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Molecular Mechanisms of the Decision between Life and Death: Regulation of Apoptosis by Apoptosis Signal–Regulating Kinase 1

Atsushi Matsuzawa*,† and Hidenori Ichijo*,[1]

Laboratory of Cell Signaling, Graduate School, Tokyo Medical and Dental University, 1-5-45, Yushima, Bunkyo-ku, Tokyo 113-8549; and †Central Research Laboratories, Kissei Pharmaceutical Co. Ltd., 4365-1 Kashiwabara, Hotaka, Minamiazumi, Nagano 399-8304

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Coordination and balance between cell survival and apoptosis is crucial for normal development and homeostasis of multicellular organisms. Defects in control of this balance may contribute to a variety of diseases including cancer, autoimmune and neurodegenerative conditions. Although a large number of pro- and anti-apoptotic factors acting for or against the final death event have been and are being discovered at an extraordinary pace with the recent progress in this area, the molecular mechanisms determining whether a cell lives or dies are not fully understood. Phosphorylation and dephosphorylation of intracellular effector molecules are the most common and important regulatory mechanisms in signal transduction and control a variety of cellular events from cell growth to apoptosis. Apoptosis signal-regulating kinase 1 (ASK1) is a member of the mitogen-activated protein (MAP) kinase kinase kinase family, which activates both the SEK1–JNK and MKK3/6–p38 MAP kinase pathways and constitutes a pivotal signaling pathway in cytokine- and stress-induced apoptosis. This review provides recent findings on the molecular mechanisms which determine cell fate such as survival, proliferation, differentiation or apoptosis, with special focus on the regulatory mechanisms of ASK1-mediated apoptosis.

Key words: apoptosis, ASK1, death receptor, MAP kinase, mitochondria.

Cells are continuously exposed to multiple opposing "death" and "survival" triggers. Dysregulation of the delicate balance between death and life is associated with the pathogenesis of a wide array of diseases, including cancer, neurodegeneration, autoimmune diseases, heart disease, diabetes, and other disorders. Apoptosis is a highly regulated and organized death process that serves critical functions in the deletion of autoreactive lymphocytes, elimination of virally-infected and malignant cells, and development of complex multicellular organisms. For example, the most exquisitely controlled apoptotic signaling occur during normal development in the central nervous system, where the correct number of postmitotic cells and synaptic connections must be established in precise spatial and temporal sequence, dictating the exact cell mass for formation of different compartments in the central nervous systems (*1*). While many components of regulatory networks controlling apoptosis have been determined, the molecular mechanisms of action and the patterns of interaction of these factors remain controversial.

Protein kinases are involved in various intracellular signaling pathways. Protein kinases and other associated signaling proteins are perfectly suited to regulate life and death decisions made in response to extracellular signals. Mitogen-activated protein (MAP) kinase cascade is evolutionarily well conserved in all eukaryotic cells and is typically composed of three kinases that establish a sequential activation pathway comprising a MAP kinase kinase kinase (MAPKKK), MAP kinase kinase (MAPKK), and MAP kinase (MAPK) (Fig. 1). JNK (c-Jun N-terminal kinase), p38 MAP kinase and ERK (etracellular signal-regulated kinase) are well-characterized subgroups of a large MAP kinase family. These kinase pathways are structurally similar, but functionally distinct. While ERK is rapidly activated by a variety of cell growth and differentiation stimuli and plays a central role in mitogenic signaling, JNK and p38 are primarily activated by various environmental stresses, including osmotic shock, UV radiation, heat shock, oxidative stress, protein synthesis inhibitors, stimulation of Fas, and proinflammatory cytokines such as tumor necrosis factor α (TNFα) and interleukin-1 (IL-1). Specific inhibitors of JNK pathways and p38, or dominant-negative mutants of JNK and p38 suppress various types of stress-induced apoptosis (*2*). In the JNK3 knockout mouse and the JNK1/JNK2 double knockout mouse, glutamate-induced hippocampal cell death and UV radiation-induced apoptosis are prevented to remarkable extents (*3*, *4*). Thus, it has been suggested that JNK and p38 play critical roles in signal transduction of stress-induced apoptosis.

