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Regulatory Mechanisms of RIPK1 in Cell Death and Inflammation

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Abstract

Receptor Interacting Protein Kinase 1 (RIPK1) and RIPK3 are key adaptors that play critical roles in inflammatory and cell death signaling. Work in recent years have shown that their activities are tightly regulated by ubiquitination, phosphorylation and proteolysis. In addition to these post-translational modifications, the expression and activities of these kinases can further be tuned by environmental changes in pH and oxygen content. Proper control of these regulatory processes is crucial for the RIP kinases to execute their functions in immune responses and tissue homeostasis. In this review, we discuss recent advance in our understanding of the molecular mechanisms that regulate the activities of the RIP kinases. We will also discuss how the different regulatory mechanisms contribute to the functions of RIPK1 and RIPK3 in different pathophysiological settings.

Keywords

Ubiquitination; phosphorylation; Caspase; Inflammation; Apoptosis; Necroptosis

1. Introduction

Cell death is an important strategy employed by multicellular organisms to maintain tissue homeostasis and to defend against pathogen challenge. Members of the Receptor Interacting Protein Kinases (RIPKs) family are key regulators of cell death and inflammation. Recent studies showed that RIPK1 is a pleiotropic cell death adaptor that not only control apoptosis and necroptosis but also directly regulate inflammatory cytokine expression. In contrast to apoptosis, which is generally non-inflammatory and tolerogenic, lytic cell death such as necroptosis promotes inflammation. Necroptosis and other types of lytic cell death such as pyroptosis and ferroptosis are marked by cell swelling and rapid loss of plasma membrane integrity. Membrane rupture leads to the release of cellular contents such as IL1α, HMGB1, uric acid, ATP and DNA into the extracellular space. Because these cellular components are potent stimulants for inflammation, they have been referred to as "damage-associated molecular patterns" (DAMPs). Because of the prominent roles of RIPK1 and its associate kinase RIPK3 in cell death and inflammation, extensive work has been done in recent years to decipher the molecular mechanisms that regulate their activities. These regulatory

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processes are diverse in nature (e.g. post-translational modifications versus protein-protein interactions). Collectively, they tune the functions of these kinases to maintain tissue homeostasis and to promote functional differentiation of immune cells. As such, disease ensues when these regulatory processes are disrupted. Here, we will review recent findings on the mechanisms that regulate RIPK1 activities.

2. The activity of RIPK1 is controlled by different post-translational mechanisms

2.1 A ubiquitin scaffold restricts RIPK1 death-inducing activity

The biochemical events that regulate RIPK1 activity is best studied in TNFR1 signaling. TNF binding to its cognate receptor TNFR1 induces formation of a membrane signaling complex termed complex I [1]. RIPK1 is recruited to this complex via death domain (DD)-DD interaction with the cytoplasmic tail of the receptor. RIPK1 is heavily ubiquitinated in this complex. In addition to RIPK1, another DD-containing adaptor TRADD also binds to TNFR1. TRADD recruitment to complex I promotes RIPK1 ubiquitination through recruitment of the adaptors TRAF2, TRAF5 and the E3 ubiquitin ligases cIAP1 and cIAP2, which ubiquitinates RIPK1 and other adaptors in the complex. Hence, in *Tradd*$^{-/-}$ cells, recruitment and ubiquitination of RIPK1 to TNFR1 was severely impaired [2, 3] (Fig. 1). This led to the popular view that the ubiquitin scaffold on RIPK1 stabilizes complex I.

The key E3 ligases that regulate RIPK1 ubiquitination are cIAP1, cIAP2 and the linear ubiquitin assembly complex (LUBAC). The IAPs were once thought to promote predominantly K63-linked RIPK1 ubiquitination. It is now clear that RIPK1 is modified by other types of ubiquitin linkages [4]. For example, using a cIAP1 mutant that is defective in the ubiquitin-associated (UBA) domain, it was shown that cIAP1 could stimulate K48-linked ubiquitination of RIPK1 [5]. Besides stabilizing the TNFR1 complex, early studies suggest that ubiquitinated RIPK1 also imposes steric hindrance to prevent transition of the membrane-bound complex I to the death-inducing complex II in the cytosol [6]. Consistent with the notion that cIAP-mediated RIPK1 ubiquitination inhibits cell death, combined deficiency of cIAP1 and cIAP2 or XIAP led to embryonic lethality that could be delayed by single allele loss of *Ripk1* [7] (Table 1). Similar rescue of cell death and inflammation by *Ripk1* heterozygous loss was also observed in mice with skin epidermal deletion of cIAP1 and cIAP2 [8].

