1. Phylogeny  
   TBCK (TBC domain‐containing protein kinase‐like protein), also known as FERRY1 (UniProt Q8TEA7), is a multidomain member of the TBC family of Rab GTPase‐activating proteins. Its evolutionary history can be traced back to the Last Eukaryotic Common Ancestor (LECA), as the TBC domain is one of the most ancient and conserved modules within eukaryotic cells (cagwin2025decodingtbckfrom pages 2-4, gabernetcastello2013evolutionoftre2bub2cdc16 pages 1-2). Orthologs of TBCK have been identified in a wide range of species—from vertebrates to lower eukaryotes—although variations exist; for example, some organisms display truncated versions lacking the full complement of domains present in human TBCK (cagwin2025decodingtbckfrom pages 2-4). TBCK is classified within the TBC domain‐containing kinase subfamily, sometimes referred to as the TBC‐K subclass, in which proteins exhibit additional domains such as an N-terminal pseudokinase domain and a C-terminal rhodanese-like domain. These additional regions distinguish TBCK from other TBC proteins that function exclusively as Rab GTPase-activating proteins and suggest that TBCK has acquired novel regulatory functions during evolution (cagwin2025decodingtbckfrom pages 1-2, gabernetcastello2013evolutionoftre2bub2cdc16 pages 3-5).

Comparative sequence analyses demonstrate that the TBC domain in TBCK is highly conserved, which reflects its critical role in modulating Rab-mediated vesicular trafficking. Meanwhile, the pseudokinase and rhodanese-like domains appear less conserved, with some sequence divergence likely corresponding to the acquisition of additional protein–protein interaction surfaces and regulatory functions in complex cellular contexts such as neuronal signaling (cagwin2025decodingtbckfrom pages 2-4, gabernetcastello2013evolutionoftre2bub2cdc16 pages 1-2). In summary, TBCK is positioned within a deeply conserved evolutionary branch of TBC proteins, and its unique multidomain structure is found throughout metazoans, indicating an ancient origin augmented by series of lineage-specific acquisitions that enhance its regulatory potential.

1. Reaction Catalyzed  
   Unlike canonical active kinases that transfer a phosphate group from ATP to protein substrates, TBCK is defined by its lack of intrinsic catalytic activity within its degenerate kinase and rhodanese-like domains. Instead, the biochemical reaction attributable to TBCK is believed to occur via its TBC domain, which functions as a Rab GTPase-activating protein (Rab-GAP). In this capacity, TBCK facilitates the hydrolysis of GTP bound to Rab GTPases. The reaction may be summarized as follows:  
     GTP + Rab–GTP → GDP + Pi  
   This reaction is catalyzed through the stabilization of the transition state by conserved catalytic motifs in the TBC domain (including an arginine “finger” and a corresponding glutamine residue) that accelerate the intrinsic GTP hydrolysis activity of targeted Rab proteins (cagwin2025decodingtbckfrom pages 4-6, cagwin2025decodingtbckfrom pages 16-17).
2. Cofactor Requirements  
   Many enzymes that catalyze phosphoryl transfer or GTP hydrolysis require divalent metal ions to stabilize the negative charges present on nucleotides. Although TBCK’s pseudokinase domain does not display conventional kinase activity, its TBC domain-driven Rab-GAP activity is typically dependent on such cofactors. Evidence from related TBC proteins and the conserved catalytic mechanism suggests that TBCK likely utilizes Mg²⁺ ions as a cofactor to facilitate the proper orientation and stabilization of the γ-phosphate of GTP during hydrolysis (cagwin2025decodingtbckfrom pages 2-4). No experimental data to date specifically confirms additional metal ion requirements; however, the canonical requirement of Mg²⁺ for nucleotide hydrolysis reactions provides a basis for including it as the principal cofactor.
3. Substrate Specificity  
   The substrate specificity of TBCK is primarily determined by its central TBC domain. This domain harbors all the conserved catalytic motifs found in Rab GAPs, such as the R finger (exhibiting an IX₂DX₂R motif) and the Q finger (with a YXQ motif), which are essential for accelerating GTP hydrolysis by Rab GTPases (cagwin2025decodingtbckfrom pages 4-6). Although reports on the direct interaction with specific Rab proteins have been somewhat variable, available evidence supports that TBCK is involved in modulating the activity of small Rab GTPases, and several studies point to a functional relationship with Rab5 in particular (cagwin2025decodingtbckfrom pages 8-9, cagwin2025decodingtbckfrom pages 16-17). While the exact consensus substrate motif for TBCK-mediated GTPase activation is not fully defined, the presence of these catalytic features implies that TBCK’s substrate specificity is dictated by its ability to interact with its Rab target(s) through recognition of structural determinants conserved within the Rab superfamily. Hence, TBCK is considered to have substrate specificity largely directed toward Rab GTPases involved in early endosome dynamics.
4. Structure  
   TBCK is a multidomain protein composed of three major regions. The N-terminal region contains a pseudokinase domain (~273 amino acids) that resembles serine/threonine kinases but lacks key catalytic residues—such as those found in the VAIK, HRD, and DFG motifs—rendering it catalytically inactive. Although it does not exhibit conventional kinase activity, this domain is believed to serve a regulatory or scaffolding role by facilitating protein–protein interactions (cagwin2025decodingtbckfrom pages 2-4, cagwin2025decodingtbckfrom pages 4-6).

