Journal of Biochemistry and Molecular Biology, Vol. 35, No. 1, January 2002, pp. 24-27

JOURNAL OF Biochemistry and Molecular Biology

Review

Apoptotic Signaling Pathways: Caspases and Stress-Activated Protein Kinases

Ssang-Goo Cho and Eui-Ju Choi*

National Creative Research Initiative Center for Cell Death, Graduate School of Biotechnology, Korea University, Seoul 136-701, Korea

Received 7January 2002

Apoptotic cell death is an active process mediated by various signaling pathways, which include the caspase cascade and the stress-activated protein kinase pathways. The caspase cascade is activated by two distinct routes: one from cell surface and the other from mitochondria. Activation of the route from cell surface requires the cellular components that include membrane receptors, adaptor proteins such as TRADD and FADD, and caspase-8, while activation of the other from mitochondria requires Apaf-1, caspase-9, and cytosolic cytochrome *c*. On the other hand, persistent stimulation of the stress-activated protein kinase pathway is also shown to mediate apoptosis in many cell types. Gene-targeting studies with *jnk*- or *jip*-null mice, in particular, strongly suggest that this signaling pathway plays a pivotal role in the cellular machinery for apoptosis.

Keywords: Apoptosis, Caspases, Stress-activated protein kinases

Apoptosis is an active process that leads to cell death, which is mediated by programmed signaling pathways, whose activation can be initiated by a variety of extracellular or intracellular stimuli. Apoptosis describes the characteristic morphological changes that include a shrinkage of the cytoplasm, membrane blebbing, compaction of the nuclear chromatin, chromosomal DNA fragmentation, and the formation of small vesicles (called apoptotic bodies), which are eventually phagocytosed by macrophages and other neighboring epithelial cells (Arends and Wyllie, 1991). These morphological changes result from the activation of a set of apoptotic signaling pathways.

Apoptosis plays a pivotal role in a variety of normal physiological processes. These include functional self-organization processes in the immune system and the central nervous system, morphogenetic changes during the embryonic development, tissue homeostasis in adult animals, and a removal of damaged cells (Arends and Wyllie, 1991). It is also deeply involved in the pathogenesis of many human disorders, such as cancer, AIDS, and other immune disorders, cardiovascular diseases, and many neurodegenerative diseases -including Alzheimers disease, Parkinsons disease, stroke, and ischemia (Thompson, 1995). Thus, the study of apoptosis is important, not only to understand the regulatory mechanisms of normal physiological processes, but also to define the patho-physiological mechanisms of many human diseases.

*To whom correspondence should be addressed.
Tel: 82-2-3290-3446; Fax: 82-2-927-9028
E-mail: ejchoi@korea.ac.kr

Regulatory factors in apoptosis

Numerous cellular factors that are associated with apoptotic signaling pathways have been identified in the last decade. These include cell surface receptors (such as the death receptors of the tumor necrosis factor receptor family), cell cycle regulators (such as pRb, and Cdk inhibitory proteins), proteolytic enzymes (such as caspases and calpain), Bcl-2 family members, the inhibitors of apoptosis protein (IAPs), many stress-response proteins (such as heat shock proteins), and cell adhesion proteins. The cellular machinery for apoptosis appears to be conserved well among multicellular organisms from worms such as *C. elegans* to mammals, including humans. Dr. Horvitz and his colleagues demonstrated several genes, including *ced-3*, *ced-4*, and *ced-9*, which are involved in apoptotic processes during the embryonic development of *C. elegans* (Metzstein *et al*., 1998). Subsequently, it turns out that a protein that is encoded by *ced-3* has a sequence homology to a cysteine protease in mammals-the interleukin 1β-converting enzyme (ICE) (Yuan *et al*., 1993). Furthermore, mammalian cells express other ICE-like proteases, now named caspases (Cysteine proteASE cleaving after ASPartic acid). These proteases are produced as a form of pro-enzyme, and can be activated by means of proteolysis in response to diverse apoptotic stimuli. Each caspase is cleaved in order to produce one large subunit (pL)

and one small subunit (pS), thereby forming a tetrameric (pL_2pS_2) active form from two molecules of pro-enzymes (Kumar and Colussi, 1999).

The mammalian counterpart protein of *ced-9* is identified to be Bcl-2, which is a typical anti-apoptotic factor among the Bcl-2 family in mammalian cells (Hengartner *et al*., 1994). The mammalian homolog of *ced-4* is also identified as a novel gene, which encodes a new protein named Apaf-1 (apoptotic protease activating factor-1) (Zou *et al*., 1997). Apaf-1, in the presence of cytochrome c and dATP, is shown to activate caspase-9.

