## 1. Phylogeny

Ribosomal protein S6 kinase beta-1 (RPS6KB1) belongs to the AGC family of serine/threonine protein kinases, an evolutionarily conserved group that traces its origins back to the Last Eukaryotic Common Ancestor (LECA) along with other core regulators of the TOR signaling pathway such as PDK1, AKT/protein kinase B, and p90 ribosomal S6 kinases (RSKs) (malanchuk2024investigatingtheregulation pages 1-2, wilson2018newperspectivesopportunities pages 1-5). This kinase is part of the highly conserved TOR pathway gene set, indicating that RPS6KB1 and its orthologs are present in all mammalian species and likely across a broad range of eukaryotes. Evolutionary studies suggest that gene duplication events in early animals and fungi produced distinct yet functionally interrelated kinases such as S6K and RSK, both regulated by the upstream kinase PDK1 and modulated by mTORC1 signaling (malanchuk2024investigatingtheregulation pages 1-2, cronin2021theroleof pages 1-2).

## 2. Reaction Catalyzed

RPS6KB1 catalyzes the transfer of a phosphate group from ATP to specific serine or threonine residues on its substrates. The reaction can be summarized as:  
  ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺  
This phosphorylation reaction is central to downstream signaling in response to growth factors and nutrients, altering the activity, stability, or subcellular localization of target proteins involved in mRNA translation, cell growth, and survival (malanchuk2024investigatingtheregulation pages 1-2, wilson2018newperspectivesopportunities pages 5-9).

## 3. Cofactor Requirements

The catalytic activity of RPS6KB1 is dependent on the presence of Mg²⁺ ions, which are essential for coordinating the binding of ATP in the kinase active site and facilitating the transfer of the phosphate group to target proteins. The requirement for Mg²⁺ is typical for serine/threonine kinases and is critical for effective enzymatic function (wilson2018newperspectivesopportunities pages 1-5).

## 4. Substrate Specificity

RPS6KB1 displays substrate selectivity that is determined both by the amino acid sequence surrounding the phosphorylation site and by the three-dimensional conformation of the substrate proteins. Physiologically, RPS6KB1 phosphorylates a variety of substrates that are key regulators of protein synthesis and cell survival. Notable substrates include:  
• Ribosomal protein S6 (rpS6), which is integral to the 40S ribosomal subunit and whose phosphorylation stimulates mRNA translation (malanchuk2024investigatingtheregulation pages 1-2, malanchuk2024investigatingtheregulation pages 2-3).  
• Eukaryotic initiation factor 4B (EIF4B), a component of the cap-binding complex that is important for the initiation of translation (malanchuk2024investigatingtheregulation pages 15-16).  
• Eukaryotic elongation factor 2 kinase (EEF2K), whose phosphorylation leads to activation of EEF2 and promotes translation elongation in response to IGF1 (malanchuk2024investigatingtheregulation pages 1-2).  
• The pro-apoptotic protein BAD, whose inhibitory phosphorylation by RPS6KB1 contributes to cell survival by suppressing apoptotic signals (malanchuk2024investigatingtheregulation pages 1-2).  
Furthermore, consensus substrate motifs for S6K have been described with a preference for an RxRxxp[ST] motif, in which the presence of arginine residues upstream of the phosphorylated serine or threonine is critical for substrate recognition (malanchuk2024investigatingtheregulation pages 1-2, poomakkoth2016p90ribosomals6 pages 2-4).

## 5. Structure

RPS6KB1 is characterized by a modular organization typical of AGC kinases. It possesses a conserved central kinase domain that is divided into an N-terminal lobe responsible primarily for ATP binding and a larger C-terminal lobe that mediates substrate recognition and catalytic activity. Key structural features include:  
• A regulatory TOS (TOR signaling) motif near the N-terminus, which is necessary for binding to the RAPTOR component of mTORC1 and is crucial for mTOR-dependent phosphorylation and activation (malanchuk2024investigatingtheregulation pages 2-3).  
• An activation loop containing critical residues such as Thr229, which is phosphorylated by PDK1, and adjacent regions including Cys217 that have been identified as sites for novel post-translational modifications like CoAlation (malanchuk2024investigatingtheregulation pages 3-6, malanchuk2024investigatingtheregulation pages 9-11).  
• A hydrophobic motif, notably incorporating Thr389 (in S6K1) that is phosphorylated by mTORC1, which plays a key role in relieving autoinhibition and promoting full kinase activation (malanchuk2024investigatingtheregulation pages 6-9).  
Structural studies using molecular docking and dynamics simulations have provided insights into the binding of Coenzyme A (CoA) in the nucleotide-binding pocket, revealing stable interactions that involve hydrogen bonds with residues in the kinase hinge region (malanchuk2024investigatingtheregulation pages 9-11, malanchuk2024investigatingtheregulation pages 16-18).  
Additionally, the overall 3D conformation of RPS6KB1, as observed in crystal structures and predicted by AlphaFold models, shows typical AGC kinase features with regulatory regions that are intrinsically disordered, allowing flexibility for interacting with various substrates and regulatory proteins (wilson2018newperspectivesopportunities pages 1-5).

