## 1. Phylogeny

BRSK2 belongs to the eukaryotic AMPK‐related kinase (ARK) family and is evolutionarily related to a set of serine/threonine kinases that include BRSK1, NUAK1/2, SIKs, MARKs, MELK, and the classical AMPK catalytic subunits. Within this family, BRSK2 and its paralog BRSK1 represent the brain‐selective branch and can be traced to conserved ancestral kinases that emerged early in vertebrate evolution, with a distinctive set of regulatory cysteine residues playing roles in redox control. Phylogenetic analyses demonstrate that despite high overall sequence identity between BRSK1 and BRSK2, BRSK2 has accumulated unique redox‐sensitive features—such as a conserved T-loop +2 cysteine and a unique substitution in the activation segment (CPE motif instead of the canonical APE motif)—which are rare among eukaryotic protein kinases, underscoring its specialized functional adaptation in neuronal contexts (bendzunas2024redoxregulationof pages 3-7, bendzunas2025redoxregulationand pages 2-3).

## 2. Reaction Catalyzed

BRSK2 catalyzes the transfer of a phosphate group from ATP to specific serine or threonine residues on substrate proteins. In mechanistic terms, the reaction can be described as follows: ATP + [protein substrate] → ADP + [phospho-protein] + H⁺. This phosphorylation event is critical for modulating the activity, conformation, and protein–protein interactions of substrates such as microtubule-associated proteins (e.g., MAPT/TAU), cell cycle regulators (e.g., CDC25C, WEE1), and proteins involved in insulin secretion (e.g., CDK16 and PAK1) (bendzunas2024redoxregulationof pages 12-15, tamir2020gainoffunctiongeneticscreen pages 1-3).

## 3. Cofactor Requirements

The catalytic activity of BRSK2 is dependent on the presence of divalent metal ions—most notably Mg²⁺—which act as cofactors by assisting in the stabilization of ATP and the phosphate transfer reaction. In addition, BRSK2 requires ATP as the phosphate donor and is activated upon phosphorylation by upstream kinases such as LKB1, a modification essential for the full catalytic competence of the kinase domain (bendzunas2025redoxregulationand pages 1-2, tamir2020gainoffunctiongeneticscreen pages 3-5).

## 4. Substrate Specificity

BRSK2 phosphorylates several physiologically relevant substrates in neuronal and metabolic pathways. Key substrates include:  
– MAPT/TAU, where phosphorylation at specific residues (e.g., Thr-529 and Ser-579) is implicated in neuronal polarization and axonogenesis;  
– CDC25C, a regulator of cell cycle progression;  
– WEE1, whose phosphorylation at Ser-642 down-regulates its activity in postmitotic neurons;  
– CDK16 and PAK1, both of which are involved in the regulation of insulin secretion.  
Kinase activity assays often employ the AMARA peptide—a generic substrate utilized to measure ARK family phosphorylation—with the activity of BRSK2 being modulated by the redox state and phosphorylation status of conserved residues in its activation loop (bendzunas2024redoxregulationof pages 12-15, bendzunas2024redoxregulationof pages 20-23, tamir2020gainoffunctiongeneticscreen pages 1-3).

## 5. Structure

BRSK2 is organized into several functionally distinct domains. The N-terminal region contains the catalytic kinase domain, which is responsible for its enzymatic activity. This domain includes highly conserved motifs such as the HRD motif, an activation loop (T-loop) where phosphorylation is required for activation, and a unique T-loop +2 cysteine residue that is essential for redox regulation. Following the kinase domain is a Ubiquitin-Associated (UBA) domain, which may play roles in protein–protein interactions and possibly autoinhibition. Further towards the C-terminus, BRSK2 contains a Proline-Rich Region (PRR), a Kinase-Associated (KA1) domain, and an Autoinhibitory Sequence (AIS), all of which contribute to the fine-tuning of its catalytic activity and subcellular localization. Unique to BRSK2 is the substitution within the activation segment—where the canonical APE motif is replaced by a CPE sequence—facilitating the formation of reversible disulfide bonds and redox-dependent conformational changes that modulate kinase function. Three-dimensional models based on AlphaFold predictions suggest that the kinase domain adopts an active-like conformation, with critical redox-sensitive cysteine residues (e.g., C176 and C183) positioned strategically to influence inter-lobe interactions and allosteric communication (bendzunas2024redoxregulationof pages 51-58, bendzunas2025redoxregulationand pages 3-5, bendzunas2024redoxregulationof pages 15-18).

