1. Phylogeny  
   Inactive tyrosine‐protein kinase PRAG1, also referred to as SgK223 or SGK223, is a member of the pseudokinase subfamily that includes closely related proteins such as SgK269 (PEAK1) and C19orf35, which together form a distinct clade within the kinase superfamily characterized by a preserved kinase fold yet lacking the canonical catalytic residues required for enzymatic activity (orourke2018thepseudokinasessgk269 pages 1-2).  
   Sequence‐structure comparisons and evolutionary trace analyses reveal that orthologs of PRAG1 are present in multiple mammalian species—including human, rat, and mouse—with conservation of the pseudo‐kinase domain and the unique dimerization region highlighting its evolutionary significance as a scaffolding molecule rather than a catalytically active enzyme (orourke2018thepseudokinasessgk269 pages 2-3).  
   Phylogenetic studies based on the protein kinase complement in the human genome have established that PRAG1 and its closely related members diverged from their active kinase ancestors early in vertebrate evolution and have subsequently evolved to support non‐catalytic regulatory functions, with the conserved dimerization interface representing a critical evolutionary adaptation (orourke2018thepseudokinasessgk269 pages 2-3).
2. Reaction Catalyzed  
   PRAG1, despite retaining a kinase‐like domain, does not catalyze the transfer of phosphate from ATP to substrate proteins, as its catalytic cleft is occluded by an inhibitory triad and key residues necessary for ATP binding are substituted (orourke2018thepseudokinasessgk269 pages 2-3).  
   Consequently, no chemical reaction involving ATP hydrolysis or substrate phosphorylation is carried out by PRAG1, and its function is fulfilled through scaffolding and regulatory mechanisms instead of conventional enzymatic phosphotransfer (lecointre2018dimerizationofthe pages 11-15).
3. Cofactor Requirements  
   Typical protein kinases require divalent metal ions, such as Mg²⁺, as cofactors to coordinate ATP within the catalytic pocket; however, owing to the structural alterations in its nucleotide‐binding site, PRAG1 does not exhibit ATP binding and does not depend on such cofactors for its function (lecointre2018dimerizationofthe pages 5-8).  
   Thus, while Mg²⁺ is critical for the catalytic activity of conventional kinases, no cofactor requirement is observed for PRAG1 because its role is primarily non‐enzymatic (orourke2018thepseudokinasessgk269 pages 2-3).
4. Substrate Specificity  
   Classical substrate specificity in kinases is defined by consensus motifs that mediate the selective phosphorylation of target serine, threonine, or tyrosine residues; in contrast, PRAG1 lacks intrinsic catalytic activity and, as such, does not phosphorylate substrates or exhibit a canonical substrate recognition motif (orourke2018thepseudokinasessgk269 pages 2-3).  
   Notably, while PRAG1 is itself phosphorylated at specific tyrosine residues—most prominently at Y391, which is required for the recruitment of binding partners such as CSK—the protein does not modify other proteins through phosphotransfer and instead exerts its regulatory influence via protein–protein interactions (lecointre2018dimerizationofthe pages 11-15).
5. Structure  
   PRAG1 is composed of a central pseudo‐kinase domain that retains the overall bilobal kinase fold but diverges in key catalytic motifs, including a markedly degenerated glycine‐rich loop, a noncanonical activation loop, and a disrupted DFG motif; these features render the ATP‐binding site inaccessible and preclude catalytic activity (lecointre2018dimerizationofthe pages 5-8).  
   A striking structural characteristic of PRAG1 is the presence of an “inhibitory triad” that surrounds the catalytic lysine, effectively sequestering it and stabilizing the inactive conformation of the kinase fold (lecointre2018dimerizationofthe pages 11-15).  
   In addition to its pseudo‐kinase domain, PRAG1 harbors a unique dimerization module located in its C‐terminal region; this dimerization module, composed of a bundle of α‐helices, is essential for mediating homotypic interactions that facilitate the allosteric regulation of associated kinases such as CSK (lecointre2018dimerizationofthe pages 25-29).  