Apoptosis signal-regulating kinase 1 (ASK1) is a member of the MAPKKK family and activates both the SEK1–JNK and MKK3/MKK6–p38 signaling cascades (*5–9*). ASK1 is activated in cells treated with inflammatory cytokines and subjected to various types of stress. Overexpression of wild-

[1] To whom correspondence should be addressed at the present address: Cell Signaling, Department of Hard Tissue Engineering, Division of Bio-Matrix, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549.
Tel: +81-3-5803-5471, Fax: +81-3-5803-0192, E-mail: ichijo.csi@tmd.ac.jp

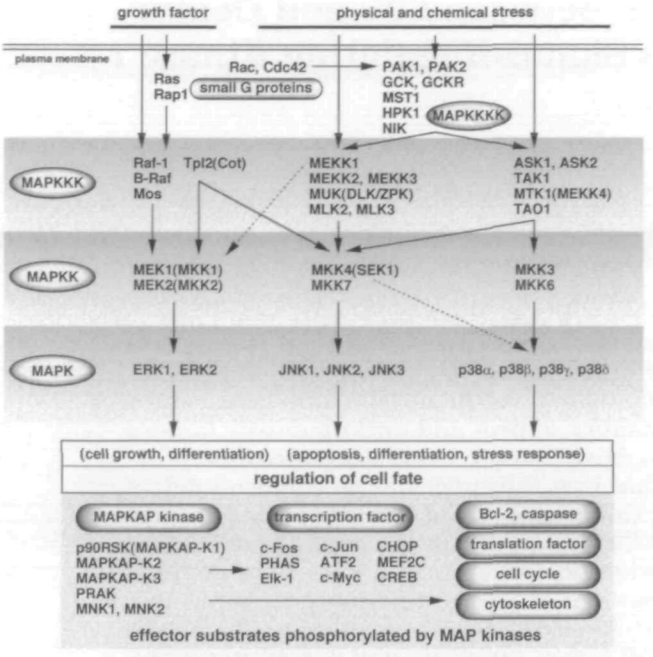

Fig. 1. **The mammalian MAP kinase modules.** Mitogen-activated protein (MAP) kinase cascade is typically composed of three kinases that establish a sequential activation pathway comprising a MAP kinase kinase kinase (MAPKKK), MAP kinase kinase (MAPKK), and MAP kinase (MAPK). In mammals, three major subgroups of MAP kinase, ERK, JNK, and p38, have been identified, which are structurally similar, but functionally distinct. MAP kinases regulate and determine cell fate, such as growth, differentiation or apoptosis, in response to environmental changes, through phosphorylation of a variety of downstream substrates: kinases, transcription or translation factors, cytoskeletal proteins, and regulators of the cell cycle and apoptosis.

type ASK1 or the constitutively active mutant induced apoptosis in various cell types. Furthermore, various stress-induced cell death, such as TNFα and oxidative stress, is remarkably reduced by the disruption of ASK1 gene in mouse, indicating that ASK1 is a key element in cytokine- and stress-induced apoptosis.

In this review, we describe some of the key pathways that regulate pro- and anti-apoptotic signals and demonstrate the molecular mechanisms which determine cell fate such as survival or apoptosis, with special focus on the mechanisms regulating ASK1-mediated apoptosis.

Protein–protein interactions: Mechanisms for silencing of apoptotic molecules

Even minor changes in the delicate balance between anti- and pro-apoptotic factors could have profound implications for the well-being of the organism. Strict regulation of apoptosis must thus be enforced by the organism to insure that proper life/death decisions are made, and leakage of unexpected pro-apoptotic signals must be completely excluded. Protein–protein interactions are the most general machinery for shutting out and silencing of antagonistic molecules. The well-known anti-apoptotic factors, IAPs (inhibitors of apoptosis), directly bind to and inhibit caspases and procaspases which are the major executioners of apoptosis (*10*). In turn, Smac/DIABLO, a recently identified pro-apoptotic molecule, is released from the mitochondria and promotes apoptosis by binding to and inhibiting IAPs (*11*, *12*). CAD (caspase-activated deoxyribonuclease) causes nuclear DNA fragmentation by its apoptosis signal-specific DNase activity. While its inhibitor ICAD constantly forms a complex with CAD to inhibit its DNase activity, ICAD is cleaved by caspase-3 upon apoptotic stimuli and unbound from CAD, allowing CAD to enter the nucleus and degrade chromosomal DNA (*13*). As described below, TNF receptor I and DR3 (death receptor 3), which mediate both survival and death signals, are constantly associated with an adaptor molecule, SODD (silencer of death domains) (*14*). SODD is dissociated from the intracellular death domain of TNF receptor upon ligation of TNF receptor by TNFα, which is followed by the formation of a death signaling complex and apoptosis signal transduction. SODD may modulate the duration of TNF signaling as a negative regulator of TNF signaling. One of the unique features of Bcl-2 family proteins is heterodimerization between anti- and pro-apoptotic members, which is believed to silence the biological activity of their partners (see below) (Fig. 2).

