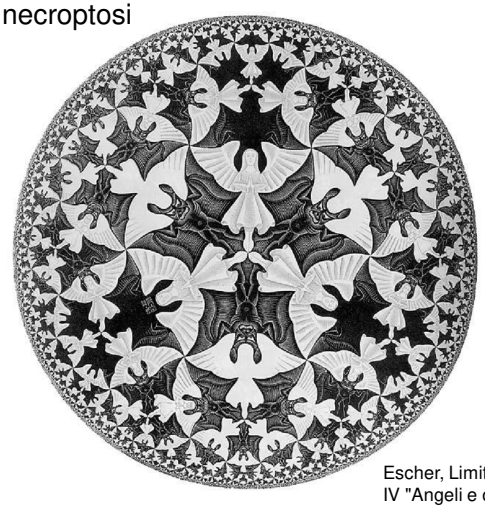
NFκB e necroptosi



Escher, Limite del cerchio
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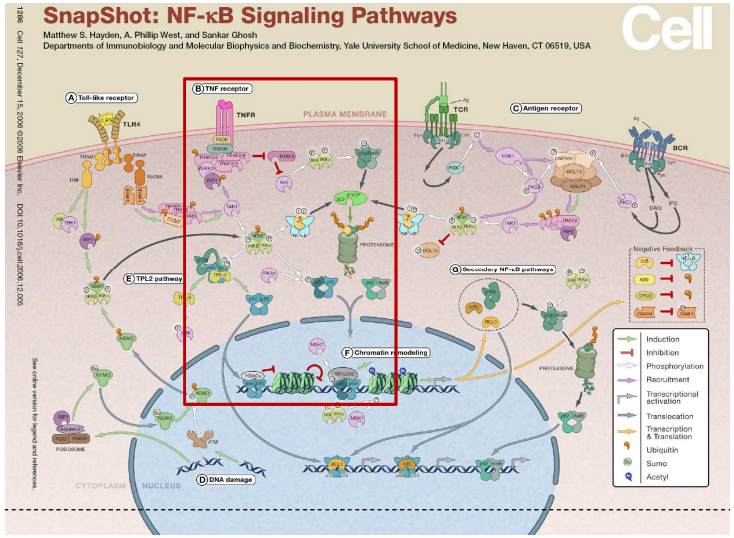


Figure Legend

(A) Toll-like receptor signaling. A Toll-like receptor (TLR) bound to ligand recruits TIR-containing adaptor molecules, MyD88 and/or TRIF, which bind to downstream effectors IRAK1–4 and RIP1, respectively. Active IRAKs bind to TRAF6 and TAK1, which subsequently activate the IKK complex. Ubiquitination of TRAF and the regulatory subunit of the IKK complex, NEMO, facilitates activation of the catalytic IKKβ subunit. This results in the phosphorylation and proteasomal degradation of IκB and the release of heterodimers of the master transcription factor NF-κB.

(B) TNF receptor signaling. Signaling via members of the TNF receptor superfamily, such as TNFR1, leads to recruitment of the adaptor proteins FADD and TRADD, several TRAF family members (TRAF2, 5, and 6), and the kinase RIP1. The TRAF/RIP complex recruits and activates TAK1, which induces activation of the IKK complex and downstream signaling via the NF-κB pathway. When stimulated, a subset of TNFR superfamily members that bind to TRAF3 (CD40, LTβR, BAFFR) induce TRAF3 degradation resulting in accumulation of the kinase NIK, which undergoes constitutive degradation in the absence of stimulation. Accumulated NIK phosphorylates and activates IKKα. IKKα, in turn, induces processing of the NF-κB family member p100 into p52 and, thus, activation of p52-containing NF-κB complexes (prototypically RelB:p52).

(C) Antigen receptor signaling. Signaling via the T cell receptor (TCR), or analogously through the B cell receptor (BCR), recruits the Src family tyrosine kinases, ZAP70 and Syk, leading to production of inositide-3-phosphate (IP3) and diacylglycerol (DAG). Activation of PI3K induces activation of PDK1, which recruits and activates protein kinase C (PKC) family members and the CARMA1/BCL10/MALT1 (CBM) complex. PKC phosphorylates CARMA1 resulting in activation and oligomerization of the CBM complex, recruitment of TRAF6, and stimulation of the classical TAK1 to IKK signaling pathway. Active IKK may also exert negative feedback through phosphorylation of BCL10.

(D) DNA damage response. DNA damage triggers activation of the PIDDosome (containing RIP1/PIDD/RAIDD) leading to sumoylation of NEMO and its translocation to the nucleus. DNA damage also activates the kinase ATM, leading to phosphorylation and ubiquitination of NEMO, which returns to the cytoplasm and mediates IKK activation.

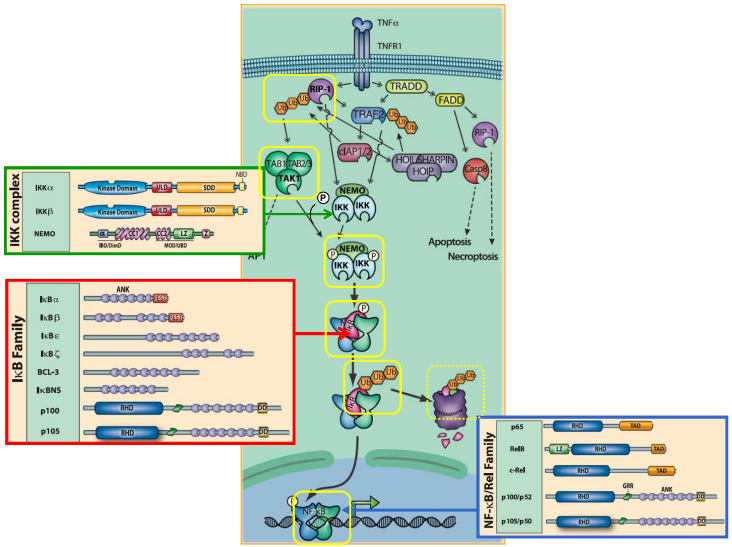
(E) TPL2 pathway. Active IKK phosphorylates p105 resulting in the release of p50 homodimers, which translocate to the nucleus and active TPL2, which then activates the ERK signaling pathway.

(F) Chromatin remodeling. NF-κB activation leads to expression of target genes by regulating chromatin structure. Unphosphorylated p65-containing NF-κB heterodimers or p50 homodimers bind to repressive histone deacetylases (HDACs) and suppress transcription. In the cytoplasm, p65 can be phosphorylated by IKK and protein kinase A (PKA), and in the nucleus by MSK1. This enables recruitment of histone acetyl transferases (HATs) including CBP/p300 resulting in the acetylation of histones, relaxing of chromatin structure and activation of gene expression. Phosphorylation of histones by IKKα or MSK1 directly may also promote transcription.