   The three‐dimensional organization of PRAG1 also features an intrinsically disordered N‐terminal region juxtaposed with the structured kinase domain, as evidenced by integrative analyses involving crystallography and AlphaFold2 modeling; this partitioning may allow for conformational flexibility essential for its scaffolding functions (ye2025prag1condensationdrives pages 5-8).  
   Overall, the structural architecture of PRAG1—characterized by a repurposed kinase domain and a highly conserved dimerization interface—underpins its role as a non‐catalytic regulator in intracellular signaling networks (orourke2018thepseudokinasessgk269 pages 1-2).
6. Regulation  
   Regulatory control of PRAG1 is predominantly executed through post‐translational modifications and dimerization‐dependent mechanisms rather than through modulation of enzymatic activity (lecointre2018dimerizationofthe pages 15-18).  
   Phosphorylation at the conserved tyrosine residue Y391 is a critical regulatory modification that creates a binding site for the SH2 domain of CSK, thereby facilitating the formation of a functional complex involved in regulating Src family kinase activity (orourke2018thepseudokinasessgk269 pages 2-3).  
   Mutational analyses have demonstrated that disruptions in the dimerization interface of PRAG1 lead to a reduction in its capacity to fully activate CSK, highlighting the dependence of its regulatory function on the ability to form stable homodimers, even though the physical association with CSK remains intact (lecointre2018dimerizationofthe pages 25-29).  
   In addition, emerging evidence indicates that PRAG1 undergoes dynamic condensation into intracellular puncta—a process that is likely modulated by post‐translational modifications and contributes to its role as a scaffold by organizing signaling complexes spatially within the cell (ye2025prag1condensationdrives pages 20-21).
7. Function  
   PRAG1 functions primarily as a scaffold protein that orchestrates intracellular signaling by modulating protein tyrosine phosphorylation through the regulation of CSK subcellular localization (orourke2018thepseudokinasessgk269 pages 2-3).  
   It serves as an effector for the small GTPase RND2, thereby stimulating RhoA activity and influencing key cellular processes such as cell morphology, adhesion, and motility, which are critical for processes that include neurite outgrowth and cell migration (ye2025prag1condensationdrives pages 20-21).  
   Furthermore, PRAG1 is implicated in the regulation of Notch signaling as a coactivator, linking its scaffolding function to pathways that govern cell differentiation and proliferation (orourke2018thepseudokinasessgk269 pages 1-2).  
   Expression studies have revealed that PRAG1 is detectable in various tissues, notably in the brain, kidney, spleen, colon, and small intestine, and its dysregulated expression has been associated with oncogenic processes that involve enhanced cell invasion and migration (orourke2018thepseudokinasessgk269 pages 2-3, subbannayya2020theproteomiclandscape pages 3-7).
8. Other Comments  
   In light of its catalytically inactive nature, no classical ATP‐competitive inhibitors have been developed specifically to target PRAG1; instead, therapeutic strategies may focus on modulating its protein–protein interactions or its dimerization interface (kung2019prospectsforpharmacological pages 9-11).  
   The unique dimerization modality of PRAG1 offers a potential intervention point, as compounds that disrupt or modulate the formation of its homodimers could alter the downstream activation of CSK and subsequently affect Src family kinase signaling (mace2021there’smoreto pages 11-13).  
   Moreover, PRAG1 has been implicated in cancer as an oncogenic driver, given its role in controlling cell adhesion, migration, and the activation of signaling pathways such as Notch; its dysregulation is documented in several tumor types and is associated with increased aggressiveness (orourke2018thepseudokinasessgk269 pages 2-3).  
   Although evidence points to the importance of phosphorylation in regulating PRAG1 activity, current studies do not report ubiquitination as a regulatory mechanism, and other forms of post‐translational modification, such as protein methylation, have been noted in related kinases but not definitively in PRAG1 (bade2021proteinmethylationand pages 108-108).
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