## 6. Regulation

The activity of RPS6KB1 is tightly regulated through both activating and inhibitory post-translational modifications (PTMs) to ensure precise control of cell growth and metabolism. Major regulatory mechanisms include:  
• Phosphorylation: Activation of RPS6KB1 requires sequential phosphorylation events. mTORC1 phosphorylates the hydrophobic motif (Thr389 in S6K1), which is critical for full activation and dissociation from complexes such as the EIF3 translation initiation complex under mitogenic stimulation (malanchuk2024investigatingtheregulation pages 1-2, malanchuk2024investigatingtheregulation pages 16-18). Subsequently, the kinase is phosphorylated on the activation loop (Thr229) by PDK1, a modification that is essential for achieving maximal catalytic activity (malanchuk2024investigatingtheregulation pages 6-9).  
• CoAlation: Under oxidative stress conditions, a novel regulatory PTM termed “CoAlation” occurs at Cys217 in the activation loop. This covalent modification by coenzyme A results in approximately 40% inhibition of kinase activity by interfering with the phosphorylation status of Thr229 and potentially protecting the cysteine from irreversible oxidation (malanchuk2024investigatingtheregulation pages 3-6, malanchuk2024investigatingtheregulation pages 9-11).  
• Other PTMs: Additional modifications such as acetylation, ubiquitination, and O-GlcNAcylation have been reported to modulate RPS6KB1 activity. For instance, acetylation at the C-terminus can inhibit mTOR-dependent phosphorylation, while ubiquitination affects protein turnover independently of phosphorylation status (malanchuk2024investigatingtheregulation pages 15-16, wilson2018newperspectivesopportunities pages 34-39).  
These regulatory mechanisms collectively ensure that RPS6KB1 integrates nutrient signals and mitogenic cues to appropriately regulate downstream processes, such as mRNA translation and cell survival (malanchuk2024investigatingtheregulation pages 1-2, cronin2021theroleof pages 10-11).

## 7. Function

RPS6KB1 functions as a central effector of mTOR signaling in response to growth factors and nutrient availability. Its biological roles are multifaceted and include:  
• Regulation of Protein Synthesis: By phosphorylating substrates such as ribosomal protein S6, EIF4B, and EEF2K, RPS6KB1 enhances both the initiation and elongation phases of protein synthesis. This leads to increased ribosome biogenesis and overall mRNA translation, thus supporting cell growth and proliferation (malanchuk2024investigatingtheregulation pages 1-2, malanchuk2024investigatingtheregulation pages 15-16).  
• Cell Cycle Progression and Growth: Phosphorylation events mediated by RPS6KB1 promote cell cycle progression and cell growth by activating pathways that lead to increased mRNA translation and by modulating the activity of proteins involved in cell cycle checkpoints (malanchuk2024investigatingtheregulation pages 1-2, poomakkoth2016p90ribosomals6 pages 2-4).  
• Feedback Regulation of mTOR Complexes: RPS6KB1 participates in negative feedback loops that regulate both mTORC1 and mTORC2. It phosphorylates components such as MAPKAP1/SIN1, MTOR, RICTOR, and DEPTOR, which in turn modulate the activity of AKT1 and other signaling pathways involved in cell survival and metabolism (malanchuk2024investigatingtheregulation pages 1-2, malanchuk2024investigatingtheregulation pages 9-11).  
• Cell Survival and Apoptosis: In addition to promoting protein synthesis, RPS6KB1 enhances cell survival by phosphorylating the pro-apoptotic protein BAD, thereby suppressing its apoptotic function. Moreover, the kinase’s influence on mitochondrial proteins such as URI1 contributes to the regulation of apoptosis via feedback mechanisms involving phosphatases like PPP1CC (malanchuk2024investigatingtheregulation pages 1-2, malanchuk2024investigatingtheregulation pages 9-11).  
• Metabolic Regulation: RPS6KB1 is implicated in metabolic reprogramming by phosphorylating IRS1, which leads to its degradation and contributes to insulin resistance, and by regulating enzymes involved in pyrimidine biosynthesis such as CAD (malanchuk2024investigatingtheregulation pages 1-2, malanchuk2024investigatingtheregulation pages 15-16).  
• Translation Initiation Complex Dynamics: Under nutrient deprivation, the inactive form of RPS6KB1 associates with the EIF3 initiation complex; however, upon mitogenic stimulation and phosphorylation by mTORC1, it dissociates from this complex to activate translation (malanchuk2024investigatingtheregulation pages 1-2).  
In summary, RPS6KB1 integrates signals from nutrient availability and growth factor stimulation into diverse cellular outcomes that include increased protein synthesis, cell growth, metabolic adjustment, and inhibition of apoptosis, making it a pivotal regulator of cell proliferation and survival (malanchuk2024investigatingtheregulation pages 1-2, cronin2021theroleof pages 10-11).

## 8. Other Comments

Several small molecule inhibitors have been developed to target RPS6KB1, motivated by its central role in cell growth, metabolism, and oncogenic signaling. For example, PF-4708671 is a selective inhibitor that is more potent against S6K1 relative to other closely related kinases such as RSK isoforms, while compounds like LY2584702 have progressed to clinical trials (bain2007theselectivityof pages 9-10, wilson2018newperspectivesopportunities pages 34-39). In addition to its role in normal cellular physiology, dysregulation of RPS6KB1 has been implicated in various disease contexts, including several cancers (where aberrant mTOR signaling drives tumor progression), obesity, and insulin resistance (malanchuk2024investigatingtheregulation pages 1-2, cronin2021theroleof pages 10-11). The kinase’s involvement in feedback loops that modulate both mTORC1 and mTORC2 signaling further underscores its potential as a therapeutic target, with ongoing research directed toward understanding its regulatory mechanisms—especially redox regulation via CoAlation—and developing more selective inhibitors (malanchuk2024investigatingtheregulation pages 9-11, bain2007theselectivityof pages 9-10). Furthermore, additional layers of control, such as acetylation and ubiquitination observed in RPS6KB1 regulation, suggest that combinatorial targeting strategies may be necessary to fully modulate its activity in disease settings. Notable mutations or alterations in expression levels of RPS6KB1 have been observed in cellular models of cancer, emphasizing the need for integrated phospho-proteomic and genomic analyses to unravel its complex role in oncogenesis (wilson2018newperspectivesopportunities pages 34-39, cronin2021theroleof pages 10-11).

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