## Phylogeny

Receptor-interacting serine/threonine-protein kinase 3 (RIPK3) belongs to the receptor-interacting protein kinase family, a distinct branch of the serine/threonine kinase superfamily conserved across vertebrates (Fay et al., 2025; Lv et al., 2022). Orthologs are present in diverse mammalian species. While RIPK3 retains core catalytic features shared with RIPK1, it has diverged to acquire a C-terminal RIP homotypic interaction motif (RHIM) that enables selective interactions with other RHIM-containing proteins and underpins its specialized roles in innate immunity and programmed cell death (Dardick & Ronald, 2006; Fay et al., 2025).

## Reaction Catalyzed

ATP + [protein]-L-serine/threonine ⇌ ADP + H⁺ + [protein]-O-phospho-L-serine/threonine (Wu et al., 2012; Zu et al., 2021).

## Cofactor Requirements

Mg²⁺ is essential for ATP binding and efficient phosphoryl transfer (Wu et al., 2012; Zu et al., 2021).

## Substrate Specificity

RIPK3 phosphorylates serine/threonine residues on substrates assembled within death-signaling complexes. Its best-characterized target is the pseudokinase MLKL, whose phosphorylation initiates necroptosis. RIPK3 also undergoes reciprocal phosphorylation with RIPK1. A strict linear consensus motif has not been defined; substrate selection is largely governed by 3-D context within RHIM-mediated assemblies (Johnson et al., 2023; Licheva et al., 2022; Martens et al., 2020).

## Structure

The protein comprises an N-terminal bilobal kinase domain and a C-terminal RHIM. The kinase domain contains the canonical catalytic Lys and a DFG motif within the activation loop; conformational changes in this loop and the C-helix control activity. Crystallographic and AlphaFold models reveal that the RHIM forms amyloid-like contacts with partner proteins (e.g., RIPK1) to build the necrosome (Lopez et al., 2019; Mace & Murphy, 2021; Johnson et al., 2023).

## Regulation

Activity is modulated by multi-layered mechanisms:  
• Auto- and trans-phosphorylation between RIPK3 and RIPK1 drive necrosome assembly (Du et al., 2021; Martens et al., 2020).  
• PLK1 phosphorylates S369 during G2/M, linking kinase activity to the cell cycle (Gupta & Liu, 2021).  
• Interactions with metabolic enzymes (GLUL, GLUD1, PYGL) provide metabolic feedback (Mace & Murphy, 2021).  
• Kinase-inactive RIPK3 can scaffold apoptotic complexes with RIPK1, FADD and CASP8 (Mace & Murphy, 2021; Moriwaki & Chan, 2017).

## Function

RIPK3 is a central hub for necroptotic and inflammatory signaling. Upon stimulation by TNF-family ligands or viral ZBP1 sensing, RIPK3 phosphorylates MLKL, whose oligomerization disrupts plasma-membrane integrity, causing inflammatory cell death (Liu et al., 2021; Zu et al., 2021). Kinase-dead RIPK3 can promote apoptosis via RIPK1-FADD-CASP8 complexes. In Zika-virus-infected neurons, RIPK3 cooperates with ZBP1 to induce ACOD1/IRG1 expression and itaconate production, thereby restricting viral replication. Binding to GLUL, GLUD1 and PYGL may stimulate the TCA cycle and elevate reactive oxygen species, linking metabolic state to inflammatory responses (Dardick & Ronald, 2006; Liu et al., 2021).

## Inhibitors

Small-molecule inhibitors that block RIPK3’s ATP-binding pocket are under development; most efforts parallel those targeting RIPK1 (Martens et al., 2020). Broader necroptosis inhibitors have been reviewed, highlighting therapeutic potential in inflammatory and neurodegenerative diseases (Zhuang & Chen, 2020).

## Other Comments

Mutations in the DFG motif or RHIM disrupt necrosome formation and downstream signaling. Aberrant RIPK3 activation contributes to acute tissue injury, chronic inflammation and viral pathogenesis, underscoring the need to balance catalytic inhibition with preservation of essential scaffold functions (Martens et al., 2020; Mace & Murphy, 2021).

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