Two signaling pathways to activate the caspase cascade

The most-studied and best-understood signaling pathway in apoptosis is the caspase cascade. One route to activate the caspase cascade is the activation that is initiated from the cell surface by apoptotic stimuli (Kaufmann and Hengartner, 2001). For instance, TNF and Fas-L have been shown to induce the caspase cascade by binding and activating their membrane receptors, TNF receptor-1 (TNFR1), and Fas, respectively. These receptors belong to the TNF receptor (TNFR) family in which other related receptors, such as TNFR, Fas/Apo-1/CD95, DR3, DR4, the p75 nerve growth factor receptor, and CD40, are also included (Ashkenazi and Dixit, 1999). Many of the TNFR family members contain the death domain in the cytoplasmic side. Binding of TNF or Fas-L to its specific receptor is shown to induce the formation of the homotrimeric complex of ligand-bound receptors. This structural change appears to enhance the interaction between the death domains of the receptors and the cytoplasmic death domain-containing proteins (Kaufmann and Hengartner, 2001). For example, TNFR1 associates with the TNFR-associated death domain protein (TRADD) through the death domain-death domain interaction in the cytoplasmic side. Similarly, Fas associates with the Fas-associated death domain protein (FADD) through the death domain-death domain interaction. FADD has another important domain, the death effector domain (DED). Fas-bound FADD can physically associate with caspase-8, which also contains DED, through the DED-DED interaction. The interaction of caspase-8, an initiator caspase, with FADD results in the activation of caspase-8, thereby leading to the activation of the downstream caspases, including caspase-3. Besides the FADD-caspase-8 activation, Fas can also interact with Daxx (Yang *et al*., 1997). The activated Daxx, in turn, associates with and activates the apoptosis signal-regulating kinase 1 (ASK1) that functions as a MAPKKK in the c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and p38 MAPK signaling pathways. Thus, Fas induces the JNK and p38 MAPK signaling pathways through Daxx (Chang *et al*., 1998). The persistent activation of the JNK and p38 MAPK signaling pathways is thought to cause apoptotic cell death. In the TNF signaling, TNFR1-bound TRADD interacts with FADD, which subsequently activates caspase-8 in a manner that is similar to the Fas-FADD-caspase-8 signaling (Baker and Reddy, 1998). The TNFR1-TRADD complex can also interact with TRAF2 (TNFR-associated factor 2), and the resulting TNFR1-TRADD-TRAF2 complex can induce the activation of the NF-κB signaling, as well as the JNK/SAPK signaling.

Mitochondria are another site for apoptotic stimuli to initiate intracellular signaling that mediates caspase activation. Xiaodong Wang and his colleagues first showed that a protein complex that is obtained from the S-100 cytosolic fractions of HeLa cells could activate caspase-3 *in vitro* (Liu *et al*., 1996). The protein complex was composed of three distinct polypeptides, which were named Apaf-1, Apaf-2, and Apaf-3, respectively (Liu *et al*., 1996; Li *et al*., 1997; Zou *et al*., 1997). Apaf-1 is a novel protein and it turned out to be a mammalian counterpart of *ced-4* in *C. elegans* (Zou *et al*., 1997). Apaf-2 was identified to be cytochrome c (Liu *et al*., 1996). These findings first suggested that mitochondria plays a central role in the mechanism of apoptosis, and that cytochrome *c* is released from the intermembrane space of the mitochondria into the cytoplasm in response to apoptotic stimuli. Apaf-3 was identified to be caspase-9, and this observation suggests that caspase-9 functions as an initiator caspase that activates the downstream effector caspases, such as caspase-3 (Li *et al*., 1997). Based on these findings, the following is proposed: when the mitochondria is exposed to apoptotic stimuli, cytochrome c is released from the mitochondria to the cytoplasm. This cytochrome c in the cytoplasm associates with Apaf-1 in the presence of dATP or ATP, thereby forming the apoptosome and activating caspase-9.

