1. Phylogeny  
   Phosphoinositide 3‐kinase regulatory subunit 4 (PIK3R4), also designated as VPS15 or the PI3‐kinase p150 subunit, is the regulatory component of the Class III phosphoinositide 3‐kinase complex and is evolutionarily conserved from yeast to mammals (cook2025structuralpathwayfor pages 1-3). Orthologs of PIK3R4, originally identified in Saccharomyces cerevisiae as Vps15, have been maintained across diverse eukaryotic lineages including metazoans and plants, indicating a preservation of essential cellular functions from the Last Eukaryotic Common Ancestor (philippon2015evolutionaryhistoryof pages 1-2). Sequence comparisons and domain architecture analyses reveal that PIK3R4 shares a common evolutionary origin with other core regulators of phosphoinositide metabolism and is grouped uniquely within the Class III PI3K complex, which is distinct from the multiple isoforms and subtypes found in Class I and II kinases (foster2003thephosphoinositide(pi) pages 1-2). Phylogenetic studies have demonstrated that, while the catalytic subunit VPS34 (PIK3C3) is singular and highly conserved, the regulatory partner PIK3R4 retains characteristic motifs that are conserved among its orthologs in both lower and higher eukaryotes (philippon2015evolutionaryhistoryof pages 14-15). Comparative analyses show that PIK3R4 does not belong to a large multigene family but rather forms an integral part of a relatively invariant enzyme complex essential for membrane trafficking and autophagy (reidick2017theclassiii pages 1-2). The evolutionary relationships between PIK3R4 and other kinases in the core phosphoinositide pathway underscore its indispensable role within the ancient signaling modules that have been refined during eukaryotic evolution (cook2025structuralpathwayfor pages 1-3).
2. Reaction Catalyzed  
   The Class III phosphoinositide 3‐kinase complex, for which PIK3R4 functions as the regulatory subunit, catalyzes the phosphorylation of phosphatidylinositol (PtdIns) at the 3‐hydroxyl position of the inositol ring using ATP as the phosphate donor, thereby generating phosphatidylinositol 3‐phosphate (PtdIns3P) and ADP (cook2025structuralpathwayfor pages 8-9, foster2003thephosphoinositide(pi) pages 3-4). This enzymatic reaction is fundamental to the production of a lipid signaling molecule that orchestrates downstream protein recruitment and membrane trafficking events (foster2003thephosphoinositide(pi) pages 3-4).
3. Cofactor Requirements  
   The phosphorylation reaction catalyzed by the VPS34 complex requires Mg²⁺ as a cofactor that facilitates ATP binding and phosphate transfer, a requirement that is consistent with the enzymology of PI3K family members (foster2003thephosphoinositide(pi) pages 3-4).
4. Substrate Specificity  
   The substrate specificity of the Class III PI3K complex is directed exclusively toward phosphatidylinositol, with the enzyme catalyzing the phosphorylation solely at the D3 position to produce PtdIns3P (foster2003thephosphoinositide(pi) pages 3-4, ohashi2021activationmechanismsof pages 2-3). This specificity is essential for the recruitment of PI3P‐binding effector proteins that mediate vesicular trafficking and autophagic processes (ohashi2021activationmechanismsof pages 2-3).
5. Structure  
   PIK3R4 is characterized by a multi‐domain architecture that supports its role as a regulatory scaffold within the Class III PI3K complex. Its N-terminal region comprises a pseudokinase domain that adopts a canonical kinase fold while exhibiting distinct deviations in conserved motifs that render it catalytically inert; notably, this domain binds GTP with high specificity through an unusual conserved arginine residue at the gatekeeper position, for instance Arg103, thereby contributing to an autoinhibitory mechanism of the VPS34 complex (cook2025structuralpathwayfor pages 1-3, ohashi2021activationmechanismsof pages 3-4). Following the pseudokinase domain, PIK3R4 contains an array of HEAT repeats that form a helical solenoid; these repeats are critical for maintaining structural integrity and serve as a platform for interacting with the VPS34 lipid kinase domain (rostislavleva2015structureandflexibility pages 3-4). The C-terminal portion of PIK3R4 is occupied by multiple WD40 repeats that assemble into a β-propeller structure, which is responsible for mediating protein–protein interactions with additional subunits of the VPS34 complex such as Beclin 1 and Vps30; these interactions are vital for proper complex assembly and functional regulation (craene2017phosphoinositidesmajoractors pages 15-17). High-resolution cryo-electron microscopy studies have delineated how the pseudokinase domain of PIK3R4 interfaces with VPS34, modulating the mobility and conformational dynamics of the catalytic site, a feature crucial for autoinhibition in the cytosolic state and activation upon membrane engagement (cook2025structuralpathwayfor pages 6-8, ohashi2021activationmechanismsof pages 7-9). Moreover, the presence of an N-myristoylation signal located near the N-terminus facilitates membrane tethering and proper spatial orientation of the VPS34 complex, further underscoring the integrated structural role of PIK3R4 in orchestrating catalytic function (cook2025structuralpathwayfor pages 1-3, rostislavleva2015structureandflexibility pages 1-3).
