1. Phylogeny  
   RIPK2 (also known as CARDIAK, RICK, or RIP2) belongs to the receptor‐interacting protein (RIP) kinase family, which is part of the tyrosine kinase-like (TKL) group and is classified as a non-RD kinase because it lacks the conserved arginine residue in the RD motif in its catalytic domain (dardick2006plantandanimal pages 2-3). Orthologs of RIPK2 have been identified in diverse vertebrate species, and its domain architecture is conserved across species that employ innate immune signaling pathways. In evolutionary terms, RIPK2 clusters with other RIP kinase family members—including RIPK1, RIPK3, and RIPK4—and is further related to kinases implicated in innate immunity such as IRAK family members. Its evolutionary conservation underscores its essential role as an effector in intracellular pathogen-recognition receptor signaling (lv2022comparativeandevolutionary pages 3-4).
2. Reaction Catalyzed  
   RIPK2 is a dual-specificity kinase that catalyzes the phosphorylation of serine, threonine, and tyrosine residues. The general reaction it mediates is: ATP + [protein]-(L-serine or L-threonine/tyrosine) → ADP + [protein]-(phosphorylated serine/threonine/tyrosine) + H⁺. In this reaction, ATP serves as the phosphate donor and the protein substrate – which may be RIPK2 itself (autophosphorylation) or other downstream targets – is phosphorylated, thereby modulating its activity (pellegrini2017structuresofthe pages 1-2).
3. Cofactor Requirements  
   The kinase activity of RIPK2 depends on divalent cations. In structures and in vitro kinase assays, Mg²⁺ is consistently used to coordinate and stabilize the ATP phosphates at the catalytic site, ensuring efficient phosphotransfer. The crystallographic and biochemical studies employed buffers containing MgCl₂ to facilitate nucleotide binding and kinase activity, and no experimental evidence has indicated a requirement for Mn²⁺ in the catalytic mechanism of RIPK2 (pellegrini2017structuresofthe pages 5-7, lethier2022structuralanalysisshows pages 7-10).
4. Substrate Specificity  
   Large-scale kinase substrate profiling has placed RIPK2 within a specific substrate motif cluster of the human serine/threonine kinome. Although the precise consensus sequence for RIPK2 is derived from high-throughput studies, evidence indicates that kinases in its group, including RIP kinases, have a substrate motif enriched with basic residues flanking the phosphorylated serine or threonine, accompanied by a preference for an aromatic residue at the +3 position relative to the phospho-acceptor residue (johnson2023anatlasof pages 2-3). This suggests that RIPK2, when selecting phosphorylation sites, favors a microenvironment that stabilizes the bound substrate via electrostatic interactions and hydrophobic contacts.
5. Structure  
   RIPK2 is organized into two primary domains: an N-terminal kinase domain and a C-terminal CARD (caspase recruitment domain) (gong2018structuralbasisof pages 1-2, pellegrini2017structuresofthe pages 1-2). The N-terminal kinase domain adopts a typical bilobal architecture seen in eukaryotic protein kinases, with an N-lobe primarily composed of β-strands and a C-lobe rich in α-helices. Critical catalytic features within this domain include the activation loop, which contains multiple autophosphorylation sites that facilitate the transition from inactive to active conformations; the DFG motif, whose aspartate coordinates a Mg²⁺ ion essential for ATP binding; and the αC-helix, whose inward displacement is necessary for forming the salt bridge between an invariant lysine (analogous to Lys47 in RIPK2) and a conserved glutamate (dardick2006plantandanimal pages 2-3, pellegrini2017structuresofthe pages 11-13). The C-terminal CARD domain is responsible for mediating protein–protein interactions, particularly with the CARD domains of NOD1 and NOD2. This domain facilitates the oligomerization of RIPK2 into filamentous structures (the “RIPosome”), which is essential for the propagation of downstream signaling events (gong2018structuralbasisof pages 1-2, lethier2023structureshowsthat pages 1-2). Moreover, structural studies have revealed that RIPK2 can dimerize via its kinase domain, forming an antiparallel configuration that is stabilized by interactions involving distinct interface regions, including the αC-helix and adjacent loops. These dimerization events are critical for proper positioning of the active site and for subsequent recruitment of ubiquitin ligases, such as XIAP, which bind across the kinase dimer interface (lethier2022structuralanalysisshows pages 1-4, lethier2023structureshowsthat pages 3-4).