Cytochrome c is not the only protein that is released from the mitochondria to the cytoplasm during apoptosis. A new protein named Smac (second mitochondria-derived activator of caspase), or DIABLO (direct IAP-binding protein with low pI), was recently identified. It can be released from the mitochondria when cells are exposed to apoptotic stimuli (Chai *et al*., 2000; Du *et al*., 2000). Smac/DIABLO is shown to bind and inhibit IAP, thereby accelerating apoptosis. As mentioned previously, many apoptotic stimuli induce a release of cytochrome c from the mitochondria into the cytoplasm and enhance the formation of the cytochrome c/Apaf-1/caspase-9 apoptosome, which leads to apoptotic cell death. However, there have been several reports that show that apoptosis does not occur in certain types of cells, even when a release of cytochrome c is observed. An explanation for these observations may be that these cells express a sufficient amount of IAP, which binds to caspases, thereby inhibiting caspase activation. If apoptotic stimuli induce the release of both cytochrome *c* and Smac/DIABLO into the cytoplasm, then cell death will increase, even in the presence of IAP.

AIF (apoptosis-inducing factor) is another mitochondrial protein that is released into the cytoplasm during apoptosis (Susin *et al*., 1999). AIF, which is identical to PDCD8 (programmed cell death 8), is a 57 kDa flavoprotein that is

homologous to oxidoreductases in bacteria. It contains two mitochondrial localization sequences, as well as two nuclear localization signal sequences. AIF resides in the intermembrane space of the mitochondria, and (when apoptotic signals arrive at the mitochondria) it can move to the cytoplasm and nucleus. In the nucleus, AIF is thought to increase the chromatin condensation and the large-scale DNA fragmentation by unknown mechanisms. AIF also seems to be involved in an increase in the release of cytochrome c, a dissipation of mitochondrial transmembrane potential, and an exposure of phosphatidylserine to the cell surface. These effects of AIF are not inhibited by inhibitors of caspases, suggesting that AIF induces a caspase-independent apoptosis.

As described previously, two different pathways that were initiated from either cell surface or the mitochondria can activate the caspase cascade. The signal transmission of the two pathways for caspase activation can sometimes be interconnected. For instance, caspse-8 cleaves not only the downstream caspases, but also other substrate proteins, including Bid. The truncated Bid (t-Bid) that is produced by caspase-8-mediated proteolysis then moves to the mitochondria, and there stimulates a release of cytochrome c into the cytoplasm. This is followed by an enhancement of the apoptosome formation and the activation of caspase-9 (Kaufmann and Hengartner, 2001). The signaling pathways that mediate the activation of the caspase cascade are also modulated by other cellular factors. For instance, c-FLIP (cellular FLICE inhibitory protein) is shown to inhibit the activation of caspase-8 by FADD (Hengartner, 2000). c-FLIP is also named as CFLAR (caspase-8 and FADD-like apoptosis regulator), I-FLICE (inhibitor of FLICE), CASPER (caspase-eight-related protein), FLAME1 (FADD-like antiapoptotic molecule 1), CASH (caspase homolog), CLARP (caspase-like apoptosis regulatory protein), or MRIT (MACH-related inducer of toxicity). c-FLIP contains a module that is homologous to the DED of FADD, as well as a domain that is homologous to the protease domain of caspase-8. c-FLIP, however, has no proteolytic activity. c-FLIP appears to regulate apoptosis by binding to other apoptosis-regulating factors, such as FADD, caspase-8, caspase-3, TRAF1, and TRAF2 (Hu *et al*., 1997; Inohara *et al*., 1997; Irmler *et al*., 1997; Shu *et al*., 1997).

The stress-activated protein kinase signaling pathway

Mitogen-activated protein kinase (MAPK) signaling pathways mediate intracellular signaling, which is initiated by a variety of extracellular stimuli. This leads to diverse cellular activities, which include cell proliferation, differentiation, and apoptosis (Chang and Karin, 2001). The MAPK signaling pathways include three components: MAP3Ks (MAP kinase kinase kinases), MAP2Ks (MAP kinase kinases), and MAPKs. An activated MAP3K phosphorylates and stimulates MAP2K, which in turn stimulates MAPK through phosphorylation (Chang and Karin, 2001). The mammalian MAPK family includes three subgroups: extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases/stress-activated protein kinases (JNKs/SAPKs), and p38 MAPK. The ERK signaling pathway is often involved in the signal transduction in cell proliferation and differentiation. The JNK/SAPK signaling pathway can be stimulated by a variety of stresses. These include genotoxic stress, heat shock, osmotic shock, and metabolic stress, as well as proinflammatory cytokines, such as TNF and interleukin 1-beta (Davis, 2000). This signaling pathway may be involved in diverse cellular activities that include cell growth, differentiation, and cell death. The persistent activation of the JNK/SAPK pathway often mediates intracellular signaling that leads to cell death. The p38 MAPK signaling pathway is also stimulated by cellular stress and proinflammatory cytokines in a way that is similar to the JNK/SAPK signaling pathway. Cellular components in the JNK/SAPK signaling pathway include JNK/SAPK, its MAP2Ks such as SEK1/MKK4/JNKK1 and MKK7/JNKK2, and MAP3Ks (such as MEKK1, ASK1, and TAK1) (Davis, 2000). JNK/SAPK phosphorylates many substrate proteins, which includes transcription factors, such as c-Jun, ATF2, Elk1, DPC4, p53, and NFAT4.