## 6. Regulation

BRSK2 is regulated by a combination of phosphorylation events and redox-based modifications. Its activation is initiated by the upstream kinase LKB1 through phosphorylation of a conserved residue in the T-loop (e.g., Thr-174 in the kinase domain), a modification that primes BRSK2 for substrate phosphorylation. In addition to this classical phospho-activation, BRSK2 is subject to redox regulation through reversible oxidation of conserved cysteine residues. Specifically, oxidation of the T-loop +2 cysteine and the cysteine within the CPE motif can lead to the formation of intramolecular disulfide bonds, which alter the conformation of the kinase domain and modulate its catalytic activity. Experimental data show that under oxidative conditions (e.g., H₂O₂ treatment), BRSK2 activity is inhibited—a state that can be reversed by reducing agents like DTT or glutathione (GSH), highlighting its role as a sensor of cellular redox status. Moreover, specific mutations in these cysteine residues can either enhance or impair kinase activity, demonstrating the finely tuned balance between phosphorylation-dependent activation and redox-based modulation. This dual regulatory mechanism not only influences enzyme activity but also modulates substrate recognition and downstream signaling pathways, such as those involved in insulin secretion and neuronal polarity (bendzunas2024redoxregulationof pages 12-15, bendzunas2025redoxregulationand pages 14-15, tamir2020gainoffunctiongeneticscreen pages 5-6).

## 7. Function

BRSK2 serves multiple critical roles in both neuronal function and metabolic regulation. In the brain, BRSK2 is instrumental in establishing and maintaining neuronal polarity and promoting axonogenesis, primarily through the phosphorylation of microtubule-associated proteins such as MAPT/TAU. This function is especially important during cortical development, where proper neuron polarization is necessary for the formation of functional neural circuits. In parallel, BRSK2 regulates cell cycle progression by modulating key cell cycle regulators—including phosphorylation of CDC25C—and it influences the mitotic onset by affecting the activity of WEE1 through phosphorylation at Ser-642, thereby ensuring proper cell cycle progression in postmitotic neurons. In addition to its neurodevelopmental functions, BRSK2 plays a critical role in the regulation of insulin secretion, where its activity is modulated by distinct phosphorylation events: phosphorylation at Thr-174 tends to inhibit insulin secretion, whereas phosphorylation at Thr-260 appears to promote insulin release. This dualistic regulation underscores the complex role of BRSK2 in metabolic processes. Furthermore, BRSK2 is implicated in the reorganization of the actin cytoskeleton and may participate in the apoptotic response under endoplasmic reticulum (ER) stress, wherein it contributes to the cellular stress response through mechanisms that likely involve both its kinase activity and redox-sensitive regulatory domains. Expression of BRSK2 is predominantly brain-selective, with notable expression in pancreatic tissues as well, aligning with its roles in neuronal signaling and metabolic regulation (bendzunas2024redoxregulationof pages 3-7, deng2022deleteriousvariationin pages 1-2, hu2023casereporta pages 5-6).

## 8. Other Comments

A number of inhibitors have been identified that target BRSK2 or its close relatives in the AMPK-related kinase family. Notably, the compound GW296115 appears as a promising chemical starting point due to its low-nanomolar inhibitory potency and cell-active properties; it has been shown to effectively inhibit BRSK2-mediated phosphorylation events in cellular assays while exhibiting a favorable selectivity profile across a broad kinome panel. These inhibitor studies provide important tools for dissecting BRSK2 function and may eventually contribute to therapeutic strategies targeting conditions such as neurodevelopmental disorders and metabolic diseases. In addition, genetic studies have linked deleterious variation in BRSK2 to neurodevelopmental conditions including autism spectrum disorder, underscoring its significance in neural development and function. Epigenetic regulation, such as DNA methylation at specific CpG sites within BRSK2, has also been associated with disease risk in contexts like idiopathic pulmonary fibrosis, further highlighting the kinase’s potential as a biomarker and therapeutic target. Overall, the combined insights from biochemical, structural, genetic, and inhibitor studies underscore BRSK2’s role as a multifunctional kinase whose activity is intricately controlled by dual modes of regulation—phosphorylation and redox-dependent cysteine modifications—positioning it as a critical node in pathways governing neuronal polarity, cell cycle progression, and insulin secretion (tamir2020pkisdeepdive pages 1-3, chen2025geneticvariationreveals pages 7-10, tamir2020gainoffunctiongeneticscreen pages 25-29).

## 9. References

bendzunas2024redoxregulationof pages 3-7; bendzunas2024redoxregulationof pages 12-15; bendzunas2024redoxregulationof pages 15-18; bendzunas2024redoxregulationof pages 20-23; bendzunas2024redoxregulationof pages 51-58; bendzunas2025redoxregulationand pages 1-2; bendzunas2025redoxregulationand pages 2-3; bendzunas2025redoxregulationand pages 14-15; bendzunas2025redoxregulationand pages 17-18; bendzunas2025redoxregulationand pages 25-26; bendzunas2025redoxregulationand pages 26-26; tamir2020gainoffunctiongeneticscreen pages 1-3; tamir2020gainoffunctiongeneticscreen pages 5-6; tamir2020gainoffunctiongeneticscreen pages 25-29; tamir2020pkisdeepdive pages 1-3; tamir2020pkisdeepdive pages 3-5; tamir2020pkisdeepdive pages 5-6; tamir2020pkisdeepdive pages 6-9; deng2022deleteriousvariationin pages 1-2; hu2023casereporta pages 5-6; chen2025geneticvariationreveals pages 7-10; OpenTargets Search: -BRSK2; sugiyama2019largescalediscoveryof pages 6-8.

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