In contrast to the IAPs, LUBAC exclusively modify RIPK1 via M1-linked linear ubiquitination [9, 10]. LUBAC is composed of the catalytic subunit HOIP, HOIL-1 and the regulatory subunit SHARPIN. Disruption of any of the subunits of LUBAC causes hypersensitivity to TNF-induced cell death [11, 12]. Similar to the cIAP1/cIAP2 co-deletion model, inhibition of RIPK1 kinase activity modestly delayed embryonic lethality of *Hoip*$^{-/-}$ and *Hoil1*$^{-/-}$ mice [11, 13]. Inhibition of RIPK1 kinase activity was also insufficient to reverse skin inflammation in mice with skin epithelium specific deletion of *Hoip* or *Hoil1* (*Hoip*$^{E\text{-}KO}$ and *Hoil1*$^{E\text{-}KO}$) [12]. Strikingly, RIPK1 kinase inhibition did significantly inhibit lethal skin inflammation in *Hoil1*$^{E\text{-}KO}$ mice when TNFR1 was also deleted [12]. These

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results indicate that LUBAC-mediated ubiquitination did regulate RIPK1 kinase activity, although this effect is likely mediated by receptors other than TNFR1.

In contrast to the *Hoip*$^{-/-}$ and *Hoil1*$^{-/-}$ mice, the *chronic proliferative dermatitis* (*cpdm*) mice, which contain a spontaneous mutation that leads to frame-shift and premature non-sense mutation of the *Sharpin* gene, are born alive but suffer from dermatitis and other immune inflammatory manifestations. Deletion of *Tnf* or genetic inactivation of RIPK1 kinase activity via the kinase inactive *Ripk1*^{K45A} allele fully rescued the inflammatory disease [8, 9, 14] (Table 1). This is in stark contrast to mice lacking *Hoip* or *Hoil1*, whose inflammatory phenotypes were not restored by inhibition of RIPK1 kinase activity [11, 12]. Could the discrepant RIPK1 phenotypes in the different LUBAC mutant mice be caused by residual E3 ligase activity in *cpdm* mice? Regardless of the mechanism, these results do support the notion that the ubiquitin scaffold of complex I has a critical role in restricting the death-inducing activity of RIPK1 (Fig. 1). As we shall discuss later, a possible mechanism by which LUBAC inhibits the pro-death activity of RIPK1 is through the recruitment of kinases such as TBK1 and IKK [15–21].

Mass spectrometry revealed that many sites on RIPK1 were modified by ubiquitination [10]. Experimental evidence indicates that K377 of human RIPK1 (K376 in mouse RIPK1) is particularly important in controlling RIPK1 activity [22]. For example, reconstitution of K377RRIPK1 mutant in RIPK1-deficient cells led to strong induction of cell death [23]. Moreover, mice expressing the K376R-RIPK1 mutant were hypersensitive to TNF-induced cytotoxicity and died during embryogenesis around E13.5 [24, 25]. Consistent with an important role for the ubiquitin scaffold in recruiting inhibitory kinases to complex I, the K376R mutation appears to restrict inhibitory phosphorylation of RIPK1 by the kinase TAK1 [17, 25].

The concept that disruption of RIPK1 post-translational modification may be a pathogen sensing mechanism is illustrated in enteric bacterial infection. The LUBAC-generated ubiquitin scaffold has a critical function in “trapping” and restricting Salmonella Typhimurium growth [26]. This protective mechanism is disrupted by *Shigella flexneri*, which encodes two E3 ligases, IpaH1.4 and IpaH2.5, that directly binds to HOIL1 and triggers HOIP K48-linked ubiquitination and proteasomal degradation [27]. Disturbance of the LUBAC ubiquitin scaffold thus inhibits NF-κB activation. It is tempting to speculate that disruption of this ubiquitin scaffold will trigger RIPK1 activation and cell death (Fig. 2).

2.2 RIPK1 phosphorylation and pathogen sensing

Early mass spectrometry revealed multiple phosphorylation sites on RIPK1 [28]. The majority of these phosphorylation events do not impinge on RIPK1 activity [23, 29, 30]. However, several phospho-acceptor sites have been identified that either stimulate or inhibit RIPK1 activity. For example, phosphorylation of S166 in the kinase domain of RIPK1 has been widely used as a marker RIPK1 activation [31]. S166 phosphorylation was not detected in cells expressing kinase inactive RIPK1, indicating that this is likely an auto-phosphorylation event. S166 phosphorylation is not specific for cell death induction. In contrast, S161 phosphorylation has also been implicated to cause RIPK1 auto-activation and to promote interaction with RIPK3 during necroptosis [29]. However, the S161A-RIPK1

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mutant had little effects on TNF-induced necroptosis [23]. Thus, further work is needed to accurately define the role of these phosphorylation events in regulating RIPK1 activity.