Oxidative stress-induced activation of ASK1 leads to apoptosis. Thioredoxin (Trx) was identified as a negative regulator of the ASK1–JNK/p38 pathway, through yeast two-hybrid screening for ASK1-binding proteins (*6*). In resting cells, ASK1 constantly forms an inactive complex with Trx, but upon treatment of cells with TNFα or reactive oxygen species (ROS) such as H_2O_2, ASK1 is dissociated from Trx and activated by subsequent modifications, including oligomerization and auto- and/or cross-phosphorylation (*5*, *6*, *15*). Trx is a redox (reduction/oxidation)-regulatory protein which has two redox-sensitive cysteine residues within the active center. Only a reduced form of Trx is associated with the N-terminal regulatory domain of ASK1 and silence the activity of ASK1; oxidization of Trx results in the dissociation of ASK1 from Trx and thereby switches an inactive form of ASK1 to active kinase. The ASK1–Trx complex is thus thought to be a redox-sensor, which functions as a molecular switch of external and internal redox status for the kinase signaling module (Fig. 3).

It was recently reported that 14-3-3 proteins directly bind to ASK1, and that overexpression of 14-3-3 proteins blocked ASK1-induced apoptosis (*16*). Moreover, intramolecular interaction, probably between the N-terminal and C-terminal domains of ASK1, may be required to maintain the inactivate form of ASK1 (*8*). As a unique example of protein–protein interactions, direct interaction between two distinct MAPKKKs, ASK1 and TAK1 (transforming growth factor β-activated kinase 1), inhibits IL-1-induced NF-κB activation through the TRAF6–TAK1 pathway, where the ASK1–TAK1 interaction disrupts the TAK1–TRAF6 complex (*17*) (see below for the NF-κB pathway and TRAF family proteins). ASK1 also strongly interacts with ASK2, which has been identified as a MAPKKK with high sequence similarity to ASK1; however, the function of ASK2 and the physiological significance of this interaction have not been elucidated. Recently, protein serine/threonine phosphatase 5 (PP5) was identified as a negative regulator for activated ASK1 in response to various types of stress (*18*). PP5 binds to and dephosphorylates ASK1, enabling inactivation of ASK1 by negative feedback. Execution of apoptosis must be strictly regulated, and apoptosis signals must constantly counterbalance survival signals to maintain homeostasis. In fact, most of the pro- and anti-apop-