(G) Secondary NF-κB pathways. Active NF-κB induces expression of components of the NF-κB signaling pathway including p100, BCL3, and IκBζ. IκBζ forms transcriptionally active complexes with p50 homodimers. BCL3 may form either repressive or active complexes with p50 and p52 dimers, depending on posttranslational modifications of BCL3. Transcription of p100 allows full activation of the alternate NF-κB pathway through IKKα-induced processing of p100 to p52. NF-κB also induces negative feedback through expression of IκB, deubiquitinating enzymes A20 and CYLD, and IRAKM, which blocks phosphorylation of IRAK1.

Abbreviations

CARMA1, caspase recruitment domain-membrane associated guanylate kinase 1; CBP/p300, cyclic-adenosine monophosphate response element binding protein/p300; CYLD, cylindromatosis; ECSIT, evolutionarily conserved signaling intermediate in Toll/IL1R pathways; ERK, extracellular signal-regulated kinase; FADD, Fas-associated death domain protein; IKK, inhibitor of kappa B kinase; IκB, inhibitor of kappa-B; IRAK, interleukin-1 receptor-associated kinase; LPS, lipopolysaccharide; MALT1, mucosal-associated lymphoid tissue; MEKK, mitogen-activated protein/ERK kinase kinase; MSK1, mitogen and stress-activated kinase-1; NEMO, nuclear factor kappa B essential modifier; NF-κB, nuclear factor-kappa B; NIK, nuclear factor kappa B inducing kinase; PI3K, phosphatidylinositol 3-kinase; PIDD, p53-induced protein with a death domain; PKAc, protein kinase A catalytic subunit; RAIDD, RIP-associated ICH-1 homologous protein with a death domain; RIP, receptor-interacting protein; SCF, Skp1 cullin F box; SUMO, small ubiquitin-related modifier; TAK1, transforming growth factor-β-activated kinase 1; TBK1, TANK binding kinase-1; TICAM, TIR-containing adaptor molecule; TIR, Toll/IL1 resistance; TIRAP TIR-containing adaptor protein; TLR, Toll-like receptor; TNFR, tumor necrosis factor receptor; TPL, tumor progression locus; TRADD, TNF receptor-associated death domain-containing protein; TRAF, tumor necrosis factor receptor-associated factor; TRAM, TRIF-related adaptor molecule; TRIF, TIR-containing adaptor inducing interferon β.

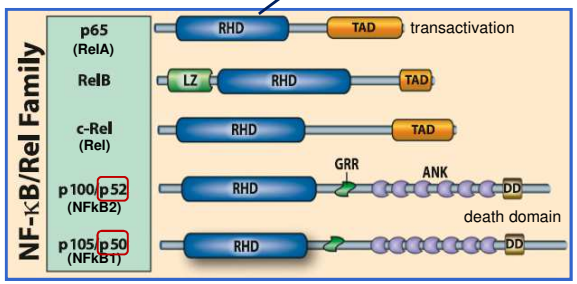


REVIEW GENES & DEVELOPMENT 26:203–234

NF-κB, the first quarter-century: remarkable progress and outstanding questions

Matthew S. Hayden[1,2,3] and Sankar Ghosh[1,3]

[1]Department of Microbiology and Immunology, [2]Department of Dermatology, Columbia University, College of Physicians and Surgeons, New York, New York 10032, USA



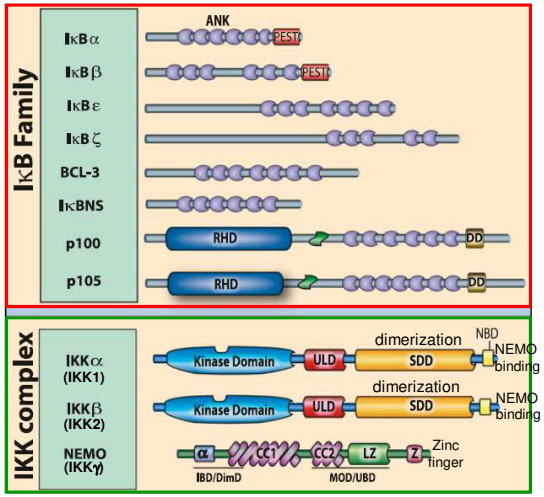
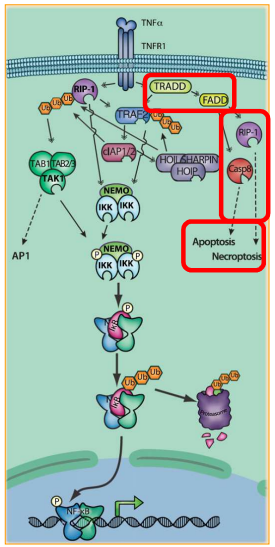


Figure 2. Components of the NF-κB pathway. The mammalian Rel (NF-κB) protein family consists of five members: p65 (RelA), RelB, c-Rel (Rel), and the precursor proteins p100 (NF-κB2) and p105 (NF-κB1), the latter giving rise to p52 and p50, respectively. The IκB family consists of eight bona fide members, IκBα, IκBβ, IκBε, IκBζ, BCL-3, IκBNS, p100, and p105, which are typified by the presence of multiple ankyrin repeat domains. Not pictured is the potential IκB family member IκBη, which is discussed in the text. The IKK complex consists of IKKα (IKK1 or CHUK), IKKβ (IKK2), and NEMO (IKKγ). Relevant domains typifying each protein family are indicated. (ANK) Ankyrin repeat domain; (DD) death domain; (RHD) REL homology domain; (TAD) transactivation domain; (LZ) leucine zipper domain; (GRR) glycine-rich region; (SDD) scaffolding and dimerization domain; (ULD) ubiquitin-like domain; (Z) zinc finger domain; (CC) coiled-coil domain; (NBD) NEMO-binding domain; (α) α-helical domain; (IBD/DimD) IKK-binding domain/dimerization domain; (MOD/UBD) minimal oligomerization domain/ubiquitin-binding domain; (PEST) proline-rich, glutamic acid-rich, serine-rich, and threonine-rich.



CELL DEATH AND AUTOPHAGY — OPINION

Regulated necrosis: the expanding network of non-apoptotic cell death pathways

Tom Vanden Berghe, Andreas Linkermann, Sandrine Jouan-Lanhouet, Henning Walczak and Peter Vandenabeele

Abstract | Cell death research was revitalized by the understanding that necrosis can occur in a highly regulated and genetically controlled manner. Although RIPK1 (receptor-interacting protein kinase 1)- and RIPK3–MLKL (mixed lineage kinase domain-like)-mediated necroptosis is the most understood form of regulated necrosis, other examples of this process are emerging, including cell death mechanisms known as parthanatos, oxytosis, ferroptosis, NETosis, pyronecrosis and pyroptosis. Elucidating how these pathways of regulated necrosis are interconnected at the molecular level should enable this process to be therapeutically targeted.