Central to TBCK is its TBC domain (approximately residues 426–710), which is characteristic of Rab GAP proteins. This domain contains the conserved catalytic motifs—the R and Q fingers—that are essential for its function in accelerating the hydrolysis of GTP bound to Rab GTPases. Structurally, the TBC domain has been modeled using advanced AI-based approaches, such as those provided by AlphaFold, which confirm the presence of a well-formed catalytic pocket with the necessary residues for Rab-GAP activity (cagwin2025decodingtbckfrom pages 4-6, cagwin2025decodingtbckfrom pages 6-8).

The C-terminal region of TBCK contains a rhodanese-like domain (approximately residues 790–893). Although domains of this type are classically associated with sulfur transfer reactions, the rhodanese-like domain in TBCK likely lacks canonical sulfurtransferase activity due to the replacement of conserved motifs and instead is thought to contribute to the overall protein fold and possibly mediate additional protein–protein interactions (cagwin2025decodingtbckfrom pages 4-6, cagwin2025decodingtbckfrom pages 6-8).

Between these structured domains, TBCK contains flexible linker regions that are enriched with short α-helices. These linker segments are the sites of multiple post-translational modifications, including phosphorylation and ubiquitination, which may influence TBCK’s conformation, stability, and interactions with other components of the FERRY complex (cagwin2025decodingtbckfrom pages 8-9, cagwin2025decodingtbckfrom pages 13-14). Overall, the modular architecture of TBCK—with a regulatory pseudokinase domain, a catalytic TBC domain, and a structural rhodanese-like domain—underpins its multifaceted roles in cellular signaling and vesicular trafficking.

1. Regulation  
   Regulatory mechanisms controlling TBCK function occur at both the post-translational and complex assembly levels. The pseudokinase domain of TBCK contains several identified phosphorylation sites, such as S118, Y153, and T169, as well as an ubiquitination site at K271; these modifications have been detected in mass spectrometry analyses and may regulate protein stability and interaction capabilities (cagwin2025decodingtbckfrom pages 4-6). In addition, TBCK protein levels are modulated by its participation in the FERRY complex. Interactions with other complex components—such as PPP1R21, C12orf4, and JIP4—appear to influence TBCK stability, as loss-of-function mutations in TBCK result in concomitant decreases in the protein levels of its FERRY complex partners (floresmendez2025tbckdeficiencyleadsto pages 40-46).