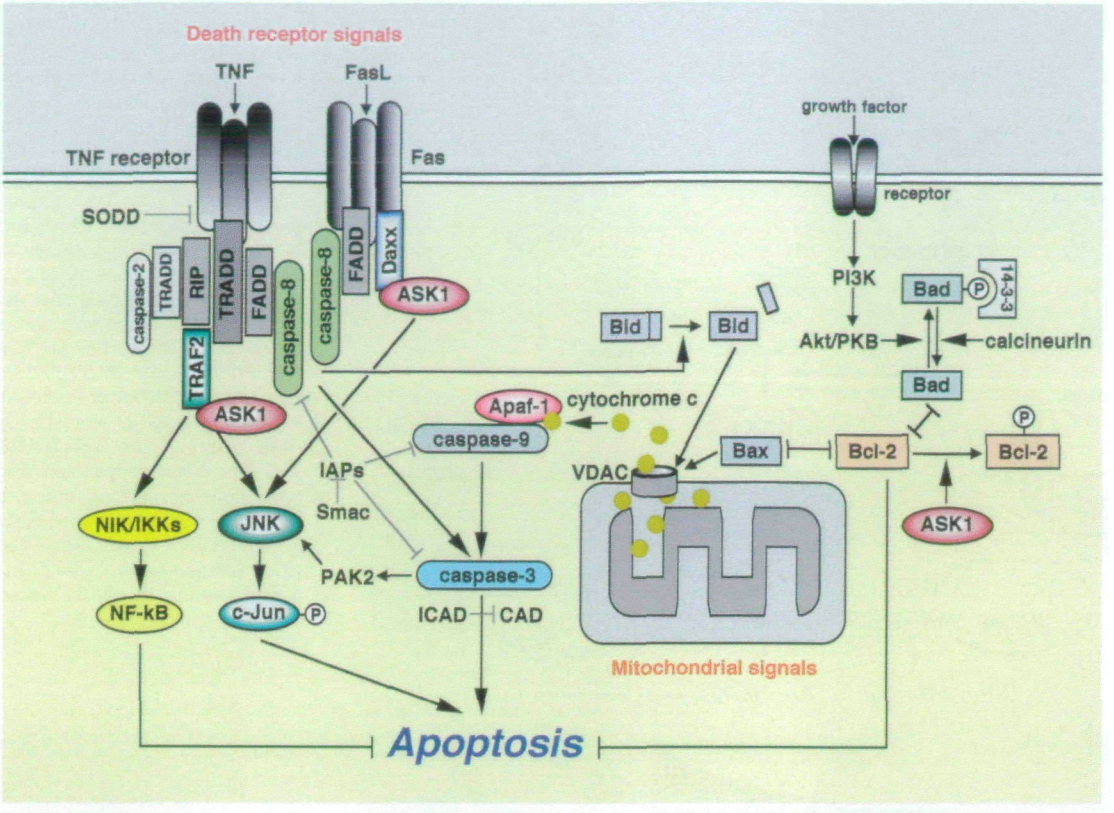

Fig. 2. **Overview of apoptosis and survival signals through the death receptor and mitochondria.** Signaling molecules shown in the figure are important inducers, determinants, and/or executioners for apoptosis, and survival factors against cell death. In response to a variety of outside and inside environmental changes, multiple signals diverge from the death receptors and are integrated in the mitochondria. These complicated and antagonistic machineries and the balance between these pro- and anti-apoptotic factors generate appropriate biological responses, such as apoptosis or survival, to diverse challenges. ASK1 also regulates cell fate at the various levels shown in the figure. The details are described in this review text.

totic molecules which play pivotal roles in apoptosis serve as a variety of negative regulators for themselves as well. ASK1 may thus possess several undiscovered antagonistic partners still more.

Death receptor signaling: TNF receptor and Fas signaling

Homeostasis in mammalian cells is dependent on the continuous integration of survival and death signals from the extracellular environment. Signaling through the TNF receptor gene family (*e.g.* Fas, TNF receptor I, DR3 and p75 neurotrophin receptor) plays a significant role in mediating pro- and anti-apoptotic cellular events. Activation of TNF receptor I mediates opposing cellular functions through the "death domain" (DD), an intracellular domain that is conserved among the death receptor family members (*19*). Upon TNFα binding, trimerization of TNF receptors occurs and results in aggregation of DDs, allowing recruitment of TRADD (TNF receptor I-associated death domain protein) (*20*). TRADD mediates recruitment of an adaptor molecule, TRAF2 (TNF receptor-associated factor 2) (*21*), which leads to activation of the JNK, p38 and NF-κB (nuclear factor for κ chain gene in B cells) pathways. TRADD also recruits FADD (Fas-associated death domain-containing protein) (*22*) and RIP (receptor interacting protein), leading to an acute execution of apoptosis and activation of NF-κB, respectively. RIP can recruit RAIDD (RIP-associated ICH-1/CED-3-homologous protein with a death domain) as well, which in turn recruits caspase-2 and induces apoptosis. Hence the TNF receptor I signaling complex is formed by interaction of TRADD, TRAF, FADD, and RIP, allowing multiple and subdivided signals to regulate both pro- and anti-apoptotic activities (Fig. 2).

ASK1 directly binds TRAF family proteins, including TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6 by overexpression, and can be activated by TRAF2, TRAF5, and TRAF6 (*7*). A dominant-negative mutant of TRAF2 blocks TNFα-induced ASK1 activation as well as JNK activation. A kinase-inactive mutant of ASK1 strongly inhibits TNFα- and TRAF2-induced JNK activation. Furthermore, ASK1 associates with endogenous TRAF2 in a TNFα-dependent manner (*7*, *23*). Thus, ASK1 is a direct downstream target of TRAF2, and recruitment of ASK1 to the active TRAF2 components is a specific mechanism by which TNFα activates both JNK and p38 pathways (Figs. 2 and 3).

TRAFs often mediate the activation of both the JNK and NF-κB pathways. A number of recent studies have linked NF-κB activation to the induction of the cell survival pathway (*24*, *25*). When TNF receptor family members activate the JNK pathway alone, the phenotypic result appears to be cell death. In contrast, the activation of both NF-κB and JNK pathways appears to result in cell survival, suggesting that the target genes of c-Jun-associated complexes could be different depending on the presence or absence of acti-