Human and murine cells have three genes for JNK/SAPK: *jnk1, jnk2*, and *jnk3*. Mutant mice that are deficient for each *jnk* gene have been constructed in recent studies. Hippocampal neurons from JNK3-knockout mice are resistant to kainate-induced neuronal cell death (Yang *et al*., 1997). These findings reveal that JNK3 is involved in kainate-induced excitotoxicity in hippocampus. Furthermore, JIP1, which functions as a scaffolding protein in the JNK signaling pathway, plays a role in the kainate- and stress-induced cell death in hippocampal neurons in a study using JIP1-null mice (Whitmarsh *et al*., 2001). A study using *jnk1/jnk2*-double knockout mice showed that JNK1 and JNK2 are important in the apoptotic process during embryonic development in the mouse brain. Another study using *jnk1/jnk2*-double knockout mice also demonstrated that JNK plays an essential role in the increase in cytochrome c release and apoptosome activation that is induced by certain types of stress, including UV (Tournier *et al*., 2000). Collectively, the studies using *jnk*-null mice strongly suggest that the JNK signaling pathway plays a crucial role in the regulation of cell death.

References

Arends, M. J. and Wyllie, A. H. (1991) Apoptosis: mechanisms and roles in pathology. *Int. Rev. Exp. Path.* **32**, 223-354.

Ashkenazi, A. and Dixit, V. M. (1999) Apoptosis control by death and decoy receptors. *Curr. Opin. Cell Biol.* **11**, 255-260.

Baker, S.J. and Reddy, E.P. (1998) Modulation of life and death by the TNF receptor superfamily. *Oncogene* **17**, 3261-3270.

Chai, J., Du, C.. Wu, J.-W., Kyin, S., Wang, X. and Shi, Y. (2000) Structural and biochemical basis of apoptotic activation by Smac/DIABLO. Nature **406**, 855-862.

Chang, H. Y., Nishitoh, H., Yang, X., Ichijo, H. and Baltimore, D.

(1998) Activation of apoptosis signal-regulating kinase 1 (ASK1) by the adapter protein Daxx. *Science* **281**, 1860-1863.

Chang, L. and Karin, M. (2001) Mammalian MAP kinase signaling cascades. *Nature* **410**, 37-40.

Davis, R. J. (2000) Signal transduction by the JNK group of MAP kinases. *Cell* **103**, 239-252.

Du, C., Fang, M., Li, Y., Li, L. and Wang, X. (2000) Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell* **102**, 33-42.

Goltsev, Y. V., Kovalenko, A. V., Arnold, E., Varfolomeev, E. E., Brodianskii, V. M. and Wallach, D. (1997) CASH, a novel caspase homologue with death effector domains. *J. Biol. Chem.* **272**, 19641-19644.

Hengartner, M.O. (2000) The biochemistry of apoptosis. *Nature* **407**, 771-776.

Hengartner, M. O. and Horvitz, H. R. (1994) *C. elegans* cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene bcl-2. *Cell* **76**, 665-676.

Hu, S., Vincenz, C., Ni, J., Gentz, R. and Dixit, V. M. (1997) I-FLICE, a novel inhibitor of tumor necrosis factor receptor-1- and CD-95-induced apoptosis. *J. Biol. Chem.* **272**, 17255-17257.

Hunot, S. and Flavell, R. A. (2001) Apoptosis. Death of a monopoly? *Science* **292**, 865-866.

Irmler, M., Thome, M., Hahne, M., Schneider, P., Hofmann, K., Steiner, V., Bodmer, J.-L., Schroter, M., Burns, K., Mattmann, C., Rimoldi, D., French, L. E. and Tschopp, J. (1997) Inhibition of death receptor signals by cellular FLIP. *Nature* **388**, 190-195.