Necroptosi

- Il termine indica una morte necrotica «programmata»
- La necroptosi è generalmente il risultato di segnali di morte sia intra- che extracellulari associati ad un blocco dell'apoptosi
- La morfologia della cellula che va in necroptosi è identica alla necrosi
- Necroptosi è importante per attivare l'infiammazione, per avere un'adeguata risposta al patogeno o per avere un'azione anti-tumorale
- La necroptosi, come la necrosi, rilascia DAMP (Damage-Associated Molecular Patterns) che attivano l'infiammazione

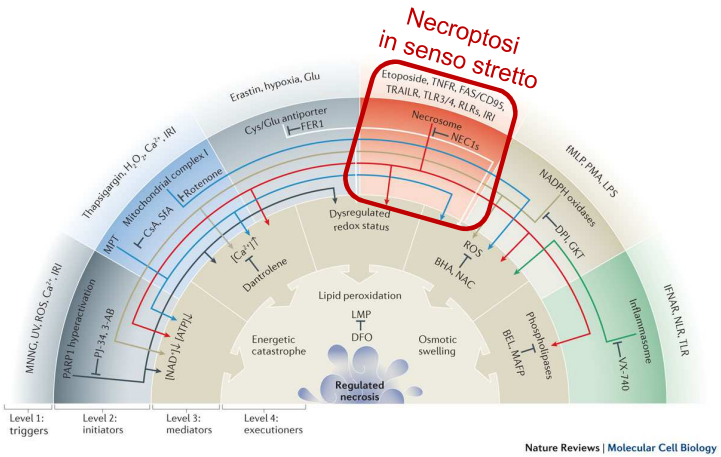
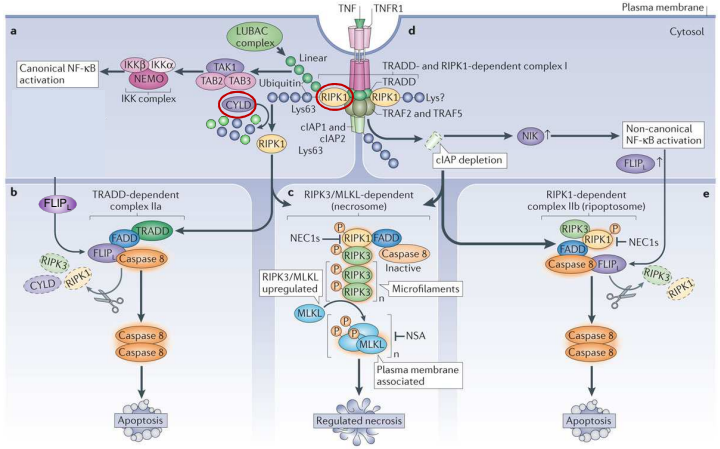


Figure 1 | **An integrated view of the emerging modes of regulated necrosis.** Regulated necrosis can be induced by poly(ADP-ribose) polymerase 1 (PARP1) hyperactivation, mitochondrial permeability transition (MPT), mitochondrial complex I, theCys/Glu antiporter, the necrosome, NADPH oxidases and the inflammasome. Diverse pathophysiological stimuli can trigger (level 1) each of these initiators (level 2), which can be blocked by the listed specific inhibitors. The coloured arrows indicate the established links between the initiator signals and various common intracellular mechanisms that mediate regulated necrosis (level 3), such as NAD^+ and ATP-depletion, Ca^{2+} overload, dysregulation of the redox status, increased production of reactive oxygen species (ROS) and the activity of phospholipases. All of these factors are mediators of regulated necrosis, and even at this level, inhibitors such as dantrolene, BHA (butylated hydroxyl anisole), NAC (*N*-acetyl-Cys), BEL (bromoenol lactone) and MAFP (methyl-arachidonyl fluorophosphonate) may interfere with necrotic signalling. Importantly, similar mediators can act downstream of various initiators, through different mechanisms. The complex interconnected effects

of the mediators on cellular organelles and membranes results in the activation of processes that execute regulated necrosis (level 4), including cellular osmotic swelling, bioenergetic breakdown that results in energetic catastrophe, lipid peroxidation and the loss of lysosomal membrane integrity through lysosomal membrane permeabilization (LMP). Note that feedback loops are not included for simplicity. 3-AB, 3-aminobenzamide; CsA, cyclosporin A; DFO, deferoxamine; DPI, diphenylen iodonium; FER1, ferrostatin 1; fMLP, *N*-formylated methionyl-leucyl-Phe; GKT, GKT137831 (an NADPH oxidase 1 and NADPH-oxidase 4 inhibitor; Genkyotex); IFNAR, IFNα/β receptor; IRI, ischaemia–reperfusion injury; LPS, lipopolysaccharide; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; NEC1s, more specific and stable variant of necrostatin 1; NLR, NOD-like receptor; PJ-34, an inhibitor of poly(ADP-ribose) polymerase; PMA, phorbol-12-myristate-13-acetate; RIG-I, retinoic acid-inducible gene 1; RLR, SfA, sanglifehrin A; TLR, Toll-like receptor; TNFR, tumour necrosis factor receptor; TRAILR, TNF-related apoptosis-inducing ligand receptor; UV, ultraviolet light; VX-740, a caspase 1 inhibitor.



(modificata)

Cell Death and Differentiation (2010) 17, 25–34

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Review

CYLD: a tumor suppressor deubiquitinase regulating NF-κB activation and diverse biological processes

S-C Sun*,1

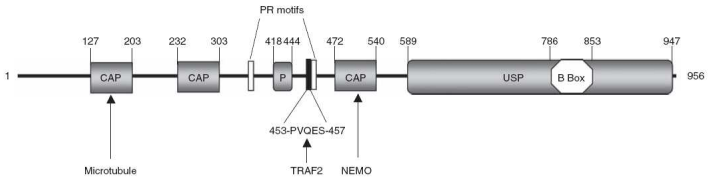

Figure 3 Structural domains of cylindromatosis (CYLD). The C-terminal portion of CYLD contains a ubiquitin-specific protease (USP) catalytic domain, with an inserted a zinc-binding B-box domain. The N-terminal portion of CYLD has three CAP-Gly domains (CAP), the third of which forms the NF-κB essential modulator (NEMO)-binding domain, and two proline-rich (PR) motifs. CYLD also contains a TRAF-binding motif (PVQES) and a phosphorylation (P) region. The C-terminal portion of CYLD (amino acids 470–957) is known to bind Bcl-3, although the precise Bcl-3-binding domain has not been defined

Cell Death and Differentiation (2017) 24, 1172–1183

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Review

CYLD, A20 and OTULIN deubiquitinases in NF-κB signaling and cell death: so similar, yet so different

Marie Lork[1,2], Kelly Verhelst[1,2] and Rudi Beyaert*,[1,2]

Polyubiquitination of proteins has a pivotal role in the regulation of numerous cellular functions such as protein degradation, DNA repair and cell signaling. As deregulation of these processes can result in pathological conditions such as inflammatory diseases, neurodegeneration or cancer, tight regulation of the ubiquitin system is of tremendous importance. Ubiquitination by E3 ubiquitin ligases can be counteracted by the activity of several deubiquitinating enzymes (DUBs). CYLD, A20 and OTULIN have been implicated as key DUBs in the negative regulation of NF-κB transcription factor-mediated gene expression upon stimulation of cytokine receptors, antigen receptors and pattern recognition receptors, by removing distinct types of polyubiquitin chains from specific NF-κB signaling proteins. In addition, they control TNF-induced cell death signaling leading to apoptosis and necroptosis via similar mechanisms. In the case of A20, also catalytic-independent mechanisms of action have been demonstrated to have an important role. CYLD, A20 and OTULIN have largely overlapping substrates, suggesting at least partially redundant functions. However, mice deficient in one of the three DUBs show significant phenotypic differences, indicating also non-redundant functions. Here we discuss the activity and polyubiquitin chain-type specificity of CYLD, A20 and OTULIN, their specific role in NF-κB signaling and cell death, the molecular mechanisms that regulate their activity, their role in immune homeostasis and the association of defects in their activity with inflammation, autoimmunity and cancer.