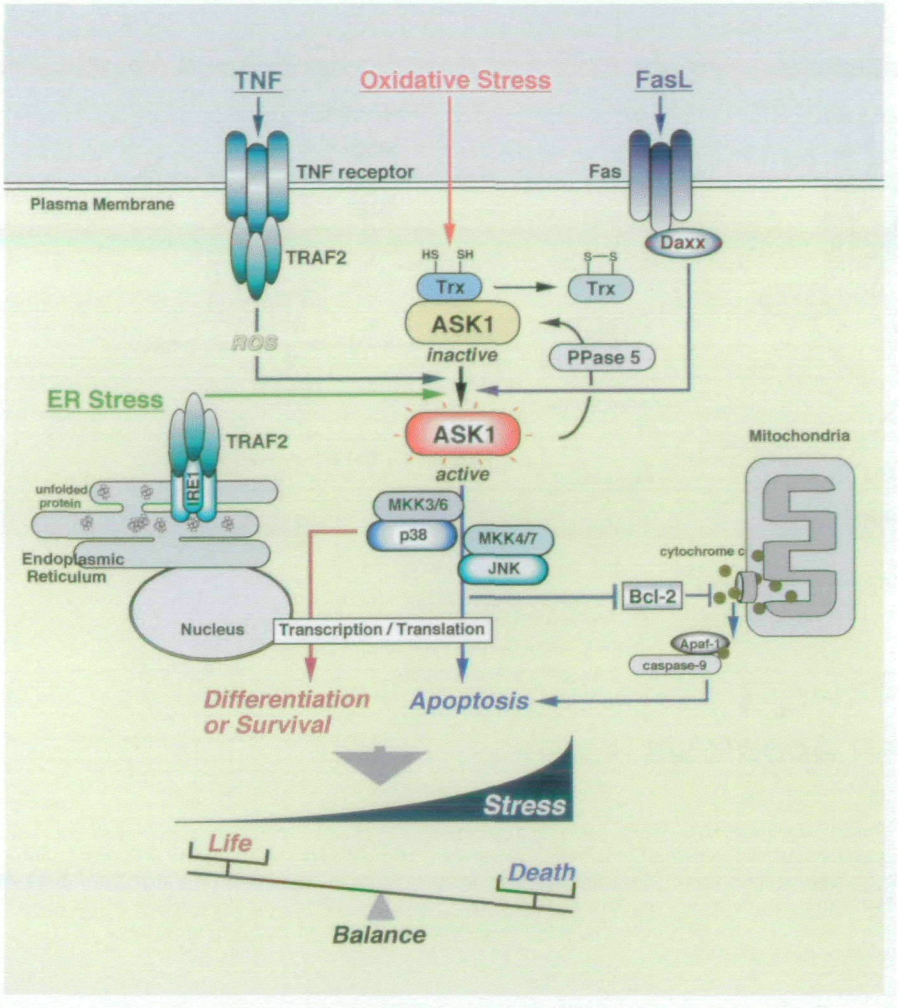

Fig. 3. **ASK1 regulates the balance between life and death.** Various stimuli, such as TNFα, Fas, and oxidative stress, activate ASK1, which is positively regulated by TRAF2 and Daxx and negatively regulated by Trx and PPase 5. The activated ASK1 induces apoptosis or stress responses through both JNK and p38 pathways. Especially, the ASK1–Trx complex is thought to be a redox-sensor, which functions as a molecular switch of external and internal redox status for the kinase signaling module. Multiple death signals, from not only the cell surface receptors but also various intracellular organelles such as endoplasmic reticulum, are integrated into ASK1. In turn, the signals are transduced to multiple organelles, such as mitochondria and nucleus, through the ASK1–JNK/p38 pathway. The balance between life and death may be determined by timing, duration, extent, and pattern of the ASK1-mediated JNK and p38 activations, to make appropriate responses to various types of extracellular and intracellular stress.

vated NF-κB, and that opposing mechanisms may exist between TRAF2–NF-κB and TRAF2–JNK pathway (*26*). For example, c-IAP1 and c-IAP2, new IAP family members, which interact with TRAFs and interfere with the activation of caspases-8, can be transcriptionally regulated by NF-κB (*27*). Bcl-2, Bcl-XL, and Bfl-1/A1, pro-survival members of the Bcl-2 homologue, are up-regulated by NF-κB in response to TNFα (*28*, *29*). These molecules newly induced by NF-κB activation may contribute to cell survival. On the other hand, TNFα-induced ASK1–TRAF2 interaction may lead to apoptosis through JNK activation. In fact, mouse embryonic fibroblasts (MEFs) derived from ASK1-deficient mice were partially but significantly resistant to TNFα-induced apoptosis accompanied by remarkable reduction of JNK and p38 activations (*30*). Mice harboring a mutation of the c-Jun locus that removes a subset of JNK phosphorylation sites (Ser63Ala, Ser73Ala) are protected from kainate-induced apoptosis in the hippocampus, as in the case of JNK3 knockout mice (*31*), suggesting that c-Jun transcription factor acts as an inducer of apoptosis and that JNK-induced phosphorylation of c-Jun is crucial for the induction of apoptosis. However, the target genes responsible for c-Jun-mediated apoptosis remain obscure. Additional studies aimed at understanding the target molecules of these two important pathways that mediate survival and death will provide important clues regarding cell proliferation, differentiation and death following activation of the death receptors.

Fas, a well-characterized member of the death receptor family, can enhance apoptosis in certain types of cells. Activation of Fas by Fas-ligand recruits FADD, permitting the acute execution of apoptosis by caspase-8 activation (*32*). While caspase-mediated activation of PAK2, one of the MAPKKK (or MAPKKKK) family members, may enhance apoptotic body formation through JNK activation (*33*), ASK1 activation by Fas appears to occur through the activation of a second pathway which involves another adaptor protein for Fas, called Daxx (*34*) (Figs. 2 and 3). Daxx binds to the N-terminal noncatalytic domain of ASK1 in a ligand-dependent manner and thereby activates JNK, which may sensitize cells to caspase-induced apoptosis (*8*). However, the inhibitory effects of dominant-negative forms of Daxx and ASK1 on Fas-induced apoptosis are much weaker than those of caspase inhibitors. We observed that Fas-induced JNK and p38 activations were strongly suppressed in thymocytes from ASK1-deficient mice, but that the sensitivities to Fas-induced apoptosis of ASK1+/+ and −/− thymocytes were indistinguishable (*30*), suggesting that the Daxx–ASK1–JNK/p38 axis is not required for Fas-induced apoptosis, at least in thymocytes.

