which normally retain NF-κB within the cytoplasm of unstimulated cells. The past 5 years have witnessed tremendous advances in our understanding of this branch of the TNF signaling network. Especially noteworthy was the identification of the multiprotein IκB kinase (IKK) complex that mediates phosphorylation of IκB in a TNF-dependent manner (*4*). The core of the IKK complex consists of two catalytic subunits, IKKα and IKKβ, and a regulatory subunit, NF-κB essential modulator (NEMO, or IKKγ). In addition, the IKK complex contains a kinase-specific chaperone consisting of Cdc37 and Hsp90 that plays a role in shuttling the complex from the cytoplasm to the membrane. The IKK complex is also recruited to TNF-R1, where it becomes activated within minutes of TNF treatment. This activation depends on RIP, indicating that the IKK activation within the receptor complex likely occurs through a RIP-dependent intermediate factor, perhaps a kinase. Gene knockout studies in mice have established essential roles for IKKβ in TNF-induced activation of NF-κB, and for NEMO in regulation of IKK complex activation in response to numerous upstream signals. In contrast, IKKα plays only a minor role in TNF-induced activation of NF-κB, but it has other important functions, such as serving as a NF-κB2/p100 kinase in B cells.

An interesting feature of the TNF signaling network is the existence of extensive cross talk between the apoptosis, NF-κB, and JNK signaling pathways that emanate from TNF-R1. In the absence of NF-κB activity, cellular susceptibility to TNF- induced apoptosis increases, whereas enforced activation of NF-κB protects against apoptosis. Similarly, TNF-induced JNK activation is stronger and more prolonged in cells lacking NF-κB, and the products of several NF-κB–activated genes inhibit activation of JNK by TNF. Moreover, NF-κB activation prompts the resynthesis of IκB and other inhibitory molecules, such as the cIAPs, thereby adding another layer of regulation of the duration and amplitude of TNF signaling.

To date, most of the players in the TNF pathway have been validated by both biochemical and genetic means, thus providing a rich source of potential drug targets for the development of a new generation of anti-inflammatory agents. However, many questions remain unanswered. For example, what MAPKKK initiates the kinase cascade that activates JNK, and how is this kinase recruited to TNF-R1 and activated within the receptor complex in response to TNF? In the case of IKK complex activation, the possibility remains that an intermediate factor or kinase is required between RIP and NEMO. Unraveling the molecular details of how the enzymes like caspase-8 and the IKK complex become activated within the TNF-R1 complex will be key to a full understanding of the dynamic nature of TNF signaling. Finally, the molecular basis for cross talk between TNF-mediated apoptosis, NF-κB, and JNK signaling pathways is not well understood. Deciphering these puzzles will greatly help interpret how a specific outcome of TNF signaling is achieved in distinct biological contexts.

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VIEWPOINT

The Fas Signaling Pathway: More Than a Paradigm

Harald Wajant*

Apoptosis and related forms of cell death have central importance in development, homeostasis, tumor surveillance, and the function of the immune system. Apoptosis is initiated by two principal pathways. The intrinsic pathway emerges from mitochondria, whereas the extrinsic pathway is activated by the ligation of death receptors. This Viewpoint introduces the basic mechanisms of the extrinsic pathway, using the example of the prototypical death receptor Fas and its role in apoptosis, but it also points out the increasingly understood importance of this receptor as a non-apoptotic signal transducer.

Fas (also called Apo-1 or CD95) is a death domain–containing member of the tumor necrosis factor receptor (TNFR) superfamily. It has a central role in the physiological regulation of programmed cell death and has been implicated in the pathogenesis of various malignancies and diseases of the immune system (*1*, *2*) [see Fas Signaling Pathway, http://stke.sciencemag.org/cgi/cm/CMP_7966 (*3*) and Fas Signaling Pathway in Cardiomyocytes, http://stke.sciencemag.org/cgi/cm/CMP_9993 (*4*)]. Although the Fas ligand (FasL)–Fas system has been appreciated mainly with respect to its death-inducing function, it also transduces proliferative and activating signals through pathways that are still poorly defined (*1*, *2*).

