## Phylogeny

Receptor-interacting protein kinase 1 (RIPK1) is one of five human RIPK family members and resides in the Tyrosine Kinase-Like (TKL) group of the human kinome (Manning et al., 2002; Martens et al., 2020; Wegner et al., 2017). The family shows a human-specific expansion that is absent from Drosophila and C. elegans kinomes (Manning et al., 2002). Murine Ripk1 is the direct orthologue of human RIPK1 (Degterev et al., 2019).

## Reaction Catalyzed

ATP + protein substrate ⇌ ADP + phosphoprotein substrate (Chen et al., 2022).

## Cofactor Requirements

Catalysis requires a divalent cation, specifically Mg²⁺, which supports ATP binding and phosphoryl transfer (Mifflin et al., 2020; Martens et al., 2020).

## Substrate Specificity

RIPK1 phosphorylates Ser or Thr residues. The preferred motif contains basic residues flanking the phospho-acceptor site on both N- and C-terminal sides, with a strong bias for an aromatic residue at the +3 position (Johnson et al., 2023).

## Structure

RIPK1 is a multidomain protein comprising an N-terminal kinase domain (aa 1–312), an intermediate domain (aa 313–531) bearing a RIP homotypic interaction motif (RHIM), and a C-terminal death domain (aa 582–671) (Degterev et al., 2019; Du & Wang, 2024; Yuan et al., 2019).

• Kinase domain: contains the activation loop beginning with the conserved DFG motif and a catalytic triad Lys45-Glu63-Asp156; toggles between DFG-in (active) and DFG-out (inactive) conformations and harbours a unique hydrophobic allosteric pocket targeted by selective inhibitors (Martens et al., 2020; Chen et al., 2022).  
• Intermediate domain: RHIM enables homo- or hetero-oligomerisation with RHIM-containing proteins such as RIPK3, TRIF and ZBP1 (Degterev et al., 2019; Du & Wang, 2024).  
• Death domain: mediates interactions with other DD-containing proteins, e.g., TNFR1, FADD and TRADD (Degterev et al., 2019).

## Regulation

Activity is controlled by extensive post-translational modification (PTM) (Degterev et al., 2019; Mifflin et al., 2020; Shan et al., 2018).

Phosphorylation  
• Activating autophosphorylation: Ser14/15, Ser20, Ser161/166; p-Ser166 is a biomarker of activation (Degterev et al., 2019; Du & Wang, 2024; Martens et al., 2020).  
• Inhibitory phosphorylation: TAK1, IKKα/β, MK2, TBK1, AMPK, JAK1 and Src phosphorylate sites including S25, T189, S320/S335 (human), S416 and Y384 (Martens et al., 2020; Yuan et al., 2019; Du & Wang, 2024). PP1γ removes inhibitory phosphates (Du & Wang, 2024).

Ubiquitination  
In TNF-α Complex I, cIAP1/2 add K63-linked chains (e.g., Lys377) and PELI1 modifies Lys115; LUBAC attaches M1-linked chains that recruit IKK to activate NF-κB. Other linkages (K11, K48) occur. CYLD and A20 act as deubiquitinases that remove chains and facilitate transition to death complexes (Degterev et al., 2019; Shan et al., 2018; Liu & Chan, 2021; Chen et al., 2022).

Proteolytic cleavage  
Caspase-8 cleaves RIPK1 at Asp324, separating the kinase and death domains and dampening apoptosis and necroptosis signalling (Yuan et al., 2019; Martens et al., 2020).

## Function

RIPK1 serves dual, context-dependent roles (Martens et al., 2020; Mifflin et al., 2020).

• Scaffold (kinase-independent): In TNF-α-induced Complex I (with TRADD, TRAF2, cIAP1/2, LUBAC), ubiquitinated RIPK1 recruits TAK1 and IKK complexes, triggering NF-κB and MAPK activation and promoting cell survival and inflammation (Degterev et al., 2019; Du & Wang, 2024).

• Cell-death effector (kinase-dependent):  
 – Apoptosis: formation of Complex IIa (RIPK1-FADD-caspase-8) leads to RIPK1-dependent apoptosis (Shan et al., 2018).  
 – Necroptosis: when caspase-8 is inactive, RIPK1 interacts with RIPK3 via RHIM domains to form the necrosome (Complex IIb); RIPK3 then phosphorylates MLKL, inducing membrane rupture (Degterev et al., 2019; Du & Wang, 2024).

Upstream inputs: death receptors (TNFR1, Fas) and pattern-recognition receptors (TLR3/4, RIG-I/MDA-5) (Du & Wang, 2024; Udawatte & Rothman, 2021).  
Key partners: TNFR1, TRADD, FADD, caspase-8, RIPK3, MLKL, TRIF, ZBP1, TRAF2/5, cIAP1/2, LUBAC, TAK1, IKK complex, NEMO, CYLD, A20 (Wegner et al., 2017; Du & Wang, 2024).

## Inhibitors

Multiple chemical classes inhibit the RIPK1 kinase domain (Shan et al., 2018; Martens et al., 2020; Mifflin et al., 2020; Chen et al., 2022).  
• Type II/III allosteric: Necrostatin-1 (Nec-1), Nec-1s, GSK’772, GSK’547, GNE684.  
• CNS-penetrant: DNL747, DNL788.  
• Multi-kinase drugs with off-target RIPK1 activity: sorafenib, ponatinib, pazopanib, dabrafenib.  
• Type I ATP-competitive inhibitors have also been reported.

## Other Comments

RIPK1 dysregulation is linked to inflammatory, autoimmune, neurodegenerative and ischemic diseases, as well as sepsis (Mifflin et al., 2020; Martens et al., 2020).  
• Loss-of-function mutations cause immunodeficiency, gut inflammation and polyarthritis (Martens et al., 2020).  
• Cleavage-resistant D324Y/H mutations produce CRIA syndrome, characterised by autoinflammation (Liu & Chan, 2021; Martens et al., 2020).  
• Kinase-dead alleles (e.g., D138N, K45A) protect against inflammation in mouse models (Mifflin et al., 2020; Wegner et al., 2017).

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