## Phylogeny

PRAG1 (also called SGK223) is one of three members of the “New Kinase Family 3” (NKF3) pseudokinase branch together with PEAK1/SGK269 and PEAK3/C19orf35 (Ha & Boggon, 2018; López et al., 2019). The PRAG1 and PEAK1 kinase-fold domains share >45 % sequence identity, yet overall identity to catalytically competent protein kinases is low (~13–25 %) (Ha & Boggon, 2018; Lecointre et al., 2018). Because it lacks multiple catalytic motifs, PRAG1 is classified as a pseudokinase within the human kinome (Lecointre et al., 2018; López et al., 2019). The Glu-Pro-Ile-Tyr-Ala (EPIYA) motif is conserved in mammalian orthologues (Safari et al., 2011).  
NOTE: One high-throughput phospho-profiling study grouped PRAG1 with Ca2+/calmodulin-dependent kinases (CAMK) (Johnson et al., 2023); this assignment conflicts with structural/biochemical data designating PRAG1 as catalytically inactive.

## Reaction catalysed

No phosphotransferase activity has been detected. Thermal-shift, ATP-binding and in-vitro kinase assays show that PRAG1 neither binds ATP nor transfers phosphate (Lecointre et al., 2018; Tactacan et al., 2015).  
Contrasting evidence: Johnson et al. (2023) reported low-level serine/threonine phosphorylation activity in an array format.

## Cofactor requirements

None detected. The protein lacks the DFG motif needed for Mg²⁺ coordination, does not bind Mg²⁺ or ATP, and shows no cofactor dependence in biochemical assays (Lecointre et al., 2018; Senda et al., 2016).  
Contrasting evidence: Johnson et al. (2023) inferred ATP usage as phosphate donor.

## Substrate specificity

Because PRAG1 is catalytically inert, intrinsic substrate specificity has not been demonstrated; signalling effects arise through binding to active kinases such as C-terminal Src kinase (Csk) (Lecointre et al., 2018).  
Contrasting evidence: peptide-array profiling placed PRAG1 within a CAMK-like, basophilic preference class (Johnson et al., 2023).

## Structure

Multi-domain architecture:  
• N-terminal segment (1–216) of unknown fold.  
• Central PEST-rich, largely disordered linker (217–931) harbouring multiple phosphorylation sites.  
• C-terminal pseudokinase (PsK) domain (950–1292) flanked by helical extensions that form an “XL/SHED” four-helix dimerisation module (906–949 and 1293–1368) (Patel et al., 2017; Ha & Boggon, 2018; Lecointre et al., 2018).

PsK domain features: canonical bilobal kinase fold locked in a closed, inactive conformation; degenerate glycine-rich loop, absent DFG motif, truncated αC helix lacking the Glu that pairs with β3 Lys; Lys997 is sequestered by an inhibitory triad (Asp978, Tyr981, Gln1021) blocking ATP access (Patel et al., 2017; Lecointre et al., 2018).

## Regulation

1. Tyrosine phosphorylation within the central linker—major site Tyr391 (EPIYA), plus Tyr238, Tyr343, Tyr411—by Src-family kinases, Csk, HER2, Lyn and DDR1 (Senda et al., 2016; Patel et al., 2017; Lecointre et al., 2018).  
   • pTyr391/411 recruits the SH2 domain of Csk, establishing a feed-forward loop in which Csk further phosphorylates PRAG1 (Senda et al., 2016).  
   • Phospho-PRAG1 also binds Grb2, Shc and other SH2/PTB proteins (Patel et al., 2017).
2. Homodimerisation via the SHED module is essential for high-affinity Csk binding and stimulation of Csk catalytic activity; dimer-defective mutants fail to activate Csk (Patel et al., 2017; Lecointre et al., 2018).

## Function

Catalytically inactive scaffold that modulates signalling through protein–protein interactions (Lecointre et al., 2018).  
• Tissue expression: brain (cortex, hippocampus), kidney, spleen, colon, small intestine (Tanaka et al., 2006; O’Rourke & Daly, 2018).  
• Subcellular localisation: plasma membrane, cytosol, focal adhesions (O’Rourke & Daly, 2018).  
• Interactors and pathways:  
– Binds Rnd2 GTPase to enhance RhoA activity, regulating actomyosin contractility and neurite outgrowth (Tanaka et al., 2006).  
– Dimerises with and activates Csk, indirectly modulating Src-family kinase activity (Lecointre et al., 2018).  
– Associates with AMPK and components of the Notch transcriptional complex (Lecointre et al., 2018).  
Consequently, PRAG1 participates in Rnd2/RhoA, SFK, Notch and JAK/STAT signalling networks (O’Rourke & Daly, 2018; Tactacan et al., 2015).

## Other comments

PRAG1 is frequently over-expressed in pancreatic, oesophageal, lung and colon adenocarcinomas, correlating with poor prognosis in non-small-cell lung cancer; it promotes tumour cell proliferation, migration and invasion (O’Rourke & Daly, 2018; Tactacan et al., 2015). Global knockout in mice is embryonic lethal (O’Rourke & Daly, 2018). Mutations disrupting either the SHED dimer interface or the Tyr391 phosphorylation site abolish Csk activation and downstream protein tyrosine phosphorylation (Lecointre et al., 2018).

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