6. Regulation  
   RIPK2 is regulated by a series of post-translational modifications that modulate its stability and activity. Following its recruitment by NOD1 and NOD2, RIPK2 undergoes autophosphorylation in the activation segment, which, while not strictly required for the propagation of downstream NF-κB signaling, contributes to its proper conformational stability (pellegrini2017structuresofthe pages 1-2). In addition to phosphorylation events, RIPK2 is subject to Lys63-linked polyubiquitination mediated by E3 ubiquitin ligases such as XIAP, BIRC2, and BIRC3, as well as linear (Met1-linked) polyubiquitination by the LUBAC complex. These ubiquitin modifications transform RIPK2 into a scaffolding protein that recruits key downstream effectors, including IKBKG/NEMO, thereby facilitating the activation of the canonical NF-κB pathway (dardick2006plantandanimal pages 13-14, pellegrini2017structuresofthe pages 25-26). Structurally, the kinase domain interacts with the BIR2 domain of XIAP across the dimer interface, an interaction that is essential for subsequent polyubiquitination events (lethier2022structuralanalysisshows pages 1-4). Through these post-translational modifications, RIPK2 is tightly regulated in both its activation and its ability to serve as a signal-transducing hub in innate immune responses.
7. Function  
   RIPK2 plays a central role in modulating both innate and adaptive immune responses. It is a key effector in NOD1 and NOD2 signaling pathways—upon recognition of bacterial peptidoglycan fragments by these intracellular pattern recognition receptors, RIPK2 is recruited via CARD–CARD interactions and forms filamentous signaling platforms. This oligomerization facilitates its autophosphorylation, subsequent ubiquitination, and the assembly of signaling complexes that lead to activation of downstream kinases including MAP3K7/TAK1, and ultimately the NF-κB transcription factor (gong2018structuralbasisof pages 1-2, pellegrini2017structuresofthe pages 13-15). RIPK2 also contributes to adaptive immunity; for example, during T-cell receptor engagement, RIPK2 participates in BCL10 phosphorylation and NF-κB activation (information section). Moreover, RIPK2 is involved in non-canonical signaling pathways such as the tyrosine phosphorylation of guanine exchange factor ARHGEF2, which further stimulates NF-κB activity via Src family kinases, and in mediating RHOA inactivation in response to nerve growth factor receptor signaling (dardick2006plantandanimal pages 9-10). Its widespread expression in immune cells underscores its importance in orchestrating immune responses against bacterial pathogens, thereby bridging pathogen recognition with inflammatory and survival responses at the cellular level (pellegrini2017structuresofthe pages 25-26).
8. Other Comments  
   Pharmacological targeting of RIPK2 has attracted significant interest given its role in inflammatory diseases such as Crohn’s disease and other autoinflammatory syndromes. Although the kinase activity of RIPK2 is dispensable for NF-κB activation in some contexts—with its scaffolding function being the primary driver—it remains a viable target for inhibitors that aim to disrupt its protein–protein interactions or ubiquitination events. Type II kinase inhibitors, including compounds like ponatinib, have been reported to modulate RIPK2 activity and are currently under investigation as potential therapeutic agents (gong2018structuralbasisof pages 1-2). Additionally, the development of inhibitors that interfere with the CARD-mediated oligomerization or the binding interface with XIAP presents an attractive strategy to dampen excessive RIPK2 signaling in disease contexts (lethier2023structureshowsthat pages 1-2). Known mutations and dysregulation in the NOD–RIPK2 signaling pathway have been linked to a range of inflammatory conditions, reinforcing the clinical relevance of this kinase. Further elucidation of the detailed substrate specificity via phosphoproteomic mapping continues to refine our understanding of RIPK2’s signaling network (johnson2023anatlasof pages 2-3).
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