As we have alluded to in the previous section, RIPK1 ubiquitination and phosphorylation are intimately linked events. Many kinases that regulate RIPK1 activity are recruited to the ubiquitin scaffold of complex I. For example, NEMO and TAK1 binding to ubiquitinated RIPK1 brings the IKKα and IKKβ to the proximity of TAK1 to facilitate TAK1-mediated phosphorylation and activation of the IKKs. The IKKs phosphorylate RIPK1 at S25 in the kinase domain, leading to inhibition of its death-inducing activity (Fig. 1) [21]. Interestingly, this inhibitory switch appears to have a critical role in limiting bacterial pathogenesis. The Yersinia effector YopJ/P inhibits TAK1 and IKK activity to unleash RIPK1, which in turn stimulates caspase 8-dependent cleavage of the pore-forming gasdermin D (GSDMD) and cell death by pyroptosis [19, 32, 33] (Fig. 2). Mice that express the S25D-RIPK1 mutant were as defective as mice expressing the kinase inactive K45A-RIPK1 mutant in Yersinia-induced macrophage death and the control of bacterial replication [21] (Table 2). Hence, S25 phosphorylation is a key early event that restricts the death-inducing potential of RIPK1.

In addition to TAK1 and the IKKs, the MAPKK MK2 also inhibits RIPK1 cytotoxic function via direct phosphorylation. However, unlike the IKKs, MK2 phosphorylates RIPK1 at S321/S336 away from the kinase domain [34–36]. This suggests that MK2 may indirectly regulate RIPK1 kinase and death-inducing activity via allosteric effects. TAK1 has also been shown to directly phosphorylate RIPK1 at S321 [17]. Similar to the IKKs, MK2 is also a target of inhibition by the Yersinia pestis effector YopJ/P (Fig. 2) [36]. Thus, RIPK1 may sit at the center of a regulatory hub that senses different perturbations by bacterial pathogens (Fig. 2).

TBK1 is another kinase that has recently joined the rank of RIPK1 inhibitory kinases. As in the case of the other inhibitory kinases, TBK1 recruitment to complex I was also facilitated by the LUBAC-generated ubiquitin scaffold via the adaptors TANK1 and NEMO (Fig. 1) [15]. *Tbk1*$^{-/-}$ mice suffer from embryonic lethality. Surprisingly, this lethality could be fully rescued by the RIPK1 kinase inactive allele D138N [18]. TBK1 and its associated kinase IKKε phosphorylate human RIPK1 at T189 (T190 in mouse RIPK1) [15, 18]. Loss of TBK1 function has been associated with neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD) [37]. Interestingly, TAK1 expression is reduced in the aging brain [18]. When combined with heterozygous loss of *Tbk1*, this reduced TAK1 expression drives phenotypes that resemble human ALS/FTD. Remarkably, inhibition of RIPK1 kinase activity reversed these disease manifestations [18]. Thus, inhibitory RIPK1 phosphorylation events are not only relevant in pathogen defense, but also have critical roles in neuroinflammation.

2.3 Proteolytic processing – Lessons from human mutations

RIPK1 has long been known to be a substrate of caspase 8 [38, 39]. For the last decade, the dominant view has been that caspase 8 cleaves and inactivates RIPK1 to blunt necroptosis. However, this viewpoint has recently been challenged with the discovery of heterozygous human mutations that specifically inhibit RIPK1 cleavage. In these patients, the essential aspartate residue (D324) in the tetra-peptide caspase 8 cleavage site LQLD was substituted

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with another amino acid residue. Despite having a normal wild type *Ripk1* allele, these patients suffer from early onset of periodic fever syndrome and lymphadenopathy marked by elevated inflammatory cytokine expression [40, 41]. This condition, which have been termed “cleavage-resistant RIPK1-induced autoinflammatory syndrome” (CRIA) [40], was recapitulated in mice that express the corresponding cleavage-resistant D325A-RIPK1 [40, 42]. Cells from the CRIA patients produced increased inflammatory cytokines in response to LPS and were hypersensitive to TNF-induced apoptosis and necroptosis. Unexpectedly, mice that are homozygous for the D325A mutation died during embryogenesis on E10.5, which was rescued by co-deletion of *Ripk3* and *Caspase8* [42, 43]. Collectively, these results indicate that RIPK1 cleavage by Caspase 8 not only inhibits necroptosis, but it also has critical roles in restraining the cell death and pro-inflammatory functions of RIPK1 (Fig. 3).

In addition to the cleavage-resistant mutations, homozygous mutations in *Ripk1* that led to frameshift, alternative splicing, deletion of exons, or single amino acid substitutions have been identified. In these patients, RIPK1 protein expression was severely reduced or even completely lost [44–46]. Intriguingly, some of the mutations were located near the C-terminus of the RIPK1 protein, suggesting that reduced protein or mRNA stability might have contributed to the loss of RIPK1 protein expression. These patients are immunodeficient yet develop inflammatory bowel disease and polyarthritis [44–46] (Table 2). Thus, human RIPK1 mutations revealed the paradoxical nature of RIPK1 as both an inducer and an inhibitor of cell death and inflammation.