Different types of ubiquitination are recognized by specific ubiquitin-binding domain (UBD)-containing proteins that mediate downstream signaling. Ubiquitination can be reversed by deubiquitinating enzymes (DUBs). Human cells contain ~100 DUBs belonging to six families. Five families, the ovarian tumor (OTU), the ubiquitin-specific proteases (USPs), the ubiquitin C-terminal hydrolases, the Josephin domain family and the newly discovered motif interacting with ubiquitin-containing novel DUB family (MINDY) are papain-like cysteine proteases, whereas JAB1/MPN/Mov34 metalloenzyme domain family members are zinc-dependent metalloproteases.[4,5] Mutations in specific DUBs have been linked with neurodegeneration, chronic inflammation, autoimmunity, infectious disease and cancer. Several DUBs, such as CYLD, A20 and OTULIN, act as negative regulators of NF-κB signaling and have an important role in TNF-induced cell death signaling.

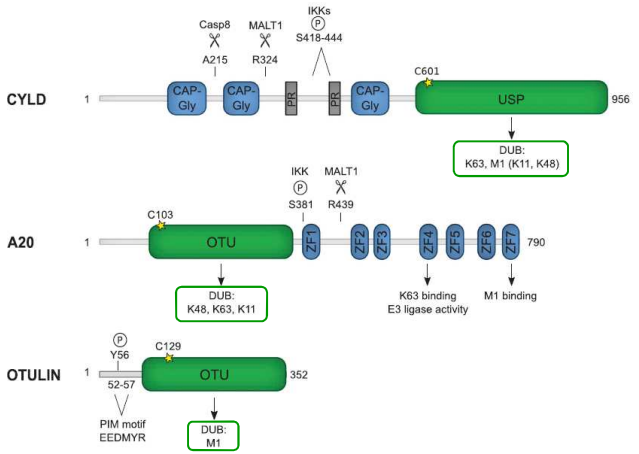

Figure 1 Domain structure of CYLD, A20 and OTULIN. CYLD contains three cytoskeleton-associated protein glycine-rich (CAP-Gly) domains that mediate microtubule binding, two proline-rich (PR) motifs and the USP domain harboring its DUB activity. A20 consists of an OTU responsible for its DUB activity and seven C-terminal zinc finger (ZF) domains. ZF4 mediates E3 ligase activity as well as binding to K63 polyubiquitin. ZF7 specifically binds M1 polyubiquitin. OTULIN is mostly made up of its OTU domain. An N-terminal PUB-interacting motif (PIM) is essential for its interaction with HOIP. Catalytic cysteines conferring the DUB activity are indicated by a star. Proteolytic processing sites by caspase-8 and MALT1, as well as specific phosphorylation sites (P), are indicated. Amino-acid numbering is for the human proteins

Table 1 Substrates that are deubiquitinated by CYLD, A20 and OTULIN in the context of NF-κB signaling and cell death

	DUB substrate	Receptor/stimulus	Pathway
CYLD	TRAF2	TNFR1	NF-κB
	NEMO	TNFR1	NF-κB
	TNFR1	TNFR1	NF-κB, cell death
	TRADD	TNFR1	NF-κB, cell death
	TAK1	TCR, TNFR1	NF-κB, JNK
	RIPK2	NOD2	NF-κB, MAPK
	RIPK1	TNF, TLR	NF-κB, MAPK, cell death
	TRAF6	TLR2, TNFR1	NF-κB, p38
	TRAF7	TLR2	NF-κB, p38
A20	RIPK1	TNFR1	NF-κB
	TRAF6	TLR4	NF-κB, MAPK
	RIPK2	NOD2	NF-κB
	NEMO	TNFR1, TCR	NF-κB
	MALT1	TCR, BCR	NF-κB
	Caspase-8	DR4, DR5	Apoptosis
	RIPK3	TNFR1 (+CHX/zVAD)	Necroptosis
	TNFR1	TNFR1	NF-κB, cell death
OTULIN	RIPK1	TNFR1, LUBAC expression	NF-κB, JNK
	NEMO	TNFR1, LUBAC expression	NF-κB, JNK
	RIPK2	NOD2	NF-κB
	TNFR1	TNFR1	
	HOIP	—	LUBAC autoubiquitination
	HOIL-1	—	LUBAC autoubiquitination
	Sharpin	—	LUBAC autoubiquitination

Abbreviations: BCR, B-cell receptor; CHX, cycloheximide; DR, death receptor; DUB, deubiquitinating enzyme; IL-1R, interleukin-1 receptor; JNK, c-Jun N-terminal kinase; LUBAC, linear ubiquitin assembly complex; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-κB; NOD2, nucleotide-binding oligomerization domain-containing protein 2; TNFR, tumor necrosis factor receptor; TCR, T-cell receptor; TLR, toll-like receptor; zVAD, carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone

Facts

- CYLD, A20 and OTULIN are key DUBs in the regulation of NF-κB and cell death signaling.
- CYLD, A20 and OTULIN target partially overlapping protein substrates.
- CYLD, A20 and OTULIN hydrolyze M1 and K63 polyubiquitin chains with different specificities.
- A20 has catalytic-independent mechanisms of action.
- Defects in CYLD, A20 or OTULIN are associated with inflammation, autoimmunity and cancer.