Fas- and TNF receptor-mediated signaling play different roles in regulating cell fate. For example, in the immune

system, TNFα contributes to the growth and differentiation of lymphocytes during early immune responses and also to the elimination of virally-infected and deviant cells at the same time; on the other hand, lymphocytes numbers are rapidly reduced to terminate the sustained immune response by up-regulation of Fas and Fas-ligand. Interestingly, the caspase-8 activity induced by TNFα was significantly less than that induced by Fas, although the magnitudes of apoptosis induced by TNFα and Fas were indistinguishable (*30*). An alternate pathway may not be required for Fas-induced cell death with sufficient activation of the FADD–caspase-8 pathway; however, weaker activation of the FADD–caspase-8 pathway by TNFα may be compensated for by simultaneous activation of the ASK1–JNK/p38 death signal. Fas and TNFα differentially utilize the ASK1–JNK/p38 pathway for regulation of apoptosis. Fas-induced apoptosis can occur in low oxygen and does not appear to require the generation of ROS, whereas TNFα-induced cell death can be inhibited by antioxidants (*35*, *36*). In fact, overexpression of TRAF2 fosters the production of ROS in transfected cells, and the interaction between TRAF2 and ASK1 is redox sensitive and can be prevented by free-radical scavengers. Overexpression of Trx in excess of coexpressed TRAF2 almost completely inhibits the TRAF2–ASK1 interaction in a process that is reversed by ROS, while overexpression of wild-type TRAF2 but not a dominant-negative form of TRAF2 removes coexpressed Trx from the ASK1–Trx complex (*37*). Generation of ROS appears to be required for the activation of ASK1 through the TNF–TRAF2 pathway (*9*, *15*). Thus, a clear difference exists between TNFα- and Fas-induced apoptosis pathways in dependency on ASK1–JNK/p38 and caspase-8 pathways. Such branching and re-integration of receptor signals could generate discrete biological responses to a variety of outside challenges, "death or life."

Mitochondrial signaling: Regulation of Bcl-2 family proteins

Based on the established interactions among the known mediators of apoptosis, two pathways of apoptotic signaling have emerged in mammalian cells. As described above, one is initiated by cell surface death receptors such as TNFα and Fas, which recruit and activate caspase-8 through adapter molecules such as TRADD and FADD. The other evokes cytochrome *c* release from mitochondria, which results in activation of Apaf-1, formation of the "apoptosome" (a large complex of apoptotic factors) and triggering of the caspase-9–caspase-3 cascade (Fig. 2). The mitochondria play a central role in many forms of apoptosis, and mitochondria-dependent apoptosis is regulated by Bcl-2 family proteins. Bcl-2 family proteins are divided into three categories: the anti-apoptotic members such as Bcl-2, Bcl-XL, Bcl-w, Mcl-1, and Bfl-1/A1; the pro-apoptotic members such as Bax, Bak, and Bok; and the pro-apoptotic BH3-only proteins such as Bad, Bid, Bik, Bim, Blk, Hrk, and Noxa (*38*). Heterodimerization between anti- and pro-apoptotic proteins inhibits the biological activity of their partners each other. The mechanisms by which Bcl-2 family proteins regulate mitochondria-dependent apoptosis are still being debated. Recently, VDAC (voltage-dependent anion channel) has been determined to be one of the functional targets for Bcl-2 family proteins (*39*). Bcl-2 family members such as Bax, Bak, Bcl-2, and Bcl-XL can associate with two components of the permeability transition (PT) pore, VDAC on the mitochondrial outer membrane and ANT (adenine nucleotide translocator) on the inner membrane (*40*). Bcl-2 and Bcl-XL close the VDAC channel, whereas Bax and Bak open it. Cytochrome *c* release from mitochondria is partly controlled by PT opening. Thus, each Bcl-2 family protein functions as a gatekeeper of the PT pore by either promoting or preventing the release of cytochrome *c*, and the balance between pro- and anti-apoptotic members in the Bcl-2 family appear to determine cell fate.

Bcl-2 family proteins are subject to covalent processing by a variety of molecules of different signaling pathways (*41*). For example, Bid is cleaved by caspase-8 in response to Fas and TNF receptor activation (*42*, *43*). Following cleavage, the C-terminal fragment of Bid translocates and binds to the mitochondria, leading to the release of cytochrome *c* and activation of downstream caspases. Thus, Bid functions as a mediator linking the death receptor signals to the mitochondria-dependent death signals.