Many large DNA viruses encode caspase 8 inhibitors and thus indirectly restrict cleavage of RIPK1 (Fig. 2). The classic example being the Cytokine response modifier A (CrmA) from Cowpox virus, which inhibits caspase 1 and caspase 8 activity [47] (Fig. 2). The related orthopoxvirus vaccinia virus encode the CrmA ortholog B13R or Spi2, which inhibits death receptor-induced apoptosis but sensitizes cells to TNF-induced anti-viral necroptosis [48–52]. However, it is noteworthy that other large DNA viruses that encode caspase 8 inhibitors also encode inhibitors that block necroptosis. These include the herpes simplex virus 1 (HSV1) ICP6 and HSV2 ICP10 (Fig. 2). Interestingly, several recent studies revealed that enteropathogenic and enterohaemorrhagic bacteria Type 3 secretion system (T3SS) encodes EspL, an atypical cysteine protease that cleaves RIPK1 at the C-terminus of the RHIM [53]. EspL also cleaves other mammalian RHIM-containing adaptors in a similar fashion (Fig. 2). As a result, necroptosis and inflammatory cytokine signaling were blunted [53]. Since another T3SS effector NleB1 has been shown to covalently modify and inhibit the caspase 8 adaptor FADD [54, 55], EspL inhibition of the RHIM-containing adaptors ensures that there is no switch from apoptosis to necroptosis. This behavior is eerily similar to that of certain herpesviruses, which also simultaneously inhibit apoptosis and necroptosis [56]. The fact that bacterial and viral pathogens have both developed similar strategies to target the same cell death programs is a strong argument that RIPK1 and its related adaptors are key sentinels in host defense.

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3. Non-PTM mechanisms that regulate RIPK1 activity

3.1 RHIM-mediated sequestration of RIPK1

The RHIM is defined by a highly conserved tetra-peptide core (e.g. IQIG or VQVG) flanked by mostly hydrophobic residues. The four mammalian RHIM-containing adaptors, RIPK1, RIPK3, the toll-like receptor 3 (TLR3)/TLR4 adaptor TRIF and the nucleic acid sensing adaptor ZBP1, are all critical signal adaptors for innate inflammation and cell death. In necroptosis, RIPK3 can be activated through RHIM-mediated binding with any of the other RHIM adaptors. This has led to the notion that RHIM-mediated interaction predominantly drives cell death (Fig. 3a). However, this dogma has recently been challenged. Mice that express a RIPK1 mutant with alanine substitutions in the RHIM core sequence (*Ripk1*^{mRHIM}) died perinatally [31, 57] (Table 2). This was a surprising observation since mice expressing kinase inactive RIPK1 were viable [14, 49]. RIPK3 phosphorylation, which indicates activation of the kinase, was abundantly detected in different tissues of the *Ripk1*^{mRHIM} mice. Moreover, a complex between RIPK3 and ZBP1 was detected in *Ripk1*^{mRHIM}-expressing cells. Deletion of *Zbp1*, *Ripk3* or *Mlkl* rescued the perinatal lethality of the *Ripk1*^{mRHIM} mice [31, 57]. Thus, genetic evidence implicates that the RHIM of RIPK1 normally restricts ZBP1 and RIPK3 to prevent necroptosis during perinatal life. Although biochemical interaction between RIPK1 and ZBP1 or RIPK3 has not been detected, these results support the notion that RIPK1 has a kinase-independent but scaffold-dependent function in cell survival (Fig. 3b).

3.2 Other regulatory mechanisms of RIPK1 activity

HSP90 is a molecular chaperone for RIPK1 [58]. Inhibition of HSP90 with geldanamycin causes rapid degradation of RIPK1 and resistance to necroptosis [39]. In addition to RIPK1, recent results indicate that HSP90 also binds to the necroptosis adaptors RIPK3 and MLKL to promote their stability and necroptosis induction [59–62]. Hence, the effect of drugs that target HSP90 likely affect necroptosis at multiple levels. Besides regulation through protein-protein interaction and post-translational modifications, RIPK1 activity can also be regulated at a transcriptional level. For example, *Ripk1* transcription was found to be inhibited under hypoxic conditions [63]. Furthermore, we recently found that RIPK1 activity is tunable in response to changes in the pH environment. At acidic pH, RIPK1 kinase activity and RIPK1-dependent apoptosis and necroptosis were both reduced [64]. Direct protonation of specific histidine residues is a common regulatory mechanism for pH-sensing channel proteins. As such, RIPK1 may be regulated by pH changes in a similar fashion. Environmental changes in pH can be a rapid and efficient way to tune RIPK1 activity during infection or in hypoxic tumor sites.