Cell

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The Deubiquitinase OTULIN Is an Essential Negative Regulator of Inflammation and Autoimmunity

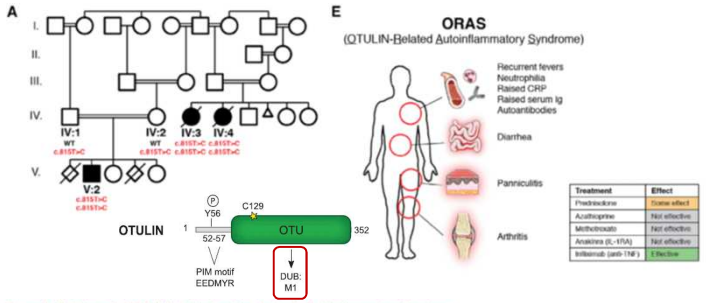
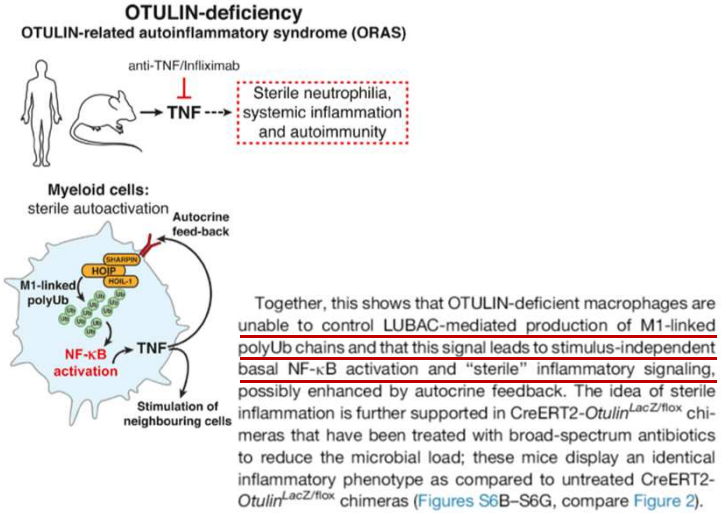


Figure 1. Mutations in *OTULIN* in Patients with a Systemic Autoinflammatory Syndrome

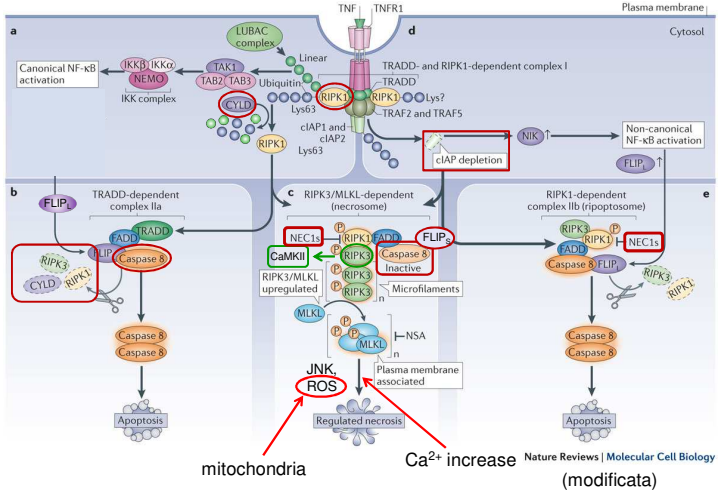
(A) Segregation of the inflammatory symptoms (filled symbols) and the *c.815T>C* substitution in *OTULIN* in the affected kindred. ○, females; □, males; double lines, consanguineous relationship; crossed symbols, deceased individuals; Δ, miscarriage; ◇, stillbirths. Roman numerals indicate generations.
(B and C) Lifetime measurements of (B) C-reactive protein (CRP) serum concentrations and (C) white blood cell (WBC, black line) and neutrophil numbers (cyan line) in blood from patients IV:3, IV:4, and V:2. Reference ranges (dotted lines) are indicated on the graphs. Patient V:2 was treated with Infliximab as indicated (orange shade).



Together, this shows that OTULIN-deficient macrophages are unable to control LUBAC-mediated production of M1-linked polyUb chains and that this signal leads to stimulus-independent basal NF-κB activation and "sterile" inflammatory signaling, possibly enhanced by autocrine feedback. The idea of sterile inflammation is further supported in CreERT2-*Otulin*$^{LacZ/flox}$ chimeras that have been treated with broad-spectrum antibiotics to reduce the microbial load; these mice display an identical inflammatory phenotype as compared to untreated CreERT2-*Otulin*$^{LacZ/flox}$ chimeras (Figures S6B–S6G, compare Figure 2).

Open Questions

- Why are (at least) three different DUBs with partially overlapping activities required to regulate NF-κB signaling and cell death?
- How is the catalytic activity or substrate binding capacity turned on or off?
- Do CYLD and OTULIN have catalytic-independent activities similar to A20?
- Is there crosstalk between the different DUBs?
- Can we modulate CYLD, A20 and OTULIN activity for therapeutic purposes?



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The FEBS Journal
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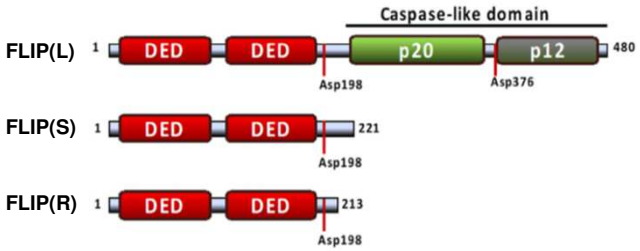
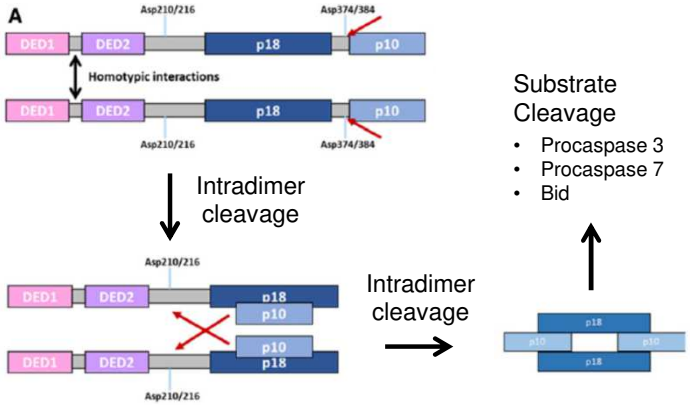
REVIEW ARTICLE

FLIP(L): the pseudo-caspase doi:10.1111/febs.15260 (2020)

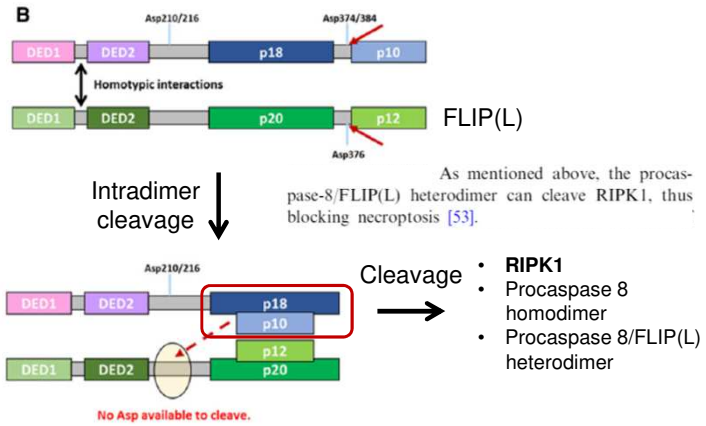
Peter Smyth, Tamas Sessler, Christopher J. Scott and Daniel B. Longley



Omodimero procaspasi 8



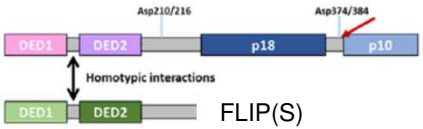
Eterodimero procaspasi 8-FLIP(L)



As mentioned above, the procaspase-8/FLIP(L) heterodimer can cleave RIPK1, thus blocking necroptosis [53].

