## Phylogeny

Orthologous RIPK4 proteins are documented in Homo sapiens, Pan troglodytes, Macaca mulatta, Mus musculus, Bos taurus, Gallus gallus, Xenopus tropicalis, Xenopus laevis and Danio rerio, with ~90 % identity in the kinase domain between human, mouse and bovine and ~62 % identity to zebrafish (huang2018crystalstructureof pages 1-3, hasnain2022insightintoripk4 pages 7-9, unknownauthors2011exploringtherole pages 137-139).  
Within the human kinome RIPK4 is assigned to the Tyrosine-Kinase-Like (TKL) group, receptor-interacting protein kinase family, and is most closely paralogous to RIPK5/ANKK1; it is evolutionarily distinct from the RIPK1-3/7 branch that carries death- or RHIM-containing C-termini (unknownauthors2011exploringtherole pages 117-122, fay2024evolutionaryandfunctional pages 1-4).  
Comparative genomic surveys across 489 jawed-vertebrate genomes show broad conservation of RIPK4 with limited evidence of recurrent positive selection, contrasting the accelerated evolution and lineage-specific loss seen for RIPK1 and RIPK3 (fay2024evolutionaryandfunctional pages 4-7).

## Reaction Catalyzed

ATP + [protein] ⇌ ADP + [protein]-O-phosphate (huang2018crystalstructureof pages 3-4).

## Cofactor Requirements

Catalysis requires Mg²⁺; enzymatic assays and crystallization employed 10 mM MgCl₂, and no Mn²⁺ dependence was reported (huang2018crystalstructureof pages 15-17).

## Substrate Specificity

A global consensus phosphorylation motif has not been defined. Experimentally confirmed substrates and sites include:  
• IRF6 Ser413/Ser424 (huang2018crystalstructureof pages 9-10)  
• Dishevelled-2 (DVL2) at characterized sites within its DEP domain (unknownauthorsUnknownyearinvestigatingthepkcηripk4 pages 14-16)  
• IKKα and IKKβ activation-loop residues leading to canonical IKK complex activation (unknownauthorsUnknownyearinvestigatingthepkcηripk4 pages 14-16)

## Structure

Domain organisation: N-terminal bilobal serine/threonine kinase domain → disordered intermediate segment → eleven C-terminal ankyrin repeats (huang2018crystalstructureof pages 1-3).  
Crystal structures of the murine kinase domain (e.g., PDB 5WNI–5WNM) reveal:  
• Canonical active-state architecture with intact Gly-rich P-loop, β3 Lys51–αC Glu69 salt bridge, DFG motif (Asp143) and HLN catalytic loop (huang2018crystalstructureof pages 3-4).  
• Side-to-side BRAF-like homodimer; interface residues R40, R79 and E284 form an extensive hydrogen-bond/salt-bridge network essential for activity (huang2018crystalstructureof pages 8-9).  
• Absence of the αJ helix found in RIPK1-3; ankyrin repeats adopt an autoinhibitory conformation that dampens NF-κB signalling when co-expressed with the isolated kinase domain (huang2018crystalstructureof pages 1-3).  
• Activation loop is constitutively ordered and does not require phosphorylation for catalytic competence, consistent with non-RD kinase behaviour (huang2018crystalstructureof pages 4-6).

## Regulation

Post-translational modifications  
• Autophosphorylation: Ser173, Ser175 and Ser179 within the activation loop enhance catalytic output (unknownauthors2011exploringtherole pages 122-124).  
• Upstream phosphorylation by PKCη: Ser103 and Thr283 are prerequisite “priming” sites that trigger subsequent autophosphorylation (unknownauthorsUnknownyearinvestigatingthepkcηripk4 pages 29-33).  
• Caspase-mediated cleavage at Asp340 and Asp378 yields a C-terminal fragment that dominantly suppresses NF-κB transcriptional activity (unknownauthors2011exploringtherole pages 122-124).  
Protein–protein/ubiquitin regulation  
• TRAF1/2/3/5 binding is required for RIPK4-driven NF-κB activation; dominant-negative TRAF1 or TRAF3 blocks this output (unknownauthors2011exploringtherole pages 122-124).  
Conformational control  
• Homodimerisation is obligatory for catalytic activity; mutations E284A or R40A/R79A/R97A disrupt the dimer and abolish ATP hydrolysis while leaving substrate binding intact (huang2018crystalstructureof pages 8-9).  
• C-terminal ankyrin repeats impose intramolecular autoinhibition of NF-κB signalling (huang2018crystalstructureof pages 1-3).

## Function

Expression: High in stratified epithelium and broadly present in non-neural tissues (huang2018crystalstructureof pages 1-3).  
Upstream regulators: PKCβ1, PKCδ and PKCη interact with the intermediate domain and enhance RIPK4 phosphorylation status (unknownauthors2011exploringtherole pages 122-124, unknownauthorsUnknownyearinvestigatingthepkcηripk4 pages 14-16).  
Downstream signalling and partners:  
• Phosphorylation of IRF6 promotes keratinocyte differentiation and epidermal barrier assembly (huang2018crystalstructureof pages 9-10).  
• Activation of NF-κB via IKKα/β phosphorylation and TRAF-dependent NEMO ubiquitination drives inflammatory gene expression (unknownauthors2017xiapmediatedinnateimmune pages 49-55).  
• Phosphorylation of DVL2 stimulates Wnt/β-catenin signalling (unknownauthorsUnknownyearinvestigatingthepkcηripk4 pages 14-16).  
• Over-expression activates JNK-AP-1 in a kinase-dependent manner (unknownauthors2011exploringtherole pages 122-124).

## Inhibitors

ATP-competitive ligands staurosporine, lestaurtinib, TG-100-115 and VX-680 bind the catalytic cleft in co-crystal structures (huang2018crystalstructureof pages 1-3).  
BRAF inhibitors vemurafenib and dabrafenib lower cellular RIPK4 protein levels in melanoma models, although direct enzymatic inhibition has not been demonstrated (madej2023vemurafenibanddabrafenib pages 23-23).

## Other Comments

Disease associations  
• Autosomal-recessive Bartsocas-Papas and lethal popliteal pterygium syndromes arise from loss-of-function mutations such as I121N, T184I and E284K that impair kinase stability or dimerisation (kalay2012mutationsinripk4 pages 3-4, huang2018crystalstructureof pages 9-10, hasnain2022insightintoripk4 pages 12-14).  
• RIPK4 knock-down increases proliferation and invasiveness of cutaneous squamous cell carcinoma cells (unknownauthorsUnknownyearinvestigatingthepkcηripk4 pages 14-16).  
• Over-expression promotes bladder urothelial carcinoma aggressiveness through NF-κB-dependent induction of VEGF-A (liu2018ripk4promotesbladder pages 11-11).

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