4. Concluding remarks

Our understanding of RIPK1 biology has undergone a major paradigm shift in recent years. It is now clear that RIPK1 is the fulcrum that balances cell survival and cell death signals. We argue that the different inhibitory mechanisms on RIPK1 have evolved to sense pathogen challenges. RIPK1 and the other RHIM signal adaptors share critical functions in innate immunity. Immune cells such as macrophages are often targeted by invading pathogens.

Thus, interference of these sensor mechanisms will directly or indirectly impinge on RIPK1. Cell death in this scenario may be desirable as it will help to limit the bacterial or viral factory. However, these defense mechanisms do come with a price at times, such as that seen in the human CRIA patients. Time will tell if our increased understanding of these regulatory maneuvers will translate to successful pharmacologic targeting of RIPK1 in various inflammatory diseases [65].

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Abbreviations:

RHIM	RIP homotypic interaction motif
LUBAC	linear ubiquitin assembly complex
ZBP1	zDNA binding protein 1
PTM	post-translation modification
DD	death domain
KD	kinase domain

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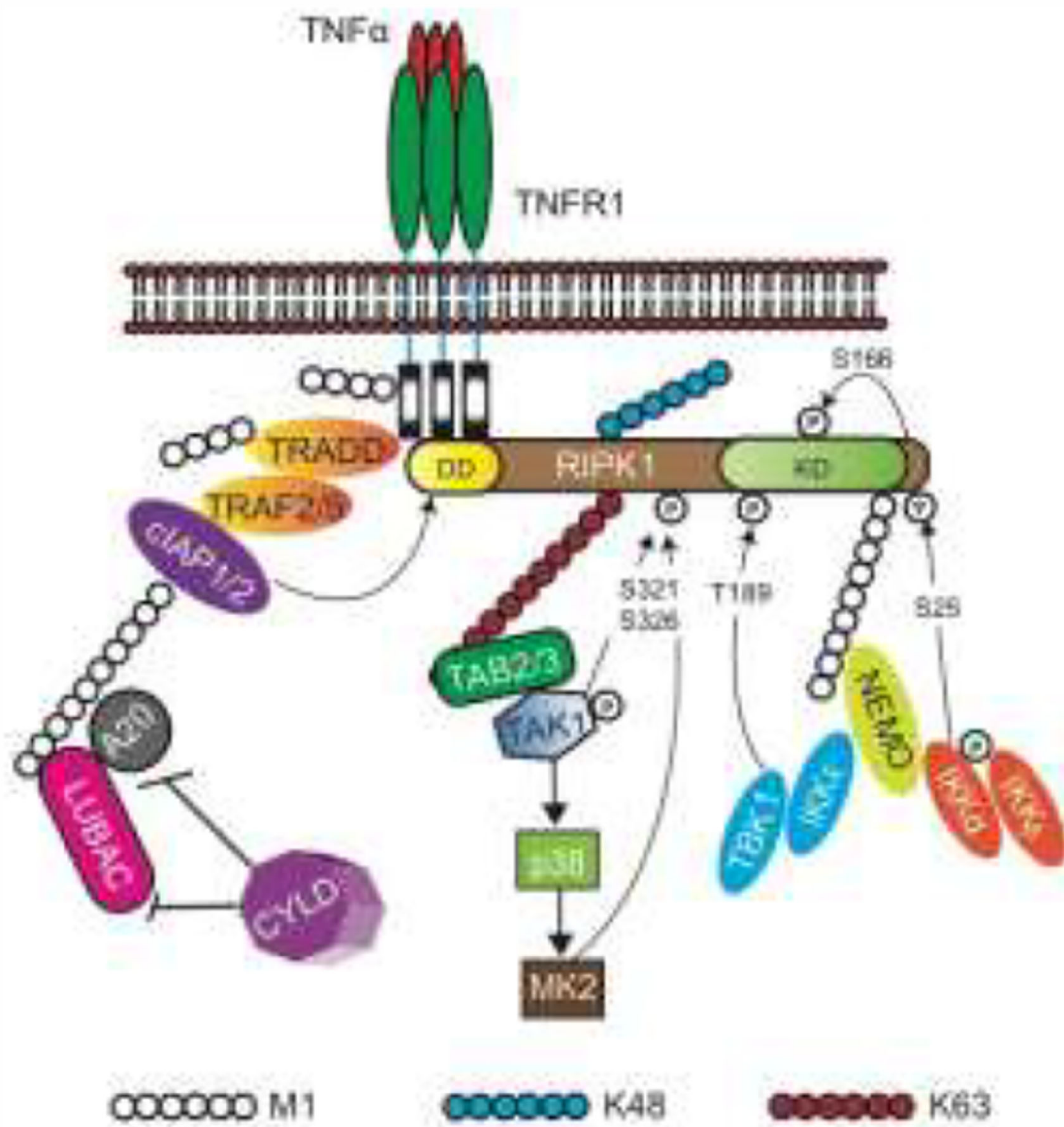

