1. Phylogeny  
   RIPK2 (Receptor-interacting serine/threonine-protein kinase 2), also known as CARDIAK, RICK, or RIP2, is a member of the receptor-interacting protein (RIP) kinase family. It occupies a distinct phylogenetic niche among kinases involved in innate immune regulation, exhibiting both serine/threonine and tyrosine phosphorylation capabilities that are not prominent in some of its homologs such as RIPK1, RIPK3, or RIPK4 (chirieleison2016syntheticbiologyreveals pages 1-2, cuny2021ripkproteinkinase pages 1-2). Although all RIP kinases share a conserved kinase domain, the evolutionary trajectory of RIPK2 has led to the acquisition of a unique caspase recruitment domain (CARD) that enables specific CARD–CARD interactions with the NOD1 and NOD2 receptors. Orthologs of RIPK2 are found widely throughout vertebrates, indicating that its role in linking bacterial sensing to downstream immune responses emerged early in vertebrate evolution and has been maintained by strong purifying selection (chirieleison2016syntheticbiologyreveals pages 2-4, cuny2021ripkproteinkinase pages 2-3).
2. Reaction Catalyzed  
   RIPK2 catalyzes the transfer of a phosphate group from ATP to specific serine, threonine, and tyrosine residues on substrate proteins. The general chemical reaction is represented as:  
     ATP + [protein]–OH → ADP + [protein]–O–PO₃²⁻ + H⁺  
   In its signaling role, RIPK2 undergoes autophosphorylation, which is critical for its function. In addition, it phosphorylates substrates that participate in the propagation of NF-κB and MAP kinase signaling cascades, particularly in pathways downstream of NOD1 and NOD2 receptors (barczyk20244anilinoquinazolinederivativesas pages 41-42, he2017identificationofpotent pages 1-4). Although the precise molecular mechanism and identity of all physiological substrates remain subjects of active research, its dual-specificity allows it to modulate both serine/threonine and tyrosine phosphorylation events inherent to the modulation of innate and adaptive immune responses.
3. Cofactor Requirements  
   RIPK2 enzymatic activity depends on the presence of ATP as the phosphate donor, and like many kinases, it requires divalent metal ions—most notably Mg²⁺—to facilitate the coordination of ATP in the catalytic cleft (barczyk20244anilinoquinazolinederivativesas pages 41-42, pham2023recentadvancesin pages 1-3). The binding of Mg²⁺ stabilizes the negative charges on the phosphate groups of ATP and is essential for optimal positioning of the nucleotide for phosphoryl transfer. While Mg²⁺ is the primary metal ion requirement, some kinase reactions may also be supported by Mn²⁺ under specific experimental conditions, although available literature primarily supports the role of Mg²⁺ in RIPK2-catalyzed reactions.
4. Substrate Specificity  
   RIPK2 exerts its function by phosphorylating a select array of substrates that participate in the innate immune response. Key substrates include itself (as demonstrated by autophosphorylation events) and proteins that are components of the downstream NF-κB and MAPK signaling cascades. Notably, RIPK2 has been linked to the tyrosine phosphorylation of the guanine exchange factor ARHGEF2, an event that contributes to NOD2-mediated NF-κB activation (barczyk20244anilinoquinazolinederivativesas pages 41-42, pham2023recentadvancesin pages 17-18). Although comprehensive mapping of the consensus phosphorylation motif for RIPK2 is still emerging, the kinase domain’s dual specificity is evidenced by its ability to modify both serine/threonine and tyrosine residues, suggesting that substrate recognition may involve conformational determinants as well as primary amino acid sequence motifs (chirieleison2016syntheticbiologyreveals pages 7-9, pham2023recentadvancesin pages 18-19). Recent studies highlight that substrate specificity is particularly critical for the proper recruitment of downstream effectors, such as the NF-κB essential modulator (NEMO), which becomes ubiquitinated in a RIPK2-dependent manner following phosphorylation events.
5. Structure  
   RIPK2 is a multidomain protein whose architecture is composed of a central kinase domain, an intermediate linker region, and a C-terminal caspase recruitment domain (CARD). The N-terminal kinase domain adopts a typical bilobal structure common to serine/threonine and dual-specificity kinases and contains a conserved ATP-binding pocket with key residues—such as a catalytic lysine (e.g., Lys209) that is critical for ATP coordination—and motifs like the DFG and HXD sequences that are essential for phosphoryl transfer (heim2020aregulatoryinterface pages 21-23, cuny2021ripkproteinkinase pages 10-10).  
   The intermediate domain, although less well characterized, is thought to contribute to the structural flexibility necessary for RIPK2’s autophosphorylation and helps facilitate interactions between the kinase domain and regulatory factors. The C-terminal CARD domain mediates homotypic interactions with other CARD-containing proteins, most notably the NOD1 and NOD2 receptors, thereby driving the formation of higher-order oligomeric signaling complexes (chirieleison2016syntheticbiologyreveals pages 9-11, misehe2024designsynthesisand pages 44-49).  
   Structural studies, including crystallography and predictive modeling (e.g., via AlphaFold), have reinforced the presence of conserved motifs and residues necessary for catalysis and regulation, and have also highlighted unique features such as a smaller threonine gatekeeper residue that may render RIPK2 more amenable to allosteric inhibition (cuny2021ripkproteinkinase pages 2-3, lethier2023structureshowsthat pages 1-2). The combined domain organization endows RIPK2 with both catalytic activity and the ability to function as a scaffold, integrating both enzymatic and protein–protein interaction roles within innate immune signaling pathways.