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In the absence of membrane-bound ligand, inactive complexes of Fas are formed by the pre–ligand-binding assembly domain of the molecule (*2*). Interaction with membrane-bound FasL (or agonistic antibodies) reorganizes these complexes and allows the formation of a death-inducing signaling complex (DISC). The Fas DISC contains the adaptor protein Fas-associated death domain protein (FADD) and caspases 8 and 10, which can initiate the process of apoptosis. FasL-induced clustering of Fas, FADD, and caspase-8 or -10 within the DISC results in autoproteolytic processing of these caspases by induced proximity and in release of the processed active proteases (Fig. 1). In type I cells, processed caspase-8 is sufficient to directly activate other members of the caspase family, whose action on defined substrates paves the way to the execution phase of apoptosis (*1*). In type II cells, proper activation of effector caspases by Fas depends on an amplification loop that relies on caspase-8–mediated cleavage of the pro-apoptotic Bcl-2 family member Bid and subsequent release of mitochondrial pro-apoptotic factors [for example, cytochrome c and second mitochondria-derived activator of caspases (SMAC, also called Diablo)] to drive the formation of the caspase-9–activating apoptosome. Active caspase-9 activates the executioner caspase-3, which in turn activates caspase-8 outside the Fas DISC, thereby completing a positive feedback loop (*1*).

Each step in Fas-mediated apoptosis can be a target of regulatory mechanisms enabling cells to show flexible responses to stimulation by Fas. Corresponding to the hierarchy of events in Fas-mediated apoptosis, these regulatory mechanisms can be specific for Fas or common to death receptors, or they can affect the apoptotic core machinery of the cell. The FasL gene is transcriptionally inactive in most cells. Thus, regulation of FasL expression itself, for example, by the transcription factors nuclear factor kappa B (NF-κB), activating protein 1 (AP1), or nuclear factor of activated T cells (NF-AT), regulates FasL/Fas-mediated effects, such as those of activation-induced cell death of $CD4^+$ T cells (*5*). To a lesser extent, regulation of Fas expression is also used to control Fas responses, for example,

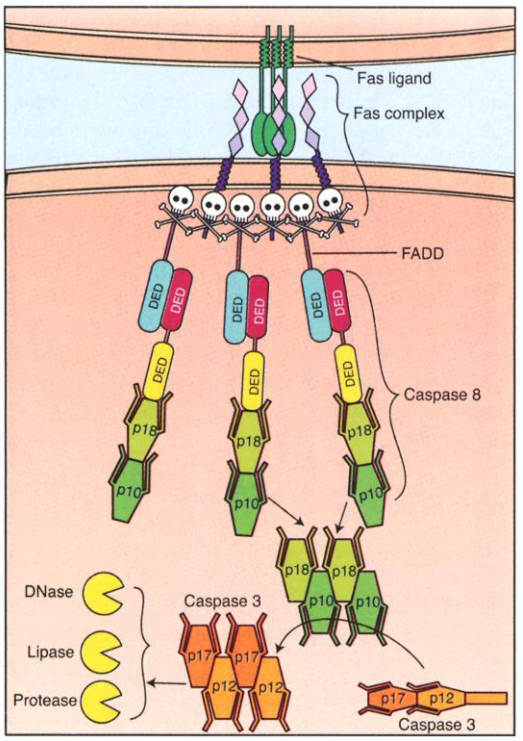

Fig. 1. Fas L-induced apoptosis occurs by autoproteolytical processing of caspase-8 (for details, see text).