Figure 1. Regulation of RIPK1 activity by ubiquitination and phosphorylation.
RIPK1 and TRADD bind to TNFR1 via DD interaction. TRADD subsequently recruits TRAF2/5 and cIAP1/2, which initiates the assembly of the ubiquitin scaffold of complex. Numerous components of complex I are modified by ubiquitination. In addition to cIAP1/2 and LUBAC, additional ubiquitinating and de-ubiquitinating enzymes such as cylindromatosis (CYLD) and A20 have also been implicated in regulating RIPK1 ubiquitination [66–71] (Table 1). Key kinases that phosphorylate RIPK1 at distinct sites are highlighted. cIAP, cellular inhibitor of apoptosis protein; IKK, inhibitor of NF-κB kinase; NEMO, NF-κB essential modulator NF-κB, nuclear factor kappa; RIPK, receptor-interacting protein kinase; TAB, TAK1-binding protein; TAK1, TGFβ activated kinase 1; TRADD, TNF-associated death domain; KD, Kinase domain.

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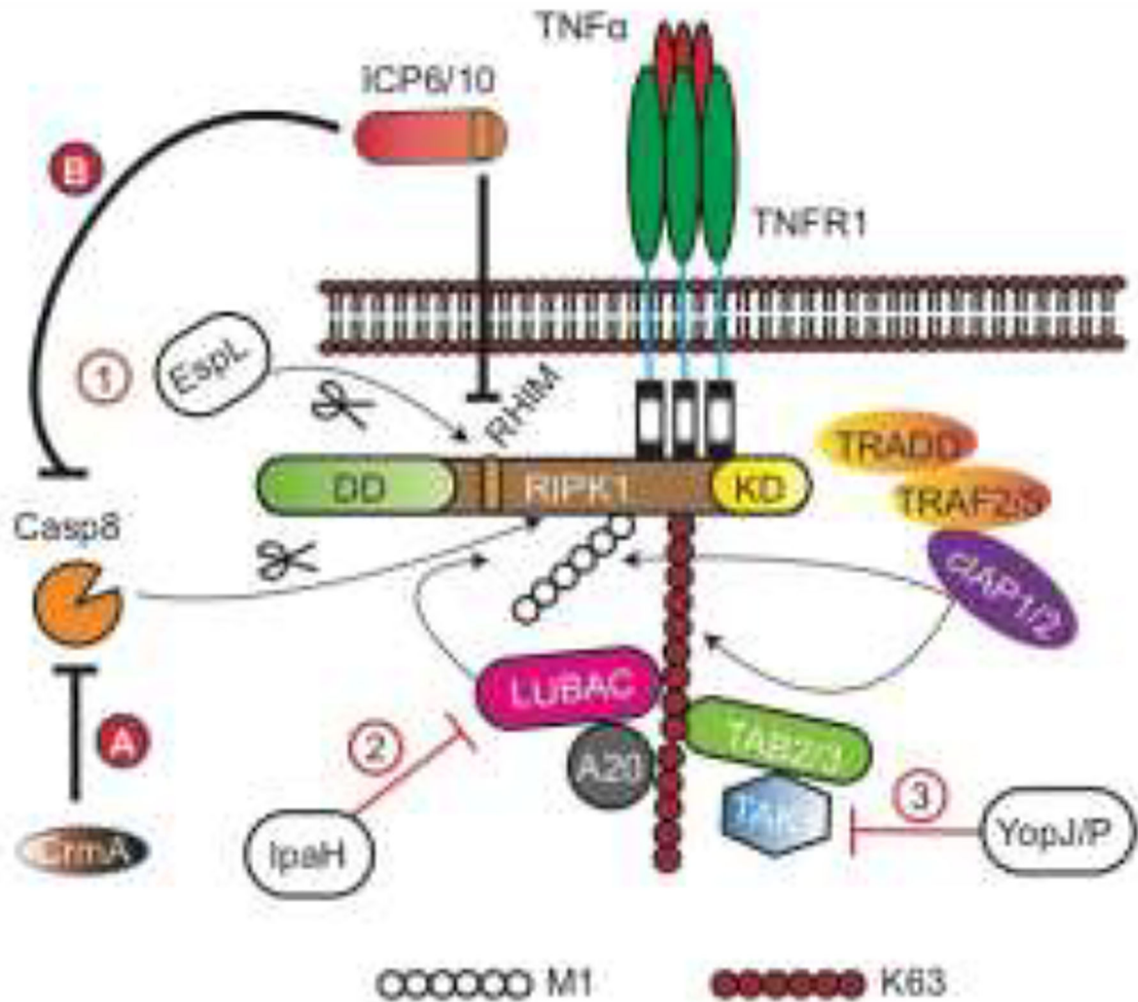

Figure 2. Pathogen targeting of RIPK1.