Furthermore, TBCK is implicated in the modulation of mTOR signaling. Altered TBCK expression correlates with changes in mTOR pathway activity, as evidenced by differential phosphorylation of mTOR downstream targets in TBCK-deficient cells, although the precise mechanisms by which TBCK regulates mTOR components remain to be fully elucidated (cagwin2025decodingtbckfrom pages 10-11, riffe2025neurogeneticdisordersassociated pages 2-3). Together, these regulatory inputs, comprising both post-translational modifications and dynamic protein–protein interactions within multiprotein complexes, govern the conformational state and functional output of TBCK in the cell.

1. Function  
   TBCK serves critical biological roles that are intricately linked to intracellular trafficking and the spatial regulation of mRNA. As a component of the FERRY (Five-subunit Endosomal Rab5 and RNA/ribosome intermediary) complex, TBCK (FERRY1) has been shown to interact directly with mRNAs and the small GTPase Rab5A. In this complex, TBCK contributes to the recruitment of mRNAs and ribosomes to early endosomes, thereby facilitating the endosomal transport and localized translation of specific mRNA subsets—a process that is particularly vital in neurons where spatially restricted protein synthesis underpins synaptic function and plasticity (cagwin2025decodingtbckfrom pages 8-9, riffe2025neurogeneticdisordersassociated pages 1-2).

In addition to its role in mRNA transport, TBCK is involved in modulating mTOR signaling. TBCK influences both the expression levels of mTOR complex components and the phosphorylation state of downstream targets, thus positioning it as a regulatory node that links vesicular dynamics with metabolic and growth control pathways. Furthermore, TBCK plays a part in the organization of the actin cytoskeleton, which is essential for cell morphology and migration. These functions are underscored by observations that mutations in TBCK disrupt endolysosomal trafficking, alter cytoskeletal organization, and impair neuronal differentiation and survival (cagwin2025decodingtbckfrom pages 1-2, riffe2025neurogeneticdisordersassociated pages 3-4).

Tissue expression studies indicate that TBCK is broadly expressed across various human tissues; however, its expression is notably high in neuronal populations, correlating with the severe neurodevelopmental phenotypes observed in TBCK deficiency syndromes. The integration of TBCK into the FERRY complex allows it to mediate a convergence of pathways – linking Rab5-regulated vesicular trafficking, mRNA localization, and mTOR signaling – which collectively contribute to the maintenance of cellular homeostasis, particularly in the central nervous system.

1. Other Comments  
   TBCK has been directly implicated in human neurogenetic disorders. Biallelic loss-of-function mutations in TBCK result in TBCK syndrome, a condition characterized by global developmental delay, profound hypotonia, seizures, and distinct dysmorphic facial features. Pathogenic variants, including nonsense and frameshift mutations that truncate the protein or disrupt its domain integrity, lead to the loss of TBCK protein as observed in patient-derived cells (cagwin2025decodingtbckfrom pages 13-14, riffe2025neurogeneticdisordersassociated pages 3-4). In addition, TBCK deficiency has been associated with impaired lysosomal function, autophagy dysregulation, and mitochondrial dysfunction, all of which contribute to the complex cellular pathology seen in affected individuals (cagwin2025decodingtbckfrom pages 10-11, floresmendez2025tbckdeficiencyleadsto pages 36-40).

Currently, no specific inhibitors targeting TBCK have been reported in the literature. Owing to its role as a pseudokinase and Rab-GAP, therapeutic approaches are more likely to focus on correcting defective endosomal trafficking or modulating downstream pathways such as mTOR signaling. The multifaceted functions of TBCK, particularly its integration into the FERRY complex and its impact on mRNA transport and cytoskeletal organization, mark it as a significant target in studies of neurodevelopmental disorders and lysosomal storage disease phenotypes. Moreover, the toxicity observed upon forced overexpression in patient-derived cell systems underscores the necessity for tightly controlled regulation of TBCK protein levels (cagwin2025decodingtbckfrom pages 13-14).

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