Phosphorylation and dephosphorylation are also important modifications of Bcl-2 family proteins. Bad executes its pro-apoptotic function by binding to anti-apoptotic proteins Bcl-2 and Bcl-XL. Upon activation by growth factors, such as EGF and IGF, a serine/threonine kinase Akt (also called PKB) phosphorylates Bad. Phosphorylated Bad is sequestered by the 14-3-3 protein in the cytoplasm away from the functional site, the mitochondria. This cytoplasmic sequestration of Bad therefore leaves mitochondrial Bcl-2 or Bcl-XL free to perform their pro-survival functions. Several kinases, including PKA, Ca^{2+}/CaMKII, Ca^{2+}/CaMKIV, and RSK, prevent apoptosis by phosphorylation of Bad (*44*, *45*). On the other hand, the Ca^{2+}-dependent protein phosphatase calcineurin promotes apoptosis by dephosphorylation of Bad (*46*). This cross-talk connects different signaling pathways such as the caspase-, Bcl-2 family-, and stress kinase-dependent apoptosis signals. Bcl-2 family proteins make it possible for a variety of death or survival signals to be integrated into a common pathway to the mitochondria.

Recently, ASK1 was reported to execute apoptosis mainly by mitochondria-dependent caspase activation. Overexpression of a constitutively active mutant of ASK1 induced cytochrome *c* release from the mitochondria and activated caspase-9 and caspase-3 but not caspase-8. Consistently, caspase-8-deficient cells were sensitive to ASK1-induced apoptosis, whereas ASK1 failed to induce caspase-3 activation and apoptosis in caspase-9-deficient cells (*47*). These results suggest that ASK1-induced apoptosis is mediated by the mitochondrial pathway, probably involving the modification of Bcl-2 family proteins. In fact, Bcl-2 was phosphorylated and inactivated by the coexpression of ASK1 and JNK. The combined expression of dominant negative forms of ASK1, MKK7, and JNK remarkably inhibited stress-induced Bcl-2 phosphorylation (*48*), suggesting that the ASK1/MKK7/JNK pathway promotes mitochondria-dependent apoptosis by Bcl-2 phosphorylation (Figs. 2 and 3). In contrast, it has also been reported that phosphorylation of Bcl-2 by the ERK pathway facilitates structural and functional stabilization of the Bcl-2 protein (*49*, *50*). The threshold for apoptosis may vary depending on cell type, stage of development and differentiation, and environment. The competition between "survival kinases" and "death kinases," such as ERK *versus* JNK, and Akt/PKB *versus* ASK1, may be a critical step in determining the susceptibil-

ity of cells to apoptosis.

MAP kinase cascade: Determinant of cell fate

As described above, responses of cells to external stimuli are regulated in part by crosstalk of intracellular signals. When conflicting signals are integrated into cells at the same time, appropriate outcomes, such as proliferation, differentiation, survival, or apoptosis, must eventually be determined by the balance of multiple opposing signals. The caspase cascade is appropriate for signal amplification and immediate execution of apoptosis, but not for induction of various biological outcomes, because of its irreversibility. In contrast, the kinase cascade not only amplifies signals and generates a threshold and sigmoid activation, but also coordinates incoming information from various signaling pathways; it can therefore transduce signals to different extents, with different timing and different duration, and allow various and appropriate response patterns.

Recently, it was demonstrated that the duration of activation of MAP kinases may contribute to determination of cell fate, such as survival, differentiation and apoptosis. The T-cell activation signals mediated through the costimulator CD28 induced rapid and transient JNK activation, which in turn stimulated cell growth, whereas UV-C or γ-radiation caused delayed and persistent JNK activation, which induced apoptosis (*51*). Early/transient and late/sustained activation of JNK and/or p38 induced by the proinflammatory cytokine TNFα or various types of stress have been reported to correlate with survival/differentiation and apoptosis, respectively (*52–54*).

In neuron-like PC12 pheochromocytoma cells, NGF withdrawal leads to sustained activation of JNK and p38 MAPK and inhibition of ERK. The dynamic balance between growth factor-activated ERK and stress-activated JNK–p38 pathways may be important in determining whether a cell survives or undergoes apoptosis (*55*). Transient and persistent activations of ERK are known to lead to different cell fates, in that early and transient activation of ERK by EGF stimulates proliferation of PC12 cells, whereas prolonged and sustained activation by NGF induces neuronal differentiation in response to NGF (*56, 57*).

