1. Phylogeny  
   SPEG‐b (Q15772), also known by the synonym Aortic Preferentially Expressed Protein 1 (APEG1), is classified within the myosin light chain kinase (MLCK) family and more specifically belongs to the Unc89/obscurin subfamily of giant muscle kinases. Phylogenetic analyses indicate that SPEG is evolutionarily conserved among vertebrates and is closely related to obscurin and UNC-89 kinases, with its origin attributed to ancestral gene duplication events in the MLCK superfamily (grogan2020doublethetrouble pages 1-2, temmerman2013structuralandfunctional pages 7-9). Multiple isoforms are produced through alternative splicing and promoter usage; notably, the SPEGα and SPEGβ isoforms are predominantly expressed in cardiac and skeletal muscle, while the APEG1 isoform (also referred to as SPEG isoform 3) is expressed in arterial smooth muscle cells (luo2021striatedpreferentiallyexpressed pages 1-2).
2. Reaction Catalyzed  
   SPEG catalyzes the transfer of a phosphate group from ATP to the hydroxyl group of serine or threonine residues on its substrate proteins. The chemical reaction can be represented as:  
     ATP + [protein]–(Ser/Thr) → ADP + [protein]–(Ser/Thr)–phosphate + H⁺  
   this reaction is characteristic of serine/threonine protein kinases (quan2019spegcontrolscalcium pages 1-2, quick2017speg(striatedmuscle pages 1-3).
3. Cofactor Requirements  
   The kinase activity of SPEG is dependent on the presence of divalent cations, with Mg²⁺ serving as the primary cofactor that facilitates proper ATP binding and subsequent phosphoryl transfer (quick2017speg(striatedmuscle pages 1-3, temmerman2013structuralandfunctional pages 7-9).
4. Substrate Specificity  
   SPEG displays substrate specificity toward serine/threonine residues in proteins that play integral roles in muscle calcium handling and structural organization. In cardiac muscle, its second kinase domain (SK2) phosphorylates the sarcoplasmic/endoplasmic reticulum Ca²⁺ ATPase 2a (SERCA2a) at threonine 484, thereby enhancing SERCA2a oligomerization and activity (quan2019spegcontrolscalcium pages 1-2, quan2019spegcontrolscalcium pages 11-13). SPEG also targets junctophilin-2 (JPH2), a key component of the junctional membrane complex responsible for maintaining T-tubule integrity, as well as other substrates such as the ryanodine receptor 2 (RyR2) and tropomyosin in muscle cells (luo2021striatedpreferentiallyexpressed pages 4-5, quick2017speg(striatedmuscle pages 13-16, liu2009disruptionofstriated pages 4-6). Although a defined consensus substrate motif has not yet been established, the substrates consistently harbor regulatory serine/threonine sites that are critical for calcium handling and muscle contractility (quan2019spegcontrolscalcium pages 7-9).
5. Structure  
   SPEG is organized into a multidomain architecture characteristic of giant muscle kinases. Its N-terminal region contains multiple immunoglobulin-like (Ig) domains and fibronectin type III (FnIII) domains, which are thought to mediate protein–protein interactions and contribute to subcellular scaffolding (grogan2020doublethetrouble pages 1-2, luo2021striatedpreferentiallyexpressed pages 1-2). This is followed by a linker region and then two tandem serine/threonine kinase domains (designated SK1 and SK2) at the C-terminus. Both kinase domains harbor conserved catalytic motifs including the DFG motif, an activation loop, and residues critical for ATP coordination; for instance, key aspartate residues (such as those highlighted in studies of SK2) are essential for catalytic function (quan2019spegcontrolscalcium pages 5-7). In addition, SPEG contains calmodulin-binding regions and potential autoinhibitory elements that may regulate its activity (fleming2021exploringobscurinand pages 7-9, grogan2020doublethetrouble pages 8-9). The structural diversity is expanded by isoform variation: while SPEGα and SPEGβ are prevalent in striated muscle, the APEG1 isoform—lacking portions of the N-terminal region—is specifically expressed in arterial smooth muscle cells, which may underlie its role in the growth and differentiation of these cells (luo2021striatedpreferentiallyexpressed pages 2-4).
6. Regulation  
   The regulatory mechanisms controlling SPEG kinase activity involve multiple layers including autophosphorylation, protein–protein interactions, and potential modulation by calcium/calmodulin. Autophosphorylation events, particularly within the inter-kinase linker regions, may relieve autoinhibition and enhance catalytic activity (fleming2021exploringobscurinand pages 5-7, grogan2020doublethetrouble pages 8-9). In cardiac muscle, SPEG-mediated phosphorylation of key substrates such as SERCA2a and JPH2 is critical for maintaining proper calcium homeostasis and structural integrity of the junctional membrane complexes; reductions in substrate phosphorylation correlate with impaired calcium handling and the development of heart failure (quan2019spegcontrolscalcium pages 7-9, quick2017speg(striatedmuscle pages 11-13). Although the precise upstream regulatory kinases or phosphatases have yet to be definitively identified, SPEG activity appears to be closely linked to the dynamics of excitation–contraction coupling in muscle cells.
7. Function  
   SPEG performs essential functions in striated muscle by regulating intracellular calcium cycling and maintaining the structural organization of muscle cells. In cardiac and skeletal muscle, the SPEGα and SPEGβ isoforms are crucial for optimizing excitation–contraction coupling; for example, SPEG phosphorylates SERCA2a at threonine 484 to promote its oligomerization and enhance calcium reuptake into the sarcoplasmic reticulum, thus ensuring efficient muscle relaxation and contraction (quan2019spegcontrolscalcium pages 1-2, quan2019spegcontrolscalcium pages 11-13). Additionally, SPEG phosphorylates junctophilin-2 (JPH2), a key component of the junctional membrane complex, thereby contributing to the maintenance of T-tubule integrity essential for coordinated calcium signaling (quick2017speg(striatedmuscle pages 1-3, luo2021striatedpreferentiallyexpressed pages 4-5). Of particular relevance to the present information, the APEG1 isoform is expressed in arterial smooth muscle cells and is proposed to regulate the growth and differentiation of these cells (luo2021striatedpreferentiallyexpressed pages 2-4, kusumoto2019speganindispensable pages 1-2). Loss-of-function models of SPEG have been linked to severe muscle pathologies, including dilated cardiomyopathy, centronuclear myopathy, and left ventricular non-compaction, underscoring its fundamental role in muscle development and calcium homeostasis (liu2009disruptionofstriated pages 2-4, quick2017speg(striatedmuscle pages 3-4).
8. Other Comments  
   To date, no selective small molecule inhibitors specifically targeting SPEG have been reported, and efforts toward developing such agents are ongoing (grogan2020doublethetrouble pages 11-11, quick2017speg(striatedmuscle pages 13-16). Mutations in SPEG have been implicated in a spectrum of muscle disorders; for example, genetic alterations that reduce SPEG activity are associated with centronuclear myopathy and various cardiomyopathies. In addition, the isoform APEG1 (SPEG isoform 3) is implicated in the regulation of arterial smooth muscle cell growth and differentiation, supporting a potential role in vascular biology (luo2021striatedpreferentiallyexpressed pages 2-4, kusumoto2019speganindispensable pages 1-2). These disease associations highlight the clinical relevance of SPEG in muscle physiology and underscore the importance of preserving its kinase activity for maintaining muscle structure and function.
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