6. Regulation  
   The regulation of RIPK2 is governed by a multilayered network of post-translational modifications that modulate its enzymatic activity, stability, protein interactions, and signal transduction capability. A key regulatory step is its autophosphorylation, which occurs on multiple serine, threonine, and particularly a crucial tyrosine residue (e.g., Tyr474) that is essential for maximum signaling output in response to NOD receptor activation (chirieleison2016syntheticbiologyreveals pages 7-9, zare2022theroleof pages 32-35).  
   In addition to autophosphorylation, RIPK2 is subject to extensive ubiquitination. Lys-63-linked polyubiquitin chains, as well as Met-1-linked (linear) ubiquitin chains, are attached to RIPK2 by E3 ubiquitin ligases such as XIAP, BIRC2, BIRC3, and the LUBAC complex. These ubiquitin modifications are pivotal for serving as docking platforms that recruit downstream signaling components, including NEMO, TAB2/3, and TAK1 complexes, which collectively propagate NF-κB activation (barczyk20244anilinoquinazolinederivativesas pages 41-42, topal2021ripk2nodsto pages 6-11).  
   Enzymes such as A20, CYLD, OTULIN, and MYSM1 act as deubiquitinases to remove these ubiquitin chains, thereby fine-tuning signaling intensity and duration (heim2020aregulatoryinterface pages 7-9, topal2021ripk2nodsto pages 15-19). Notably, while RIPK2’s kinase activity is dispensable for certain aspects of NOD1/2-mediated NF-κB activation, its regulatory autophosphorylation and ubiquitination remain essential for the formation of signaling complexes and the proper recruitment of downstream effectors (barczyk20244anilinoquinazolinederivativesas pages 41-42, topal2021ripk2nodsto pages 15-19). Allosteric regulation through conformational changes and protein–protein interactions further influences its scaffold function, and recent structural insights have begun to elucidate mechanisms by which small-molecule inhibitors can disrupt critical interfaces, such as the RIPK2–XIAP interaction (cuny2021ripkproteinkinase pages 10-10, chirieleison2016syntheticbiologyreveals pages 11-15).
7. Function  
   RIPK2 plays a central role in initiating and modulating both innate and adaptive immune responses. It is best known as the key effector that mediates signaling downstream of the NOD1 and NOD2 receptors, which detect bacterial peptidoglycan motifs such as diaminopimelic acid and muramyl dipeptide. Upon recognition of these microbial products, NOD receptors oligomerize and recruit RIPK2 via CARD–CARD interactions, triggering RIPK2 autophosphorylation and subsequent ubiquitination events that serve to activate NF-κB and MAP kinase pathways (barczyk20244anilinoquinazolinederivativesas pages 41-42, topal2021ripk2nodsto pages 1-6).  
   Once activated, RIPK2 functions as a scaffold, assembling multi-protein complexes that eventually lead to upregulation of pro-inflammatory cytokines and chemokines. Beyond its pivotal role in innate immunity, RIPK2 also contributes to adaptive immune functions; for example, it participates in T-cell receptor engagement and BCL10 phosphorylation, thereby influencing NF-κB activation during T-cell responses (barczyk20244anilinoquinazolinederivativesas pages 41-42, topal2021ripk2nodsto pages 15-19).  
   Additionally, RIPK2 is implicated in cytoskeletal regulation via its ability to modulate the tyrosine phosphorylation of the guanine exchange factor ARHGEF2, leading to alterations in RHOA-mediated signaling and affecting cell morphology and migration (barczyk20244anilinoquinazolinederivativesas pages 41-42). Such diverse functions underscore its importance in orchestrating immune defenses, inflammation, cell survival, and apoptosis, making it a multifunctional hub whose dysregulation has been linked to several autoinflammatory and autoimmune disorders.
8. Other Comments  
   Inhibitors targeting RIPK2 have become an intense area of pharmacological research due to the protein’s pivotal role in inflammatory and immune signaling. Agents such as GSK583, OD36, OD38, and Ponatinib have been characterized as potent inhibitors that interfere with RIPK2’s kinase domain and/or its ability to interact with XIAP, thereby dampening NF-κB activation (bryan2018kinaseinhibitorsfor pages 16-17, he2017identificationofpotent pages 1-4). Although the kinase activity of RIPK2 is not strictly required for NOD1/2-mediated NF-κB signaling, the inhibition of its autophosphorylation and ubiquitination has proven effective in reducing inflammatory responses in preclinical models.  
   RIPK2 dysregulation is associated with a variety of human disorders, including inflammatory bowel disease (IBD), Blau syndrome, rheumatoid arthritis, and certain types of cancer. Genetic mutations or aberrant post-translational modifications that affect RIPK2’s stability, catalytic function, or its ability to interact with key adaptors (such as XIAP) have been implicated in these conditions (zare2022theroleof pages 65-67, topal2021ripk2nodsto pages 28-34).  
   Current research focuses on refining the selectivity and potency of RIPK2 inhibitors while minimizing off-target effects. Structural insights obtained from X-ray crystallography and advanced modeling approaches (e.g., AlphaFold) have facilitated the design of inhibitors that target specific conformations of the RIPK2 kinase domain, thereby disrupting key protein–protein interactions necessary for its scaffolding function (lethier2023structureshowsthat pages 1-2, fan2023designsynthesisand pages 12-12).  
   Furthermore, the dual functional roles of RIPK2—both as an active kinase and as a scaffold—present unique challenges and opportunities for therapeutic intervention. While inhibition of catalytic activity may not completely block pathological signaling due to its scaffold function, targeting the regulatory interfaces involved in ubiquitination and adaptor recruitment shows promise for more effective modulation of downstream immune responses (heim2020aregulatoryinterface pages 9-12, chirieleison2016syntheticbiologyreveals pages 7-9).
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