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## Editorial

# The flick of a switch: which death program to choose?

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Two major switches determine cell fate. The first, and most important, is the binary decision between life and death.The second, following opening of the death circuit, determines whether death occurs by apoptosis or necrosis. What factors determine whether the switch goes up or down, and are there other subtleties that influence the selected pathway? Now, novel *in vivo* data have shed light on the molecular balance between apoptosis and necrosis, solving an enigma in the field.[1]

For a long time, necrosis was considered an accidental and uncontrolled mode of cell death. Quite recently, however, novel experimental evidence has provided unexpected models of death receptor (DR)-mediated necrosis that have profoundly changed our view and caused a new revival in cell-death research. Indeed, several DRs signal by inducing either apoptosis or programmed necrosis, often referred to as necroptosis. Necroptosis is a cell-death modality that can occur in the absence of caspase activity and is even enhanced in the presence of synthetic or natural caspase inhibitors.[2] How then does a cell select whether to die by apoptosis or by necrosis (or even to survive)?

The kinase activities of receptor-interacting protein 1 (RIPK1) and RIPK3 have been found to be essential regulators of DR-induced necrosis, while the platform function of RIPK1 is implicated in survival signaling. The recent paper by Dillon *et al.*[1] investigates the molecular balance between apoptosis and necrosis *in vivo*. In particular, why are knock-outs of *caspase-8*, *fadd* and *flip* embryonically lethal at e10.5, and therefore, are very different from knockout mice lacking other major components of the extrinsic and intrinsic apoptotic pathways such as *tnfr1*, *fasr*, *bid*, *apaf1*, *casp9* and *casp3*? This suggests that the presence of the FADD/caspase-8/FLIP platform performs a survival function that goes beyond their role in the initiation of apoptosis.

When TNF binds to its cognate DR, TNFR1, it initiates the formation of dynamic complexes, which are regulated by ubiquitylation, phosphorylation and proteolysis. The initial binding of TNF results in the formation of a membrane-associated complex I, which is highly ubiquitylated, consisting of numerous proteins (such as TRADD, TRAF2, IAP1, LUBAC, TAK1 and RIPK1) and which initiates a signaling cascade that involves the activation of MAPKs and the canonical NF-κB pathway. This eventually leads to the induction of genes, which modulate cell survival and inflammatory cytokine production. This endosome-associated survival/inflammation complex I can be switched to a cytosolic pro-death complex II (consisting of FADD, caspase-8, FLIP, RIPK1 and RIPK3) by interfering with the ubiquitylation status (for review, see Vandenabeele *et al.*[3]). A similar cytosolic complex II, called the ripoptosome, can be initiated in a TNF-dependent or -independent way by etoposide-induced DNA damage and by inhibiting or knocking down IAPs.[4] In the ripoptosome, a new decision can emerge – death by apoptosis (generally regarded as the default program) or by necroptosis (the backup program or the 'ejector seat' when apoptosis does not function properly). This necrotic ejector seat is initiated by the mutual cooperation between the two serine/threonine kinases RIPK1 and RIPK3, interacting through a homotypic RHIM domain, resulting in multiple auto- and transphosphorylations – from which the specific cellular fates have not yet been established – resulting in the formation of a necrosome complex. Because RIPK1 kinase activity under conditions of IAP1 and IAP2 deficiency or inhibition, for example, conditions of ripoptosome formation, can also lead to RIPK1-mediated apoptosis, the crucial necroptosis-initiating kinase is considered to be RIPK3. The elucidation of its direct substrates has only recently been started by Xiaodong Wang's group, implicating a RIPK3 (kinase)/MLKL (adaptor)/PGAM5 (phosphatase)/DRP1 (GTPase) signaling axis in the fission process during necroptosis.[5] Whether the latter mitochondrial process is really essential for the execution of necrosis remains to be shown.

The paradigm of the FADD, caspase-8 and FLIP trinity controlling the necrotic ejector seat program has been shown in cells[6,7] and *in vivo* by the rescue of *caspase-8*$^{-/-}$ and *fadd*$^{-/-}$ embryonic lethality by crossing with *ripk3* knockouts.[7,8] The contribution of FADD and caspase-8 to necrosis regulation and inflammatory conditions has been studied in more detail by using intestine- and skin-specific conditional knockout mice.[9–12] But what about the contribution of the third member of the anti-necrotic trinity? As elegantly shown by Guy Salvesen's group, the presence of $FLIP_L$ favors a heteromeric caspase-8/ $FLIP_L$ constellation, which prevents activated caspase-8 being released.[7] This retention of heteromeric caspase-8/ $FLIP_L$ probably allows proteolytic

