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News and Views

Induced proximity model attracts NF-κB researchers

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Changes in a cell's environment are integrated into complex signal transduction mechanisms that decide whether a cell survives or succumbs in response to a noxious stimulus. Two important determinants in this delicate life–death balance are signaling pathways leading to caspase and NF-κB activation. Both pathways are regulated by small protein–protein interaction motifs that glue signaling components together primarily by homophilic interaction and transmit signals to downstream effectors. A recent paper by Núñez and colleagues now suggests that these interaction motifs may be multifunctional and capable of controlling caspase and NF-κB activation by a common molecular mechanism, called induced proximity activation.[1]

In the apoptosis pathways three types of interaction domains have been identified: the death domain (DD), the death effector domain (DED) and the caspase recruitment domain (CARD).[2–4] The DD is relevant for receptor signaling. Receptors such as TNF-R1 and CD95 recruit DD-containing adaptors such as TRADD and FADD (Figure 1). FADD in turn binds via an additional DED motif to caspase-8 and forms thereby the death-inducing signaling complex (DISC). In the other apoptotic cascade triggered by mitochondria CARD interactions are important. In this pathway, the adaptor Apaf-1 self-associates to a large macromolecular complex instigated by binding of cytochrome *c* and dATP. The hereby induced conformational change of Apaf-1 facilitates binding of caspase-9 via CARD–CARD interaction resulting in the formation of the apoptosome. For both the DISC and the apoptosome, the model of induced proximity has been proposed to explain how the first proteolytic signal is produced after clustering of the inactive caspase precursors.[5–7] According to this model multimolecular aggregation of the adaptors enforces a locally high concentration of caspase zymogens. The close proximity of the zymogens then allows the immature proteases to self-activate due to their low intrinsic enzymatic activity.

In contrast to the apoptotic caspase cascades, the mechanisms that bring together signaling components of the anti-apoptotic transcription factor NF-κB are less understood. The critical step in activating NF-κB is the phosphorylation-induced degradation of its inhibitor IκB which is controlled by a large multi-protein complex consisting of two IκB kinases, α and β, and a non-catalytic, regulatory subunit, called IKKγ (NEMO).[8] In the TNF-RI-induced NF-κB pathway the adaptor protein RIP plays a crucial role, as targeted disruption of the RIP gene completely abolished NF-κB activation.[9] RIP contains three structural domains: a DD that binds to the receptor-associated TRADD, an intermediate domain that interacts with IKKγ in the IKK complex, and a kinase region whose enzymatic activity appears to be not required. There is also a cross-regulation between the NF-κB and caspase pathway. For instance, RIP can be cleaved by caspase-8 and this cleavage may play a role in regulating the balance between life and death in response to TNF.[10]

Very recently, several new RIP molecules such as RICK (RIP-2/CARDIAK) and RIP-3 have been implicated in NF-κB and caspase activation.[11–15] Like RIP, RICK contains an intermediate region, but unlike RIP, RICK carries a CARD instead of a DD. Intriguingly, RIP and RICK appear to be engaged in two separate NF-κB activation pathways, resembling the two caspase pathways. Whereas RIP is involved in the TNF-RI pathway, RICK seems to control receptor-independent NF-κB activation via interaction with the Apaf-1-related protein Nod1/CARD4.[16,17] Like Apaf-1, Nod1 contains an N-terminal CARD and an essential nucleotide-binding domain (NBD), but carries leucine-rich repeats at its C-terminus instead of the WD40 repeats present in Apaf-1. Interestingly, these leucine-rich repeats are also present in plant disease resistance genes, suggesting that Nod1 may play an evolutionary conserved role in stress responses. However, it is unknown how Nod1 is activated under physiological conditions.

As pointed out by Núñez and colleagues,[1] the Nod1/RICK pathway is structurally and functionally highly related to both the Apaf-1-mediated caspase cascade and the receptor-mediated NF-κB pathway. What are these similarities? *(i)* For both RIP- and RICK-induced NF-κB activation their intermediate region is required as it bridges the IKK complex to IKKγ. *(ii)* Similar to Apaf-1-induced caspase-9 activation, binding of RICK to upstream Nod1 is mediated by homophilic CARD interaction following self-oligomerization of Nod1. *(iii)* This self-association is inhibited by the C-terminal WD40 repeats in the case of Apaf-1 and the leucine-rich regions in the case of Nod1, respectively. *(iv)* In both molecules binding of ATP or other nucleotides to the NBD probably induces an unfolding which unmasks the CARD and allows binding of downstream components.

