## Phylogeny

Orthologues of KSR1/KSR2 are present from nematodes to vertebrates (Caenorhabditis elegans ksr-1/ksr-2, Drosophila D-ksr, Xenopus Ksr, Danio ksr1, Rattus Ksr1, Mus Ksr1, Homo KSR1/KSR2) (Kornfeld et al., 1995; Zhang, 2014).  
Within the human kinome the protein groups with Tyrosine-Kinase-Like (TKL) members and clusters with RAF-related pseudokinases sharing the conserved CA1–CA5 regions (Clapé-ron & Therrien, 2007; Martín-Vega & Cobb, 2023). KSR proteins are evolutionarily related to RAF MAP3Ks but have lost the canonical Ras-binding domain and catalytic β3-lysine, consistent with specialization for scaffolding and allosteric regulation (Clapé-ron & Therrien, 2007).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + L-seryl/threonyl-P-[protein] (demonstrated in vitro) (Goettel et al., 2011).

## Cofactor Requirements

ATP binding is essential for structural integrity; the requirement for a divalent metal ion (e.g., Mg²⁺) remains unresolved (Goettel et al., 2011; Roskoski, 2012).

## Substrate Specificity

The only validated substrate is MAP2K1/MEK1, which is phosphorylated on non-activation-segment serines in vitro (Goettel et al., 2011; Roskoski, 2012). No consensus phosphorylation motif has been defined (Neilsen et al., 2017).

## Structure

Domain layout:  
• CA1 – CC-SAM that binds the BRAF-specific BRS motif and aids membrane recruitment (Lavoie et al., 2018).  
• CA2 – proline-rich segment (Frodyma et al., 2017).  
• CA3 – atypical C1/CRD conferring lipid-dependent plasma-membrane localisation (Clapé-ron & Therrien, 2007).  
• CA4 – Ser/Thr-rich region containing an FXFP ERK-docking site (Frodyma et al., 2017).  
• CA5 – C-terminal pseudokinase domain where Arg637 replaces the catalytic Lys; HRD and DFG motifs are retained (Martín-Vega & Cobb, 2023).

Crystal structures of MEK-bound KSR1/2 kinase domains (PDB 5UHV, 6B8C) reveal an αC-OUT inactive conformation with an incomplete hydrophobic spine and an unphosphorylated activation loop. Helix αG contacts MEK, while the N-lobe mediates side-to-side heterodimerisation with BRAF (Chow et al., 2022; Khan et al., 2020; Maloney et al., 2022). MEK binding stabilises the KSR C-lobe and promotes BRAF–KSR heterodimer formation (Lavoie et al., 2018).

## Regulation

Phosphorylation  
• Ser297, Ser392: constitutive C-TAK1 sites that generate 14-3-3 docking motifs, retaining KSR1 in the cytosol (Cacace et al., 1999; Müller et al., 2001).  
• Thr260, Thr274, Ser443: Ras-inducible ERK sites mediating feedback (Cacace et al., 1999).  
• Ser392 is dephosphorylated by PP2A after growth-factor stimulation, enabling membrane translocation (Clapé-ron & Therrien, 2007).

Ubiquitination  
Praja2 poly-ubiquitinates KSR1, directing proteasomal degradation (Goettel et al., 2011).

Conformational/allosteric control  
MEK occupancy drives BRAF–KSR heterodimerisation and BRAF activation; the ATP-site ligand APS-2-79 locks the αC-OUT state and blocks RAF interaction (Frodyma et al., 2017; Lavoie et al., 2018).

Chaperones  
HSP90, HSP70, HSP68 and p50 CDC37 associate with the kinase domain and stabilise the protein (Stewart et al., 1999).

## Function

KSR1 is highly expressed in brain and is detectable in T cells and colonic epithelium (Frodyma et al., 2017; Goettel et al., 2011). It constitutively binds MEK1/2 and forms stimulus-induced heterodimers with BRAF and RAF1; additional partners include ERK1/2, 14-3-3, PP2A, inosine-monophosphate dehydrogenase (IMP), and HSP90 complexes (McKay et al., 2009; Stewart et al., 1999). Acting as a scaffold and MEK-dependent allosteric regulator, KSR1 modulates amplitude and duration of signals transmitted through the RAS–RAF–MEK–ERK cascade downstream of receptor tyrosine-kinase or cAMP inputs (Neilsen et al., 2017).

## Inhibitors

APS-2-79 binds the nucleotide pocket, prevents KSR1–BRAF heterodimerisation and dampens Ras-dependent ERK signalling (Neilsen et al., 2017; Chow et al., 2022).

## Other Comments

Ksr1-null mice are viable but display resistance to Ras-driven tumourigenesis (Neilsen et al., 2017). Reported oncogenic mutations include C809Y (disrupts MEK binding yet enhances ERK activation) and P505A (alters kinase-domain integrity) (Frodyma et al., 2017; Unknown Authors, 2010).

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