Fig. 3. Processing and substrates of procaspase-8 homodimers and procaspase-8/FLIP(L) heterodimers. (A) Homodimerization is initially mediated via homotypic DED interactions. Within a homodimer, the caspase-8 catalytic domains are arranged in an antiparallel fashion. This creates an enzymatic active site, which can then cleave adjacent homodimers in the region between large (p18) and small (p10) catalytic subunits. This cleavage enables the second activation step to take place, which is intradimer cleavage in the linker region between p18 and the DEDs. **The p18/p10 heterotetramers that are formed can be released from the complex and activate apoptosis** by cleaving procaspases-3/7 and BID. **(B) Formation of a heterodimer between the (pseudo)catalytic regions procaspase-8 and FLIP(L) is energetically favorable compared with caspase-8 homodimers**. This promotes formation of a FLIP(L)/caspase-8 heterodimeric enzyme that **can efficiently cleave adjacent procaspase-8 homodimers** between their large and small catalytic subunits, thereby promoting processing of these homodimers. **The heterodimer also cleaves adjacent heterodimers and RIPK1**. The lack of critical cysteine in FLIP(L)'s 'active site' and lack of a suitable target site for caspase-8 in the region between FLIP(L)'s DED2 and p20 subunit prevent intradimer cleavage of the heterodimer, which is therefore **retained in the complex**.

Eterodimero procaspasi 8-FLIP(S)



FLIP(S)

(Elaborazione personale)

FLIP(S) inhibits apoptosis via caspase-8, but cannot block necroptosis mediated by RIPK1 as it has no catalytic activity. Thus, both FLIP splice forms modulate the activity of caspase-8 in these complexes, leading to distinct signaling outcomes.

FLIP as a mediator of therapy resistance

One of the reasons behind the association between high FLIP expression and poor prognosis is that FLIP confers resistance to a number of therapeutics.

FLIP expression and drug resistance in cancer

FLIP has been shown to be overexpressed in a number of cancer types, including non-small-cell lung cancer (NSCLC) [10], colorectal cancer [120–123], pancreatic cancer [124], nasopharyngeal carcinoma [125], stomach cancer [126,127], meningiomas [128], urothelial cancer [129], prostate cancer [93], acute myeloid leukaemia [130], Burkitt's lymphoma [131], cervical carcinomas [132,133], ovarian carcinoma [134] and breast cancer [135,136]. It is also well established that the viral FLIP K13 has antiapoptotic activity and plays an important role in the pathogenesis of gamma-herpesvirus HHV-8-associated tumours, including Kaposi's sarcoma, primary effusion lymphoma and multicentric Castleman's disease [137–139]. Several themes have emerged that influence the impact of FLIP expression on cellular phenotype, including subcellular localization of the protein, splice form-specific effects and the stage of the tumour.

Table 1. Some examples of agents reported to inhibit FLIP expression.

Agents	Mechanism of action	References
Cisplatin, gemcitabine	DNA damaging agents/Anti-metabolites	[124,141,152, 153, 156]
Etoposide	Topoisomerase II inhibitor	[154]
Vorinostat (SAHA), amurensin G	Histone deacetylase inhibitor	[147, 161]
Antisense oligonucleotides and siRNAs	Blockade of mRNA translation, and RNAi	[170]
5-fluorouracil (5-FU)	Thymidylate synthase (TS) inhibitor	[124,153]
LY294002, PIK75	PI3K inhibitors	[163,165]
SNS-032	CDK9 inhibitors	[163]
KN-93	CAMK II inhibitors	[167]

- Qual è la correlazione tra sovraespressione di FLIP(L) e maggiore malignità del tumore, inducendo esso l'apoptosi? A che fine le cellule tumorali selezionano un meccanismo che ne causa la morte?

(Le citazioni sono da Smyth et al (2020) FLIP(L): the pseudo-caspase, *FEBS J.* doi:10.1111/febs.15260)

In recent years, a number of models of DISC assembly have been proposed. In 2012, it was reported simultaneously by two groups that FADD and FLIP are highly substoichiometric at the DISC relative to procaspase-8, and it was subsequently proposed that the DISC is composed of **DED chains in which procaspase-8 is the predominant protein**. In a further development, cryo-EM studies suggested that individual DED chains interact with one another to form helical filaments. It was later proposed that recruitment of FLIP(S) into procaspase-8 DED chains terminates the chains and thereby limits procaspase-8 activation, **whereas FLIP(L) promotes DED-mediated procaspase-8 oligomerization and thereby promotes caspase-8 activation**. However, **an apoptosis-promoting function for FLIP(L) is at odds with a number of siRNA-based and overexpression studies, which indicate an anti-apoptotic function**. Our recent alternative model suggests the existence of shorter procaspase-8/FLIP tandem DED chains linking FADD molecules attached to adjacent DR trimers. **This model can explain the ability of FLIP(L) to both activate and inhibit DISC-mediated apoptosis**; as depending on the levels of procaspase-8 and FLIP(L) in the short chains, there are two potential scenarios:

- **Apoptosis inhibition**: High levels of FLIP(L) will result in a predominance of FLIP(L)/procaspase-8 heterodimers; therefore, **the lack of procaspase-8 homodimers will prevent sufficient processing of procaspase-8 to its apoptosis active heterotetramer.**
- **Apoptosis activation**: Low levels of FLIP(L) will result in a predominance of procaspase-8 homodimers; in this scenario, **the FLIP(L)/caspase-8 heterodimer can promote the rate-limiting first cleavage step of adjacent procaspase-8 homodimers and thereby promote apoptosis induction.**

We have recently defined the stoichiometry of FLIP(L):procaspase-8 at the DISC at which these two opposing functions operate. **Generally, total cellular levels of procaspase-8 exceed those of FLIP(L) even in cancer cells which have elevated FLIP expression**; however, FLIP DISC recruitment is more efficient.

At low levels of receptor activation, the number of DISCs formed will be low and there will therefore be a predominance of heterodimers (**FLIP(L):procaspase-8 ratio ~ 1 : 1**) and **apoptosis will be inhibited (scenario 1)**.

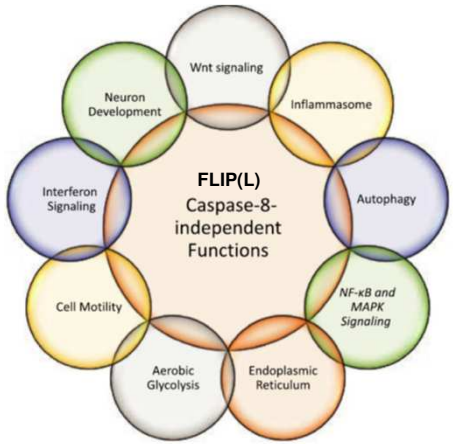
At high levels of receptor activation, FLIP(L) levels will become depleted relative to the more highly expressed procaspase-8 (**FLIP(L):procaspase-8 ratio << 1 : 1**) and there will therefore be a **predominance of homodimers, the activation of which will be accelerated by corecruited heterodimers (scenario 2)**.