Based on the structural and functional similarities, Núñez and coworkers now propose the idea that, analogously to the activation of procaspases, also NF-κB activation may be mediated by oligomerization-induced proximity activation. To test this hypothesis, the authors exchanged the interaction domains of the different molecules by tandem repeats of a derivative of the FK506-binding protein. This elegant system has been first designed by the group of Schreiber as an artificial mimic of molecular recruitment processes.[18] Indeed, in all four pathways, namely in the FADD/caspase-8, TRADD/RIP, Apaf-1/caspase-9 and the

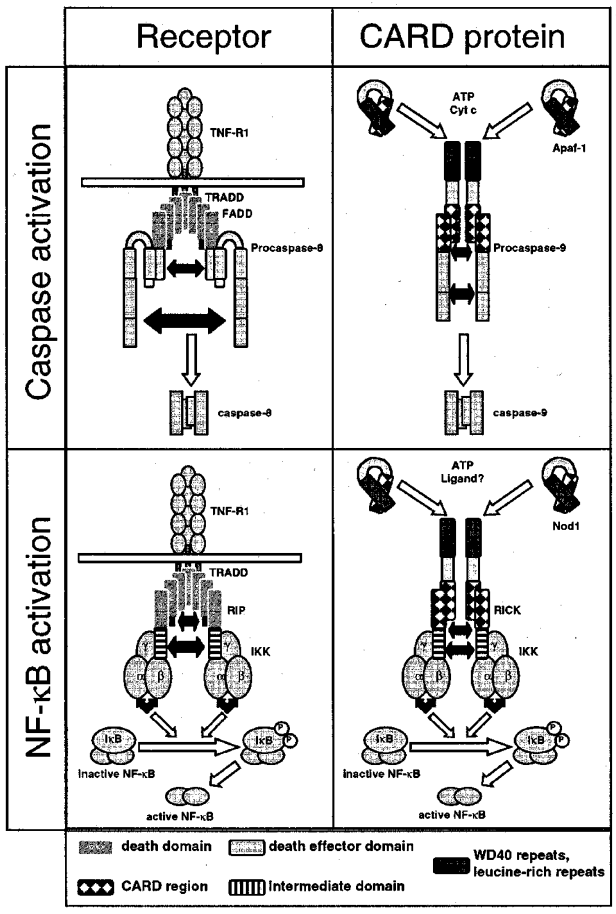

Figure 1 The model of induced proximity in the caspase and NF-κB activation pathway. In the two caspase activation pathways, clustering of a death receptor, such as TNF-R1, or dATP together with cytochrome *c* triggers the oligomerization of adaptors, such as TRADD/FADD and Apaf-1, respectively. The clustered adaptors then bring the caspase precursors into close proximity to an adjacent caspase zymogen. It is assumed that procaspase-8 and -9 are subsequently activated due to their low intrinsic activity. A similar mechanism of induced proximity activation may be functional in the two pathways leading to NF-κB activation. The adaptors RIP and RICK are recruited to upstream TRADD and Nod1, respectively. Prior to this event, the molecules are oligomerized by TNF-R1 ligand binding or an ATP-induced conformational change of Nod1, which also occurs in Apaf-1. According to the induced proximity model, the enforced oligomerization of RIP or RICK leads to binding of the IKK regulatory subunit IKKγ. This step brings the IKK complex in close proximity to the receptor or Nod1 complex, resulting in IKK and NF-κB activation.

Nod1/RICK pathway, the relevant molecules become oligomerized and activated with a dimeric form of FK506. This observation is fully consistent with the model of induced proximity activation. Apaf-1 and Nod1, respectively, associate via an oligomerization domain that is located in the NBD. Two or more molecules of caspase-9 or RICK are then brought into proximity through CARD interaction. However, whereas caspases are activated due to their low intrinsic activity, it is unclear whether RICK kinase activity is actually required for the downstream events. Kinase-dead mutants of RICK exhibit partially decreased NF-κB activation, indicating that RICK clustering may indeed induce autoactivation of its kinase activity and promote subsequent IKKγ binding.

Notwithstanding the attractiveness of the induced proximity model for NF-κB activation, a number of questions remain: for example, what is the link between the TNF-RI/TRADD/RIP or the Nod1/RICK complex and the many other adaptors and kinases that have been implicated in NF-κB activation, such as TRAF2, NIK, MEKK1 or Akt. Similarly, are those or additional molecules required to stabilize the interaction of the huge IKK signalosome with TNF-R1 and Nod1? Since Nod1/RICK interaction can promote NF-κB as well as caspase activation, it remains also unclear why two opposing pathways are induced and by which checkpoints their final outcome is controlled. In any case, it is becoming clear that signal transduction is not merely mediated by the single interaction of two molecules, but requires tremendously large multi-protein complexes. Induced proximity thus may provide a more general model for the generation of biochemical signals.

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