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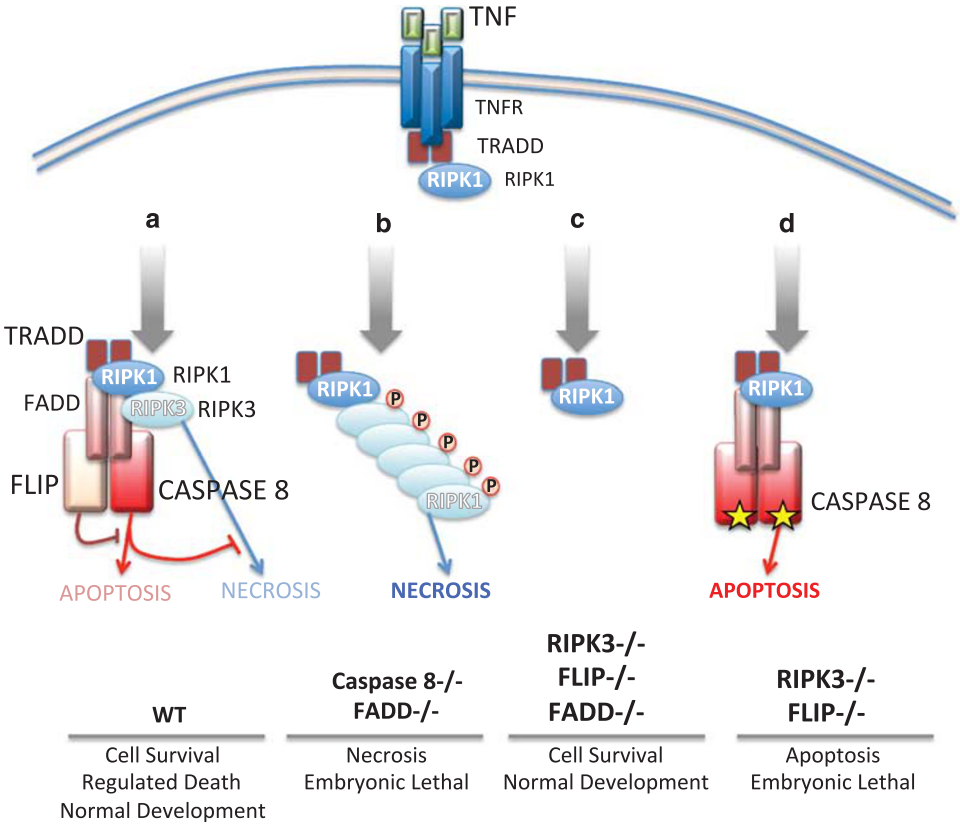


**Figure 1** Caspase-8 has a crucial role in the regulation of DRs. Downstream of DRs like TNF, caspase-8 is part of the death-inducing signaling complex, recruited by its interaction with FADD, (**a**). Here, it inhibits the activation of RIPK3 by RIPK1, thus also regulating necroptosis. At the same time caspase-8 is bound and regulated in its pro-apoptotic function by its interaction with FLIP. Consequently, both apoptosis and necrosis can result from activation of the DR in a regulated manner. (**a**) In a normal receptor, the regulated interaction of these elements allows a controlled cell death. Wild-type mice therefore show normal development with a regulated death pathway. (**b**) Knockout mice for caspase-8, *caspase-8*$^{-/-}$, FADD, *fadd*$^{-/-}$ or double knockouts for both show an embryonically lethal phenotype due to uncontrolled necrosis by active (phosphorylated) RIPK3. (**c**) Triple knockouts for *fadd*$^{-/-}$, *flip*$^{-/-}$ and *ripk3*$^{-/-}$ have a normal cell-death pathway and develop to normal birth because of absence of necrosis (driven by RIPK3) and apoptosis (driven by FADD and thus inability to recruit caspase-8). (**d**) Double-knockout mice for *ripk3*$^{-/-}$; *flip*$^{-/-}$ die during embryonic development due to uncontrolled apoptosis driven by active (stars) caspase-8. Modified from Dillon *et al.*[1]

cleavage of substrates involved in pro-necrotic signaling such as RIPK1, RIPK3 and CYLD.[13] Recent work by Doug Green's group focuses on the *in vivo* controlling function of FLIP.[1] Knocking out the *flip* gene abolishes both FLIP isoforms, that is, $FLIP_S$, a competitive inhibitor of caspase-8 recruitment, and $FLIP_L$, a competitive inhibitor of caspase-8 but which would still allow local caspase-8 activity if the heteromeric complex is formed.[7] FLIP deficiency thus favors both massive apoptosis and necrosis. In contrast to *caspase-8* and *fadd* single-knockout embryos, which are perfectly rescued by crossing with *ripk3* knockout mice, the only way to overcome embryonic lethality resulting from *flip* deficiency is by a combination of fadd and ripk3 deficiency, which prevents both apoptotic and necrotic signaling. The biological evidence for this pathway has been provided very recently by work based on multiple knockout mice[1] (Figure 1). Indeed, the figure shows the effect of different double- and triple-knockout mice lacking different components of this crucial complex.[1] These experiments demonstrate that FLIP is an important brake on both apoptotic and necrotic cell death *in vivo*.

There are many ways to die,[14] and to regulate the apoptosis–necrosis switch, for example by ATP[15] or by nitric oxide.[16] Indeed, molecular switches between apoptosis and necrosis include adenosine triphosphate-dependent steps in the activation of caspases or steps sensitive to reactive oxygen/nitrogen species, as shown in the nervous system.[15] *In vivo*, programmed necrosis occurs mainly in pathophysiological processes such as ischemia-reperfusion injury in heart, brain and kidneys, viral infection, pancreatitis and sepsis,[9] and is capable of killing tumor cells that have developed strategies to evade apoptosis.[3] Thus, detailed knowledge of DR-induced necrosis signaling may be exploitable therapeutically in novel ways. Moreover, it is crucial to establish whether the expression and activity of the brakes (such as FADD, caspase-8, FLIP and IAPs) and gears (such as RIPK3 and CYLD) of the necrotic pathway are affected in particular pathologies.

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