in p53-induced apoptosis. Stimulation of Fas by membrane-bound FasL can be antagonized by the soluble decoy receptor DcR3, by various Fas isoforms lacking the transmembrane and/or death domains, and by soluble FasL generated by proteolytic processing or alternative splicing. The caspase-8–activating capacity of the Fas-DISC is mainly regulated by FADD-like interleukin-1β–converting enzyme (FLICE)–like inhibitory protein (FLIP) (*6*). FLIP exists in several isoforms that are structurally similar to caspase-8 although lacking in enzymatic activity (*6*). FLIP can be incorporated into the DISC of death receptors, thereby disabling DISC-mediated processing and release of active caspase-8 (*6*). In addition, Fas-mediated apoptosis is controlled by a plethora of regulators of the mitochondrial pathway of cell death, for example, by Bcl-2 family members, SMAC, or inhibitor of apoptosis proteins (*1*). Fas-mediated cell death occurs not only by apoptosis but also, depending on the cellular context, by necrosis. Fas-induced necrosis requires the adaptor protein FADD and the Fas-interacting serine/threonine kinase receptor-interacting protein (RIP), whereas caspase-8 seems to be dispensable (*7*). However, the molecular mechanisms linking Fas, FADD, and RIP to the execution processes of necrosis (for example, the production of reactive oxygen species) are not yet clear.

Although Fas is recognized predominantly as a death inducer, it also transduces proliferative signals in normal human diploid fibroblasts and T cells (*2*). The signaling pathways underlying Fas-induced proliferation might be partly related to the apoptotic pathway. In fact, stimulation of T cell growth by FasL can be blocked by caspase inhibitors. In addition, caspase-8, FADD, and FLIP are implicated in Fas-induced expression of the proto-oncogene c-*fos*, and mice deficient in these molecules have a defect in T cell proliferation. To what extent the Fas-mediated proliferation is related to activation of NF-κB, another non-apoptotic response elicited by Fas, remains to be clarified. A role of c-Jun NH_2-terminal kinase (JNK) activation in Fas-mediated proliferation seems rather unlikely, because FADD is normally not required for the activation of JNK. Rather, Fas promotes JNK activation through interaction with the Fas-binding protein DAXX and apoptosis signal–regulated kinase 1 (Ask1), a member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family. Although JNK activation often correlates with Fas-mediated apoptosis, the pro-apoptotic effect of JNK activation (for example, up-regulation of FasL) normally does not directly contribute to Fas-dependent cell death. Some recent studies point to a role of JNK activation in Fas-mediated cardiac hypertrophy that occurs in response to the stress of pressure overload in the absence of apoptosis (*8*).

Since the cloning of Fas in 1991, tremendous progress has been made in the understanding of the molecular basis of apoptosis induction by this receptor. Nevertheless, many facets of Fas function are still poorly understood, in particular with respect to its non-apoptotic functions. Thus, it is easy to predict that, for now, the FasL-Fas system will remain as a death receptor paradigm; however, research in the years to come should shed more light onto other, non-apoptotic functions of these prominent members of the TNF/TNFR family.

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VIEWPOINT

G Protein Pathways

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The heterotrimeric guanine nucleotide–binding proteins (G proteins) are signal transducers that communicate signals from many hormones, neurotransmitters, chemokines, and autocrine and paracrine factors. The extracellular signals are received by members of a large superfamily of receptors with seven membrane-spanning regions that activate the G proteins, which route the signals to several distinct intracellular signaling pathways. These pathways interact with one another to form a network that regulates metabolic enzymes, ion channels, transporters, and other components of the cellular machinery controlling a broad range of cellular processes, including transcription, motility, contractility, and secretion. These cellular processes in turn regulate systemic functions such as embryonic development, gonadal development, learning and memory, and organismal homeostasis.

Heterotrimeric guanine nucleotide-binding proteins (G proteins) are signal transducers, attached to the cell surface plasma membrane, that connect receptors to effectors and thus to intracellular signaling pathways (*1*). Receptors that couple to G proteins communicate signals from a large number of hormones, neurotransmitters, chemokines, and autocrine and paracrine factors. After the first four G proteins (G_s, G_t, G_i, and G_o) were identified by biochemical purification, a large number of G proteins and their subunits were identified by cDNA cloning (*2*). G proteins consist of three subunits, α, β, and γ. When signaling, they function in essence as dimers because the signal is communicated either by

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