Thus, FLIP(L) acts in what could be considered a classic pseudoprotease manner at the DISC, functioning to regulate the rate and extent of activation of the protease from which it evolved.

The picture is much simpler for FLIP(S) and FLIP(R) as they form inactive heterodimers with procaspase-8, and so act as **straightforward inhibitors of procaspase-8 processing and apoptosis induction**.

In pratica

- **Alti livelli di FLIP(L) (1:1 con procaspasi-8) → inibizione dell'apoptosi;**
- **Bassi livelli di FLIP(L) (<< 1:1 con procaspasi-8) → attivazione dell'apoptosi.**



SCIENCE SIGNALING | REVIEW Jensen *et al.*, *Sci. Signal.* **13**, eaax8295 (2020) 18 February 2020

INFLAMMATION

SMAC mimetics and RIPK inhibitors as therapeutics for chronic inflammatory diseases

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New therapeutic approaches for chronic inflammatory diseases such as inflammatory bowel disease, rheumatoid arthritis, and psoriasis are needed because current treatments are often suboptimal in terms of both efficacy and the risks of serious adverse events. Inhibitor of apoptosis proteins (IAPs) are E3 ubiquitin ligases that inhibit cell death pathways and are themselves inhibited by second mitochondria-derived activator of caspases (SMAC). SMAC mimetics (SMs), small-molecule antagonists of IAPs, are being evaluated as cancer therapies in clinical trials. IAPs are also crucial regulators of inflammatory pathways because they influence both the activation of inflammatory genes and the induction of cell death through the receptor-interacting serine-threonine protein kinases (RIPKs), nuclear factor κB (NF-κB)–inducing kinase, and mitogen-activated protein kinases (MAPKs). Furthermore, there is an increasing interest in specifically targeting the substrates of IAP-mediated ubiquitylation, especially RIPK1, RIPK2, and RIPK3, as druggable nodes in inflammation control. Several studies have revealed an anti-inflammatory potential of RIPK inhibitors that either block inflammatory signaling or block the form of inflammatory cell death known as necroptosis. Expanding research on innate immune signaling through pattern recognition receptors that stimulate proinflammatory NF-κB and MAPK signaling may further contribute to uncovering the complex molecular roles used by IAPs and downstream RIPKs in inflammatory signaling. This may benefit and guide the development of SMs or selective RIPK inhibitors as anti-inflammatory therapeutics for various chronic inflammatory conditions.

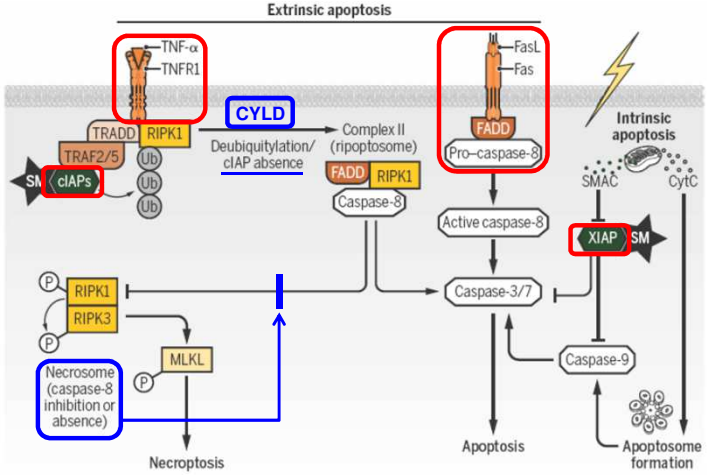


Fig. 4. Cell death pathways. Activation of TNFR1 by TNF-α stimulates canonical NF-κB signaling in a manner that depends on the assembly of complex I (TRADD, TRAF2 or TRAF5, RIPK1, and cIAPs) and on cIAP-mediated ubiquitylation (Ub) of RIPK1 (Fig. 3). If cIAPs are absent or inhibited by an SM or if RIPK1 is deubiquitylated, then stimulation of TNFR1 leads to the formation of complex II (also called the ripoptosome or death complex), resulting in cell death. This complex, comprising FADD, RIPK1, and caspase-8, can stimulate necroptosis or apoptosis. It promotes necroptosis by stimulating the formation of the necrosome (RIPK1, RIPK3, and MLKL), leading to phosphorylation and oligomerization of MLKL and disruption of the plasma membrane. Complex II promotes apoptosis by promoting the activation of caspases. Activation of death receptors, such as Fas, stimulates the recruitment of FADD and pro–caspase-8, which leads to cleavage and activation of caspase-8, thus enabling activation of the effector caspases (caspase-3 and caspase-7) that execute apoptosis. Cytokine death ligand pathways, like those induced by TNF-α or FasL, are known as extrinsic apoptosis. Intrinsic apoptosis, which is triggered by nonreceptor-mediated stimuli, such as DNA damage, involves mitochondrial release of cytochrome C (CytC) and SMAC. CytC is crucial for the formation of the apoptosome, which activates caspase-9, culminating in mitochondrial-mediated apoptosis by caspase-3 and caspase-7. XIAP inhibits caspase-3, caspase-7, and caspase-9 to prevent intrinsic apoptosis; inhibition of XIAP by endogenous SMAC or exogenous SM promotes apoptosis.

It is evident that IAPs constitute a group of functionally complex proteins because they exhibit multiple distinct functions, for instance, the ability to directly inhibit caspases, as seen for XIAP, or to act as E3 ubiquitin ligases tagging proteins either for degradation or signaling, as seen for cIAP1, cIAP2, and XIAP. cIAPs are not solely positive but are also negative regulators of NF-κB activation; their ubiquitylating activity can be degradative (for example, K48-linked ubiquitin of NIK) or function as signaling platforms (like K63-linked ubiquitin for RIPK1 or RIPK2). Some of the functions of the IAPs are redundant, or partially so, whereas some IAPs have unique functions.