Several classes of bacterial effectors target different regulatory machineries that impinge on RIPK1 activity. For example, the T3SS effector EspL cleaves RIPK1 at the boundary of the RHIM, the *Shigella flexneri* E3 ligases IpaH1.4 and IpaH2.5 trigger proteasomal degradation of components of LUBAC, and the Yersinia effector YopJ/P inhibits TAK1. Bacterial interference of RIPK1 activity can either inhibit (e.g. EspL) or enhance (e.g. IpaH and YopJ/P) RIPK1-mediated inflammation and cell death. Orthopoxviruses encode CrmA or related inhibitors that block caspase 8, which indirectly inhibits RIPK1 cleavage to sensitize cells to necroptosis. On the other hand, the HSV1 and HSV2 inhibitors ICP6 and ICP10 exhibit dual activity against caspase 8 and the RHIM to block apoptosis and necroptosis. DD: Death domain, KD: kinase domain.

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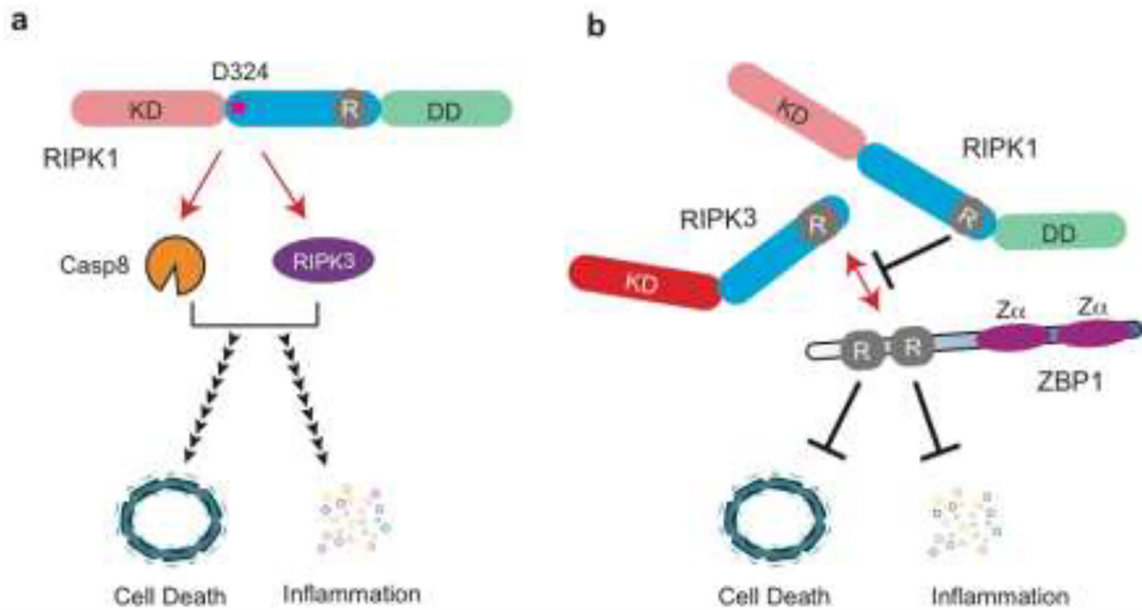

Figure 3. Cleavage and interaction with other RHIM adaptors prevent inadvertent RIPK1 activation.

(**a**) Caspase 8 (Casp8) cleaves RIPK1 at D324. Heterozygous mutations at this cleavage site causes a spontaneous inflammatory disease in human. The non-cleavable RIPK1 unleashes Caspase 8 and RIPK3, which in turn stimulate cell death and elevated inflammatory cytokine production. The red stars represent mutation in D324. KD: kinase domain, R: RHIM, DD: death domain. (**b**) Genetic evidence indicates that RIPK1 sequesters ZBP1 and/or RIPK3 via the RHIM during perinatal development. This functional interaction is also critical for prevention of cell death and inflammation, and maintenance of homeostasis of the skin epithelium. When the RHIM of RIPK1 is disrupted by genetic manipulation, excessive ZBP1-RIPK3-mediated necroptosis occurs, leading to inflammation and lethality. Zα: zDNA-binding domain.