6. Regulation  
   PIK3R4 is regulated through a series of post-translational modifications and protein–protein interactions that modulate the activity of the VPS34 complex. One notable regulatory feature is the N-myristoylation of its N-terminal region, a modification that anchors the protein to membrane compartments and becomes dynamically exposed upon activation, thus facilitating recruitment to specific intracellular sites (cook2025structuralpathwayfor pages 1-3, ohashi2021activationmechanismsof pages 25-26). In addition, the pseudokinase domain of PIK3R4 binds GTP via a unique gatekeeper arginine residue (Arg103), and mutations in this residue (such as R103K) have been demonstrated to impair GTP binding and subsequently reduce the lipid kinase activity of the VPS34 complex, highlighting the importance of nucleotide binding in the regulation of the complex (cook2025structuralpathwayfor pages 18-21, ohashi2021activationmechanismsof pages 1-2). PIK3R4 also interfaces with regulatory Rab GTPases including Rab1A and Rab5A that contribute to the recruitment of the VPS34 complex to distinct membrane domains, thereby fine-tuning the spatial control of PtdIns3P production (okkenhaug2013signalingbythe pages 3-4, ohashi2021activationmechanismsof pages 22-24). Furthermore, conformational changes induced by these interactions relieve the autoinhibitory contacts between the PIK3R4 pseudokinase domain and the VPS34 activation loop, a transition that is essential for full enzymatic activation and subsequent downstream signaling (cook2025structuralpathwayfor pages 8-9, reidick2017theclassiii pages 4-6).
7. Function  
   PIK3R4 plays a central role as the regulatory subunit of the Class III PI3K complex by mediating the synthesis of phosphatidylinositol 3‐phosphate (PI3P), a lipid second messenger that is crucial for multiple membrane trafficking pathways. In its complex with VPS34, PIK3R4 is involved in the initiation phase of autophagy through Complex I, which includes the autophagy-specific subunit ATG14L, thereby facilitating autophagosome formation (cook2025structuralpathwayfor pages 1-3, foster2003thephosphoinositide(pi) pages 3-4). In addition, within Complex II—characterized by the presence of the UVRAG subunit—PIK3R4 contributes to the maturation of autophagosomes and endocytic vesicles, thereby playing an important role in degradative endocytic trafficking and cytokinesis (cook2025structuralpathwayfor pages 8-9, foster2003thephosphoinositide(pi) pages 3-4). Through its scaffolding function, PIK3R4 stabilizes VPS34 and regulates its catalytic activity by controlling conformational transitions that are critical for the generation of PI3P at defined intracellular membranes (ohashi2021activationmechanismsof pages 7-9, reidick2017theclassiii pages 2-4). The production of PI3P by the VPS34 complex is indispensable for the recruitment of effector proteins containing FYVE and PX domains, which in turn regulate vesicular trafficking processes such as endocytosis, autophagosome biogenesis, and lysosomal protein sorting (foster2003thephosphoinositide(pi) pages 3-4, okkenhaug2013signalingbythe pages 3-4). The function of the PIK3R4/VPS15-containing complexes is therefore critical for maintaining cellular homeostasis through the proper execution of autophagy and endosomal sorting pathways (cook2025structuralpathwayfor pages 1-3, ohashi2021activationmechanismsof pages 1-2).
8. Other Comments  
   To date, there are no selective pharmacological inhibitors reported that directly target PIK3R4; however, modulation of the activity of the VPS34 complex by interfering with its regulatory interfaces, including those mediated by PIK3R4, remains an active area of investigation (cook2025structuralpathwayfor pages 3-4, okkenhaug2013signalingbythe pages 28-32). Mutations or alterations that affect the post-translational modification and nucleotide-binding properties of the PIK3R4 pseudokinase domain have been linked to defects in autophagy and disruptions in endocytic trafficking; such dysregulation has been implicated in neurodegenerative conditions and may also contribute to tumorigenesis (cook2025structuralpathwayfor pages 1-3, raess2017decipheringthefunctional pages 48-51, okkenhaug2013signalingbythe pages 28-32). The unique structural features of PIK3R4, including its non-canonical kinase fold and membrane-targeting motifs, render it a potential target for therapeutic strategies aiming to modulate PI3P production in pathological contexts; nevertheless, further elucidation of disease-associated mutations is required before direct inhibitors can be developed (ohashi2021activationmechanismsof pages 6-7, reidick2017theclassiii pages 4-6).
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