ASK1 also has divergent effects on neuronal cells. Overexpression of the constitutively active form of ASK1 activates JNK and induces apoptosis in NGF-differentiated PC12 cells and primary rat sympathetic neurons, and the dominant-negative ASK1 reduces the neuronal apoptosis induced by NGF withdrawal from these cells (*58*). On the other hand, in undifferentiated PC12 cells, moderate expression of constitutively active ASK1 induces neural differentiation through activation of p38 (*59*). These results suggest that strong activation of ASK1–JNK pathway leads to apoptosis, whereas strong activation of the ASK1–p38 pathway (with weak or no activation of JNK) leads to differentiation of neuronal cells. The mechanism by which ASK1 induces differential activation of JNK and p38 depending on cell-type and/or cellular context is unknown. ASK1-specific scaffold proteins, adaptor proteins and phosphatases may be involved in the regulation of subcellular localization, timing and duration of signals, to determine the biological outcome of the ASK1 signaling pathway.

Interestingly, when MEFs derived from ASK1-deficient mice were stimulated with TNFα or various oxidants, only the sustained phases of JNK and p38 activation were suppressed. Transient activations of JNK and p38 were not impaired in ASK1−/− MEFs (*30*). Moreover, ASK1−/− MEFs were resistant to TNFα- and oxidant-induced apoptosis. It is thus possible that TNFα- and oxidative stress–induced sustained but not transient activations of JNK and/or p38 are responsible for apoptosis, and that the ASK1–JNK/p38 pathway mainly mediates apoptosis, among TNFα- and oxidative stress-activated kinases. Transient activation of JNK/p38 (as well as ERK activation) may be mediated by other MAPKKKs, such as MEKKs or MLKs, and induce cell growth and differentiation but not apoptosis. The extent and duration of exposure to oxidative stress are important to determine subsequent cell fate, especially that of neuronal cells, because of susceptibility to redox alterations; excess or long exposure leads to cell death, whereas low or transient exposure leads to survival/differentiation (*60*). As a redox-sensor, ASK1 may drive apoptosis signaling only when cells are damaged lethally by excess and prolonged exposure to oxidative stress.

Furthermore, we observed that ASK1−/− MEFs were resistant to endoplasmic reticulum (ER) stress-induced apoptosis accompanied by drastic loss of activation of JNK and p38 (*61*). Most cellular proteins are translocated into the ER where post-translational modification, folding, and oligomerization occur. Accumulation of unfolded and misfolded proteins in the ER induces cellular stress and triggers the expression of a number of molecular chaperones, such as Bip/GRP78 and GRP94, which assist protein folding and promote cell survival. However, excess extent and long duration of ER stress eventually lead to apoptosis (*62*). ER stress signals have recently attracted considerable attention as a novel pathway responsible for pathological intracellular stress, in which the balance between survival and apoptosis signaling is a critical determinant of cell fate. Importantly, mutations or deletions of presenilin-1, which are implicated in Alzheimer's disease, influence the ER stress signaling pathway and facilitate neuronal apoptosis in patients (*63, 64*). IRE1, a transmembrane sensor protein in the ER, is one of the downstream targets of presenilin-1 and activates the JNK pathway by recruiting TRAF2 (*65*). Although the molecular mechanism of ASK1-mediated ER stress signaling is currently under investigation, it is possible that the ASK1–JNK pathway plays an important role in the progress of neurodegenerative disorders such as Alzheimer's disease.

Thus, ASK1 profoundly influences the decision of cell fate, such as survival/differentiation or apoptosis, allowing cells to make appropriate responses to an enormous variety of extracellular and intracellular stresses by balancing and integrating several different signals (Fig. 3). The MAP kinase cascade is evolutionarily well conserved from yeast to mammals, and is typically composed of three kinase modules that enable sequential and sigmoidal activation. Since apoptosis is not required for unicellular organisms such as yeast, the regulatory systems of apoptosis for multicellular organisms may be derived and developed from the stress signaling systems used by unicellular organisms to adapt themselves to changes in their surroundings. To the end, even by a single kinase pathway, a variety of distinct outcomes must have been produced by alterlating its timing, duration, extent and pattern. This implies that there is no discontinuity between various cellular responses, prolifera-

tion-survival-differentiation-apoptosis, which are determined by minor changes in the delicate balance between anti- and pro-apoptotic signalings.

Conclusions

The complicated and antagonistic machineries composed of many pro- and anti-apoptosis factors are necessary for multicellular organisms as a safeguard to prevent erroneous triggering of death or proliferation. The balancing effects of these factors at various checkpoints of signal transduction culminate in a final decision to die or to live, and the collapse of this balance leads to a variety of diseases. Although many apoptotic pathways have been identified, as described in this review, further study is required for deeper understanding of the physiological importance of the numerous molecules implicated in apoptosis. Current studies on apoptosis will lead to the development of new therapeutic strategies; to have cells die when they are unneeded and to rescue cells from death when they are needed.

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