## Phylogeny

Receptor-interacting serine/threonine-protein kinase 3 (RIPK3) is one of seven members of the RIP kinase family and belongs to the Tyrosine Kinase-Like (TKL) clade of the human kinome (Martens et al., 2020). Its N-terminal kinase domain shares ~30 % identity (60 % similarity) with RIPK1 and RIPK2 and shows structural relatedness to mixed-lineage kinases and BRAF (Raju et al., 2018; Shlomovitz et al., 2017). Orthologues are conserved in mammals, including human (518 aa) and mouse (486 aa) proteins (Li et al., 2012; Shlomovitz et al., 2017).

## Reaction Catalyzed

ATP + [protein]-L-Ser/Thr ⇌ ADP + [protein]-L-Ser/Thr-phosphate (Li et al., 2012).

## Cofactor Requirements

Catalysis requires ATP and a divalent cation coordinated by the DFG motif in the activation loop (Raju et al., 2018).

## Substrate Specificity

RIPK3 phosphorylates serine or threonine residues within downstream effectors; its consensus motif resembles that recognized by mixed-lineage kinase family members (Li et al., 2012; Raju et al., 2018).

## Structure

• Modular organisation: N-terminal kinase domain and C-terminal RIP homotypic interaction motif (RHIM); no death domain or CARD (Shlomovitz et al., 2017).  
• Key catalytic features: activation loop with DFG motif, hydrophobic C- and R-spines, and an essential Lys50 for ATP binding (Li et al., 2012; Shlomovitz et al., 2017).  
• Dimerisation: kinase domains form homodimers via the αC-helix/β4-strand interface, reminiscent of RAF kinases (Raju et al., 2018).  
• Structural models: human RIPK3 modelled on the mouse RIPK3-MLKL complex (PDB 4M69) (Choi et al., 2018).  
• RHIM facilitates assembly of higher-order amyloid-like “necrosome” complexes (Li et al., 2012).

## Regulation

• Activation: kinase-domain dimerisation followed by cis-autophosphorylation; Ser227 (human) is critical (Raju et al., 2018; Shlomovitz et al., 2017).  
• Additional phosphorylation sites in mouse (Ser199, Ser204, Thr231, Ser232) control MLKL recruitment (Shlomovitz et al., 2017).  
• Upstream kinases: RIPK1 and, in cardiomyocytes, CaMKII (Shlomovitz et al., 2017).  
• Ubiquitination: the E3 ligase Peli1 mediates K48-linked ubiquitination and proteasomal degradation of active RIPK3 (Choi et al., 2018).  
• Proteolysis: the caspase-8–FLIPL complex cleaves and inhibits RIPK3 (Li et al., 2012).  
• Allosteric control: heterodimerisation with a kinase-inactive mutant can enhance wild-type activity (Raju et al., 2018).

## Function

RIPK3 is expressed in immune and epithelial cells and in experimental lines such as myeloid cells, A375 melanoma, HEK293T, HeLa and HT-29 (Geserick et al., 2015; Shlomovitz et al., 2017).  
• Upstream signals: death receptor TNFR1, TLR3/4, interferon receptors and the sensor DAI/ZBP1 converge on RIPK3, often via RIPK1 or TRIF (Li et al., 2012; Shlomovitz et al., 2017).  
• Core complex: RHIM-mediated interaction with RIPK1 forms the necrosome that drives necroptosis (Li et al., 2012).  
• Main substrate: MLKL is phosphorylated on Thr357/Ser358, triggering oligomerisation, membrane translocation and lytic cell death (Shlomovitz et al., 2017).  
• Additional roles:  
– Apoptosis via RIPK1–FADD–caspase-8 complex formation (Shlomovitz et al., 2017).  
– Pyroptosis through activation of the NLRP3 inflammasome (Shlomovitz et al., 2017).

## Inhibitors

Small-molecule ATP-competitive inhibitors include GSK’872 and dabrafenib; the viral protein vIRA from murine cytomegalovirus also blocks RIPK3-mediated necrosis (Li et al., 2012; Mandal et al., 2014; Raju et al., 2018; Shlomovitz et al., 2017).

## Other Comments

Dysregulated RIPK3 activity is linked to inflammatory diseases, viral infection, atherosclerosis, neurodegeneration and tissue injury; elevated serum RIPK3 may serve as a myocardial infarction biomarker (Li et al., 2012; Shlomovitz et al., 2017).  
Documented loss- or gain-of-function mutants include D161N (kinase-dead, embryonic lethality), K50A/K51A (abolish activity) and interface mutants R69H, H156G/R (impair necroptosis) (Raju et al., 2018; Shlomovitz et al., 2017).

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