1. Phylogeny  
   RSK3 (also known as RPS6KA2) is a member of the p90 ribosomal S6 kinase family, a subfamily of MAPK-activated protein kinases that evolved through gene duplication events within the animal lineage. RSK3 displays approximately 73–80% sequence identity with its paralogs RSK1, RSK2, and RSK4, and all share a conserved dual kinase domain architecture that distinguishes them from other serine/threonine kinases. The presence of an N-terminal kinase domain (NTKD) and a C-terminal kinase domain (CTKD), separated by a regulatory linker region, is reflective of an ancient fusion event between two distinct kinase genes. This arrangement is conserved among mammalian orthologs and is integrated within the larger AGC kinase family, which also comprises kinases such as protein kinase A (PKA), protein kinase B (AKT), and p70 S6 kinase. The evolutionary analyses based on sequence conservation and domain structure place RSK3 within the core signaling modules of the MAPK pathway that are seen as early as in the common ancestor of eukaryotes (cargnello2011activationandfunction pages 10-12, romeo2012regulationandfunction pages 2-4, lara2013thep90rsk pages 2-4).
2. Reaction Catalyzed  
   RSK3 catalyzes the phosphorylation of protein substrates by transferring a phosphate group from ATP to available hydroxyl groups on serine and threonine residues in target proteins. The reaction is represented as follows:  
     ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺  
   This reaction is typical for serine/threonine kinases and is instrumental in modulating the activity of substrates involved in transcription, translation, and cell cycle control (pan2004ribosomals6kinase pages 1-2, smith2005identificationofthe pages 1-1).
3. Cofactor Requirements  
   RSK3 requires magnesium ions (Mg²⁺) as a cofactor for its catalytic activity. Mg²⁺ is essential to properly coordinate ATP within the kinase active site and to stabilize the transition state during the phosphoryl transfer reaction. This requirement is characteristic of kinases in the AGC family and is crucial for efficient catalytic activity (pan2004ribosomals6kinase pages 1-2, yi2021ribosomalproteins6 pages 4-6).
4. Substrate Specificity  
   RSK3 exhibits a substrate specificity that is defined by its preference for phosphorylating serine residues within a consensus sequence containing basic residues. The motif is generally of the form Arg/Lys–X–Arg–X–X–Ser/Thr, with a notable selectivity for serine phosphorylation over threonine. The arrangement of positively charged arginine or lysine residues near the phosphoacceptor site is critical for substrate recognition and proper orientation within the active site, in line with the specificities described for other members of the RSK family (romeo2012regulationandfunction pages 7-8, lara2013thep90rsk pages 4-5, pearson2001mitogenactivatedprotein(map) pages 13-15).
5. Structure  
   RSK3 is organized into two distinct kinase domains with a regulatory linker region interposed between them. The N-terminal kinase domain (NTKD) belongs to the AGC family and adopts the characteristic bilobal structure with a smaller, β-sheet-rich N-lobe and a larger, α-helical C-lobe. This domain includes an ATP-binding cleft that incorporates key catalytic elements such as the glycine-rich loop, the DFG motif, and the catalytic loop. The C-terminal kinase domain (CTKD) is more structurally related to calcium/calmodulin-dependent kinases and primarily serves a regulatory function by undergoing autophosphorylation events that facilitate the subsequent activation of the NTKD. The regulatory linker contains critical motifs such as the hydrophobic motif (HM) and the turn motif (TM), which are necessary for recruiting phosphoinositide-dependent kinase 1 (PDK1) and for stabilizing the active conformation of the NTKD. In addition, structural studies from homologous kinases (e.g., RSK2) have revealed unique features such as a novel beta-sheet insertion in the N-lobe that may modulate substrate interactions and catalytic efficiency in RSK3 (cargnello2011activationandfunction pages 10-12, romeo2012regulationandfunction pages 5-7, kurinov2009structuraldiversityof pages 3-4).
6. Regulation  
   RSK3 is activated through a hierarchical phosphorylation cascade downstream of extracellular signal-regulated kinases (ERK1/2). Activation commences when ERK1/2 binds a conserved docking domain in the C-terminal portion of RSK3 and phosphorylates residues within the CTKD activation loop. This phosphorylation event in the CTKD triggers an autophosphorylation within the regulatory linker, particularly at the hydrophobic motif, thereby creating a binding site for PDK1. PDK1 then phosphorylates a critical residue in the activation loop of the NTKD, resulting in full kinase activation. In addition, RSK3 contains regulatory regions that include nuclear localization signals and potential autoinhibitory domains, which govern both its catalytic activity and its subcellular distribution following mitogenic stimulation. These phosphorylation events and protein–protein interactions ensure that RSK3 activity is precisely coordinated with upstream MAPK signaling (cargnello2011activationandfunction pages 12-13, martinez2015rsk3aregulator pages 11-14, romeo2012regulationandfunction pages 7-8).
7. Function  
   RSK3 functions as a serine/threonine-protein kinase positioned downstream of ERK signaling. It mediates mitogenic and stress-induced signaling by phosphorylating a range of substrates that include transcription factors and components of the translational machinery. Through these phosphorylation events, RSK3 regulates gene expression, translation processes, and subsequent cellular proliferation, survival, and differentiation. Tissue expression studies have shown that RSK3 is present in skeletal muscle, heart, pancreatic tissues, and fetal tissues, thus suggesting a role in tissue-specific signaling and developmental processes. In addition, while RSK3 generally promotes survival and proliferation, some studies indicate that its activity in epithelial ovarian cancer cells may function in a tumor suppressor capacity (cargnello2011activationandfunction pages 10-12, smith2005identificationofthe pages 1-2, yi2021ribosomalproteins6 pages 4-6).
8. Other Comments  
   Small molecule inhibitors such as SL0101 and BI-D1870 have been used to inhibit RSK isoforms, including RSK3, in cellular studies, thereby demonstrating the potential for selective therapeutic targeting in conditions characterized by aberrant RSK activity. RSK3 has been associated with oncogenic signaling in several cancer types, and its role in regulating transcription factors and translation suggests that dysregulation of its kinase activity can contribute to uncontrolled cell proliferation. In certain contexts, such as in epithelial ovarian cancer cells, RSK3 may exert tumor suppressive effects. The dual kinase domain architecture and the presence of distinct regulatory motifs highlight its potential as a unique drug target within the MAPK pathway (smith2005identificationofthe pages 6-7, clark2005theserinethreonineprotein pages 7-8, yi2021ribosomalproteins6 pages 4-6).
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