mice have helped to clarify the debate over the mechanism of action of some anticancer drugs. Some reports demonstrated that anticancer drug-induced cell death may utilise the CD95 system. Experiments from FADD- and Casp-8-deficient mice, however, convincingly demonstrate that a death receptor-mediated pathway is not the principal mechanism of drug-induced apoptosis. Although embryonic fibroblasts from both mutant mice were resistant to apoptosis mediated by CD95 and other death receptors, they retained sensitivity to anticancer drugs. As for drug-mediated apoptosis, FADD or Casp-8 gene-targeted cells did not exhibit altered apoptosis sensitivity following irradiation or overexpression of c-Myc. Thus, despite the fact that cell type-specific variations could account for these discrepancies, FADD and Casp-8, although absolutely required for death receptor-mediated pathways, are dispensable in other apoptotic settings.

The ER-governed apoptotic pathway definitively requires more detailed studies. As the Casp-12 deficiency provides only partial protection against ER stress-induced apoptosis, there appears to be another, yet to be identified ER-resident caspase(s) that can also contribute to this intrinsic pathway. Another plausible explanation would be a caspase-independent signaling connection between ER and mitochondria capable of activating the mitochondria-dependent death pathways.

This “messenger” function could be fulfilled by some products naturally emerging upon ER stress, for instance certain fragments of misfolded proteins.

The Apoptosis-Unrelated Function of Caspases and Other Components of the Death Machinery

In the past few years, several caspase knockout mice have been generated. Although the phenotypes of most of them confirm an essential role of these molecules in cell death, non-apoptotic functions in development and differentiation also became visible. The well-documented functions of Casp-1/-11 in the immune system have been largely covered in earlier parts of this review. Gene targeting of the death receptor-associated Casp-8 and its adaptor molecule FADD not only confirmed the essential role of both molecules in receptor-mediated induction of apoptosis, but also provided some firm evidence for the roles of these molecules aside from cell death. It becomes increasingly evident that these two molecules, either in concert or independently, play an important role in cell proliferatory/activatory pathways. Casp-8 and FADD-null mice exhibited defective heart muscle development with thin trabeculae and ventricular musculature. In the immune system, hematopoietic precursor cells from these knock-outs reveal a strongly impaired colony-forming activity and a defect in maintaining sufficient numbers of T cell progenitors entering thymic development.

In T cells of FADD−/− mice or animals expressing a dominant-negative FADD protein, activation-induced proliferation is defective despite normal IL-2 production. T cells lacking FADD function frequently arrest at the G0/G1 transition of the cell cycle. Pro-T cells from FADD-DN on the rag-1−/− background fail to proliferate in response to CD3ε ligation. Interestingly, FADD has been recently shown to become phosphorylated at the serine 194 during the G2/M phase transition. The phosphorylation status of FADD may be critical for its function. Previous efforts to discover new interaction partners for FADD, applying a two-hybrid screening procedure, may need to be repeated in a system which assures constant FADD phosphorylation at critical serine residues, or which at least employs mutated FADD mimicking the phosphorylated form. Whether the proliferative effect of FADD requires Casp-8 activation or a different and unrelated signaling event remains to be determined. Another supportive,
indirect hint for a role of the FADD/Casp-8 pathway in cell growth is the observation that proliferation of primary T cells is inhibited by cell-permeable caspase inhibitors. Finally, it has been observed that Casp-8 is indeed cleaved in non-apoptotic cells after TCR stimulation. Thus, there is considerable evidence suggesting that FADD is involved in T cell proliferation, either in cooperation with or independent of Casp-8.

Caspases could indeed provide a checkpoint during cell-cycle progression, assuring that only healthy cells can divide. In fact, several negative regulators of cell-cycle progression, including Wee1, an inhibitor of the cell cycle-regulatory kinases CDK2 and CDK2, as well as CDC27, a component of the anaphase-promoting complex, are rapidly cleaved by caspases. Wee1 is a critical component of the G2/M cell-cycle checkpoint machinery and mediates cell-cycle arrest by phosphorylation of CDC2. Processing of Wee1 by caspases during apoptosis in Jurkat cells correlated with a 20-fold decrease in Wee1 activity and an increase in CDC2 activity. Moreover, the cyclin inhibitors p21\textsuperscript{Waf1} and p27\textsuperscript{Kip1} are targeted by caspases, resulting in increased CDC2 activity that could allow cell-cycle progression.

“Safe” activation of caspases during mitosis can only occur if mechanisms are provided that assure a selective control of their activity. This could, at least in part, be achieved by a specific subcellular compartmentalization of caspases, guaranteeing selective accessibility of cleavable substrates. It has been consistently found that, although caspases were activated and Wee1 was cleaved after TCR triggering, neither the DNA replication factor RFC140 nor DFF45, the inhibitor of caspase-activated Dnase, were proteolysed in proliferating T cells. Cleavage of RFC140 and DFF45 would lead to inhibition of DNA replication and fragmentation of genomic DNA, causing apoptosis rather than promoting cell proliferation.

The protection of cell-cycle regulatory and other vital structures from the active caspases could also be achieved by spatial and temporal co-expression of relevant inhibitors of apoptosis. Good candidates are the IAP- and Bcl-2-family members. It has been observed that Bcl-2, which, similar to FADD, is phosphorylated at the G2/M transition, delays the re-entry of resting NIH 3T3 cells into the cell cycle. Moreover, Bcl-2-transgenic mice have impaired T cell proliferation, whereas transgenic overexpression of Bax accelerates cell-cycle progression and apoptosis. Cells overexpressing Bcl-2 also contain decreased levels of phosphorylated retinoblastoma protein, a key regulator of cell-cycle progression. Finally, downregulation of Bcl-2 by antisense approaches enhances proliferation of acute myeloid leukemic cells. As all of the mutations that suppress the anti-apoptotic activity of Bcl-2 also abolish the inhibitory effect on cell-cycle transition, these two activities of Bcl-2 may be connected. Thus, these findings suggest that inactivation of pathways modulating caspase activity can disturb cell-cycle progression.

The rapid discovery of a great number of caspases together with multiple control points of their activation renews the “apoptosis only” perception of these molecules. Clearly, more experimental evidence is necessary to reveal the potential dual role of caspases in apoptosis and cell proliferation. In contrast to earlier models, suggesting that apoptosis is simply an aberrant form of mitosis, we would rather propose a different scenario, in which a subset of apoptotic molecules could play a role in mitosis. Certainly, many questions regarding the precise contribution of caspases to proliferation remain unanswered: Why are caspases generally not deleted or silenced in most tumors, despite the obvious survival advantage to be gained? How are the dual functions of caspases regulated and what are their cell cycle-relevant targets? Furthermore, is activation of caspases a normal part of the progression through the cell cycle, and which checkpoints do they control? To which extent can a cell “safely” turn on caspase activity without the risk of apoptotic implosion? Finally, is the cell cycle-specific phosphorylation of Bcl-2 and FADD important for the regulation of M-phase events? Answering these questions might provide new insights into lymphocyte proliferation and the biological role of caspases. A major obstacle of most genetically targeted mice is their prenatal lethality, which precludes manifestations of caspase functions in the adult organism. The generation of conditional and cell type-specific caspase knockout mice will certainly shed more light on these issues.

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