Vie di segnale indotte dai «recettori di morte»

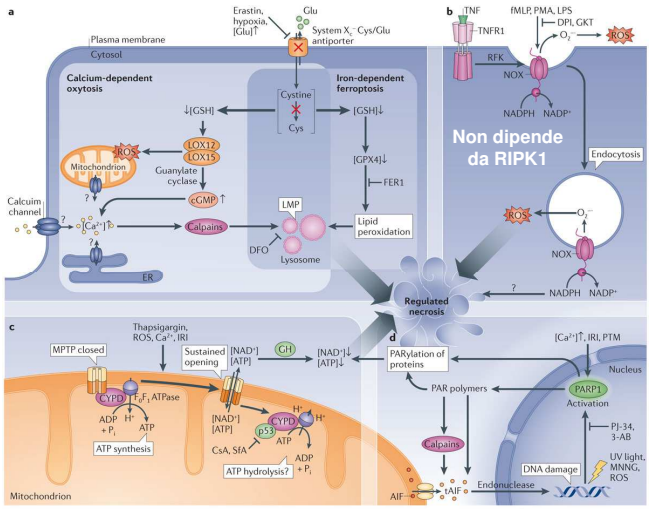
- Dopo il legame del ligando (TNFα), i recettori trimerici (TNFR1) reclutano specifiche proteine adattatrici (TRADD, TRAF2) a formare un complesso (**TNFR1 complex I**) che include cIAP1, cIAP2, RIP1K
- Le cIAP1, cIAP2 sono ubiquitin ligasi che ubiquitinano RIPK1 (M1,K63) permettendo il docking di TAK e TAB2/3, che porta ad attivazione della via canonica di NFkB
- Se la cellula produce **cylindromatosis D** (**CYLD**), che è un de-ubiquitinasi, RIPK1 viene deubiquitilata permettendo la formazione del **necrosoma, in cui viene reclutata RIPK3 e la caspasi 8 (inattiva)**

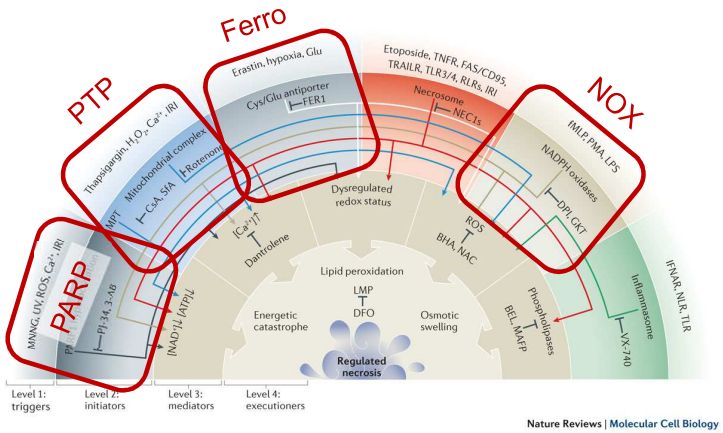
RIPK1

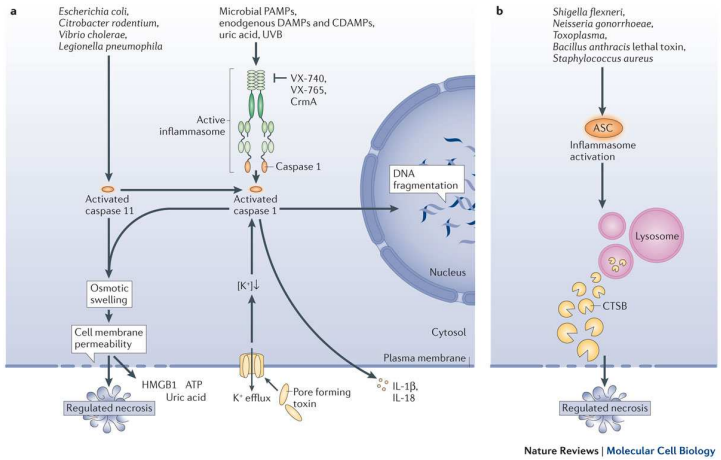
- Proteina critica per la necroptosi
- Proteina di 74 kDa, RIP1 ha un Death Domain (DD) al C-terminale
- Al N-terminale dominio Ser/Thr chinasi
- Un dominio intermedio critico per l'interazione con NFkB
- Il dominio intermedio contiene anche un **RIP homotypic interaction motif (RHIM)** che permette il legame con **RHIM di RIP3 per attivare la necroptosi**
- Necrostatin-1 (**Nec-1**) è una molecola di sintesi che inibisce RIP1

Ripoptosoma

- In seguito a diminuzione delle cIAP1 e cIAP2 (ad esempio per un danno genotossico) si creano le premesse per la formazione del ripoptosoma formato fa RIPK1, FADD, caspasi 8 e cFLIP
- Se nel complesso c'è la forma **cFLIP$_L$** si attiva caspasi 8 che degrada RIPK1 e si attua l'**apoptosi**
- Se nel complesso c'è la forma **cFLIP$_S$** RIPK1 non viene degradato, si associa con RIPK3 e si attiva la **necroptosi**






a
Escherichia coli,
Citrobacter rodentium,
Vibrio cholerae,
Legionella pneumophila
Microbial PAMPs,
enodgenous DAMPs and CDAMPs,
uric acid, UVB
VX-740,
VX-765,
CrmA
Active
inflammasome
Caspase 1
Activated
caspase 11
Activated
caspase 1
DNA
fragmentation
Nucleus
Osmotic
swelling
[K+]↓
Cytosol
Cell membrane
permeability
Plasma membrane
HMGB1 ATP
Uric acid
K+ efflux
Pore forming
toxin
IL-1β,
IL-18
Regulated necrosis
b
Shigella flexneri,
Neisseria gonorrhoeae,
Toxoplasma,
Bacillus anthracis lethal toxin,
Staphylococcus aureus
ASC
Inflammasome
activation
Lysosome
CTSB
Regulated necrosis
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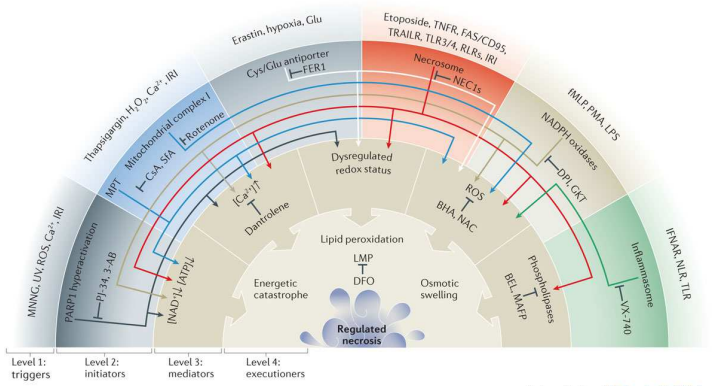
Erastin, hypoxia, Glu
Etoposide, TNFR, FAS/CD95,
TRAILR, TLR3/4, RLRs, IRI
Thapsigargin, H2O2, Ca2+, IRI
Cys/Glu antiporter
FER1
Necrosome
NEC1s
Mitochondrial complex I
Rotenone
MPT
CsA, SfA
fMLP, PMA, LPS
NADPH oxidases
DPI, GKT
Dysregulated
redox status
[Ca2+]↑
Dantrolene
ROS
BHA, NAC
MNNG, UV, ROS, Ca2+, IRI
PARP1 hyperactivation
PJ-34, 3-AB
[NAD+]↓ [ATP]↓
Lipid peroxidation
LMP
DFO
Energetic
catastrophe
Osmotic
swelling
Regulated
necrosis
Phospholipases
BEL, MAFP
Inflammasome
VX-740
IFNAR, NLR, TLR
Level 1:
triggers
Level 2:
initiators
Level 3:
mediators
Level 4:
executioners
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