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Table 1:

E3 ligase and kinase deficiencies that affect RIPK1 activity

Genetic deficiency	Viability	Phenotype	Biochemical Effect on RIPK1	Genetic resuce by RIPK1 deficiency	Reference
Ciap1$^{-/-}$***Ciap2–/–***	lethality at ~E10.5	Cardiovascular defect	Inhibition of RIPK1 ubiquitination	single *Ripkl-null* allele extends survival till ~E14.5	7
Ciap1$^{-/-}$***Xiap–/–***	lethality at ~E10.5	Cardiovascular defect	Reduction of RIPK1 ubiquitination	single *Ripk1*-null allele extends survival till weaning	7
Sharpin$^{-/-}$	Viable	dermatitis, multi-organ inflammation	Impaired S25 phosphorylation and ubiquitination. Increased RIPK1 death-inducing activity.	full rescue by kinase inactive *Ripk1*$^{K45A/K45A}$	11, 14, 21
Hoip$^{-/-}$	lethality at ~E10.5	Defective yolk sac vascularization, Excessive Endothelial Cell Death	Enhanced formation of RIPK1 death-inducing complex	survival extended to ~E14.5 by *Rjpk1*^{K45AJK45A}	11, 12
Hoil1$^{-/-}$	lethality at ~E10.5	Disrupted vascular architecture and cell death in the yolk sac endothelium	Enhanced formation of RIPK1 death-inducing complex	survival extended to ~E14.5 by kinase inactive *Ripk1*$^{K45A/K45A}$	11
Cyld$^{-/-}$	Viable	Grossly normal	Increased length of RIPK1 ubiquitin chains. Inhibition of RIPK1 death-inducing activity.	N.D.	66, 67, 71
A20$^{-/-}$	Die after 1 week	Runting, multi-organ inflammation	Enhanced RIPK1 activity and cell death	N.D.	68, 69, 70
Tbk1$^{-/-}$	lethality at E13.5 - E14.5	Severe liver degeneration	Loss of inhibitory phosphorylation at T189 (T190 for mouse RIPK1)	full rescue by kinase inactive *Ripk1*$^{D138N/D138N}$	18
***Mapkapk2* (MK2)**$^{-/-}$	Viable	No overt phenotype	Blockade of inhibitory phosphorylation at S321 and S336. Enhanced RIPK1 death-inducing activity	N.D.	34, 35, 36
Ikkα/β$^{-/-}$	embryonic lethality	liver damage	Prevents inhibitory phosphorylation of RIPK1 at S25. Increased RIPK1 death-inducing activity.	N.D.	16, 21

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Table 2:

Mutations of RIPK1 and the associated phenotypes

(N/A = Not applicable

	Genetic deficiency	Viability	Phenotype	Effect on RIPK1	Genetic Rescue	Reference
Mutations in Mice	***Ripk1**$^{K45A/K45A}$*	Viable	No overt defects	Loss of RIPK1 kinase activity	N/A	15
	***Ripk1**$^{D138N/D138N}$*	Viable	No overt defects	Loss of RIPK1 kinase activity	N/A	16
	***Ripk1**$^{K376R/K376R}$*	lethality at ~E13.5	Excessive cell death and severe inflammation	Disrupt RIPK1 ubiquitination	Co-deletion of *Fadd* and *Ripk3* or *Fadd* and *Mlkl*	23, 24
	***Ripk1**$^{S25D/S25D}$*	Viable	No overt defects	Mimics IKK inhibitory phosphorylation	N/A	20
	***Ripk1**$^{S321D/S321D}$*	Not reported	Not reported	Mimics MK2 inhibitory phosphorylation	N/A	33
	***Ripk1**$^{D325A/D325A}$*	lethality at ~E10.5	endocardial damage	Inhibit Caspase 8 cleavage of RIPK1	Co-deletion of *Fadd* and *Mlkl*	39, 41, 42
	***Ripk1**$^{RHIM/RHIM}$*	P0	Dermatitis, multi-organ inflammation	RIPK3(D161N), RHIM mutant RIPK3, RIPK3 deficiency, MLKL deficiency or Loss of ZBP1	Deletion of *Zbp1, Ripk3* or *Mlkl*	30, 56
Human mutations	***Heterozygous D324N, D324H and D324Y mutations in human***	Viable	periodic fever, recurring lymph adenopathy	Inhibit Caspase 8 cleavage of RIPK1	N/A	39, 40
	Homozygous mutations of RIPK1 (I615T, T645M, Y426*, C601Y)	Viable	Primary immunodeficiency and/or Intestinal inflammation	Substantial reduction of RIPK1 protein expression	N/A	43, 44
	Biallelic mutations (frame-shift, deletion of exons or alternative splicing)	Viable	recurrent infections, intestinal inflammation and polyarthritis	Complete loss of RIPK1 protein expression	N/A	43, 44, 45