Inohara, N., Koseki, T., Hu, Y., Chen, S. and Nunez, G. (1997) CLARP, a death effector domain-containing protein interacts with caspase-8 and regulates apoptosis. *Proc. Nat. Acad. Sci. USA* **94**, 10717-10722.

Kaufmann, S. H. and Hengartner, M. O. (2001) Programmed cell death: alive and well in the new millennium. *Trends Cell Biol.* **11**, 526-534.

Kerr, J. F., Wylie, A. H. and Currie, A. R. (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer.* **26**, 239-257.

Kuan, C. Y., Yang, D. D., Samanta Roy, D. R., Davis, R. J., Rakic, P. and Flavell, R. A. (1999) The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron* **22**, 667-676.

Kumar, S. and Colussi, P. A. (1999) Prodomains--adaptors--oligomerization: the pursuit of caspase activation in apoptosis. *Trends Biochem. Sci.* **24**, 1-4.

Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S. M., Ahmad, M., Alnemri, E. S. and Wang, X. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* **91**, 479-489.

Liu, X., Kim, C. N., Yang, J., Jemmerson, R and Wang X. (1996) Induction of Apoptotic Program in Cell-Free Extracts: Requirement for dATP and Cytochrome c. *Cell* **86**, 147157.

Liu, Z., Sun, C., Olejniczak, E. T., Meadows, R. P., Betz, S. F., Oost, T., Herrmann, J., Wu, J. C. and Fesik, S. W. (2000) Structural basis for binding of Smac/DIABLO to the XIAP BIR3 domain. *Nature* **408**, 1004-1008.

Metzstein, M. M., Stanfield, G. M. and Horvitz, H. R. (1998) Genetics of programmed cell death in C. elegans: past, present and future. *Trends Genet.* **14**, 410-416.

Shu, H.-B., Halpin, D. R. and Goeddel, D. V. (1997) Casper is a FADD- and caspase-related inducer of apoptosis. *Immunity* **6**, 751-763.

Susin, S. A., Lorenzo, H. K., Zamzami, N., Marzo, I., Snow, B. E., Brothers, G. M., Mangion, J., Jacotot, E., Constantini, P., Loeffler, M., Larochette, N., Goodlett, D. R., Aebersold, R., Siderovski, D. P., Penninger, J. M. and Kroemer, G. (1999) Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* **397**, 441-446.

Thompson, C. B. (1995) Apoptosis in the pathogenesis and treatment of disease. *Science* **267**, 1456-1462.

Tournier, C., Hess, P., Yang, D. D., Xu, J., Turner, T. K., Nimnual, A., Bar-Sagi, D., Jones, S. N., Flavell, R. A. and Davis, R. J. (2000) Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science* **288**, 870-874.

Verhagen, A. M., Ekert, P. G., Pakusch, M., Silke, J., Connolly, L. M., Reid, G. E., Moritz, R. L., Simpson, R. J. and Vaux, D. L. (2000) Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. *Cell* **102**, 43-53.

Whitmarsh, A. J., Kuan, C. Y., Kennedy, N. J., Kelkar, N., Haydar, T. F., Mordes, J. P., Appel, M., Rossini, A. A., Jones, S. N., Flavell, R. A., Rakic, P. and Davis R. J. (2001) Requirement of the JIP1 scaffold protein for stress-induced JNK activation. *Genes Dev.* **15**, 2421-2432.

Wu, G., Chai, J., Suber, T. L., Wu, J.-W., Du, C., Wang, X. and Shi, Y. (2000) Structural basis of IAP recognition by Smac/DIABLO. *Nature* **408**, 1008-1012.

Yang, D. D., Kuan, C. Y., Whitmarsh, A. J., Rincon, M., Zheng, T. S., Davis, R. J., Rakic, P. and Flavell, R. A. (1997) Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. *Nature* **389**, 865-870.

Yang, X., Khosravi-Far, R., Chang, H. Y. and Baltimore, D. (1997) Daxx, a novel Fas-binding protein that activates JNK and apoptosis. *Cell* **89**, 1067-1076.

Yuan, J., Shaham, S., Ledoux, S., Ellis, H. M. and Horvitz, H. R. (1993) The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1 beta-converting enzyme. *Cell* **75**, 641-652.

Zou, H., Henzel, W. J., Liu, X., Lutschg, A. and Wang, X. (1997) APAF-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell* **90**, 405-413.