## Phylogeny

RAF1 is a serine/threonine-protein kinase of the RAF sub-family (A-RAF, B-RAF, RAF1) within the MAP kinase kinase kinase (MAP3K) tier of the tyrosine kinase-like (TKL) group of the human kinome (García-Alonso et al., 2022; Roskoski, 2010; Simanshu & Morrison, 2022; Unknown Authors, 2023). Orthologues are highly conserved in vertebrates including human, mouse, rat, chicken, Xenopus, Takifugu and zebrafish (Razzaque et al., 2007).

## Reaction Catalyzed

MgATP + protein-OH ⇌ protein-OPO₃²⁻ + MgADP + H⁺ (Kobayashi et al., 2010; Roskoski, 2010).

## Cofactor Requirements

Requires a divalent cation, typically Mg²⁺ (preferred) or Mn²⁺, which coordinates ATP in the catalytic cleft (Kobayashi et al., 2010; Molzan et al., 2010; Roskoski, 2010).

## Substrate Specificity

RAF1 displays narrow specificity, primarily phosphorylating the dual-specificity kinases MEK1 and MEK2 on serine/threonine residues located within defined consensus motifs (García-Alonso et al., 2022; Molzan et al., 2010; Roskoski, 2010; Simanshu & Morrison, 2022).

## Structure

The polypeptide is organised into three conserved regions (CR1-CR3) (García-Alonso et al., 2022; Leicht et al., 2007).  
• CR1 (N-terminus) contains a Ras-binding domain (RBD, residues 56–131) and a cysteine-rich domain (CRD, 138–184) that binds two Zn²⁺ ions and engages membrane phospholipids (Roskoski, 2010).  
• CR2 is a Ser/Thr-rich regulatory segment harbouring the inhibitory phosphorylation site Ser259 (García-Alonso et al., 2022).  
• CR3 (residues 349–609) forms the protein kinase domain composed of a small N-lobe (with the glycine-rich P-loop, 355–363, and αC helix) and a large C-lobe. Catalysis is governed by an activation loop positioned between the DFG (486–488) and APE (523–525) motifs and containing Thr491 and Ser494 (Roskoski, 2010; Unknown Authors, 2014).

## Regulation

Activity is controlled by multisite phosphorylation, protein interactions, dimerisation and conformational change.  
• Phosphorylation  
– Ser259: inhibitory; creates a 14-3-3 docking site (García-Alonso et al., 2022).  
– Ser338 & Tyr341: activating; Ser338 by PAK/SRC, Tyr341 by SRC (Molzan et al., 2010; Roskoski, 2010).  
– Thr491/Ser494 (activation loop) and Ser621 (C-terminal 14-3-3 site) promote full activation and stabilisation (Kobayashi et al., 2010; Roskoski, 2010).  
• Protein interactions  
– 14-3-3 proteins bind pSer259/pSer621 to stabilise an autoinhibited state (García-Alonso et al., 2022; Tartaglia et al., 2010).  
– RAS-GTP engages the RBD, recruits RAF1 to membranes and disrupts pSer259-14-3-3 binding (Leicht et al., 2007).  
– HSP90–CDC37 chaperone complex binds the kinase domain and assists folding/stability (García-Alonso et al., 2022).  
• Dimerisation: side-to-side homo- or heterodimer formation (commonly with B-RAF) is required for full catalytic activity (Roskoski, 2010).

## Function

RAF1 is a central effector in the RAS-RAF-MEK-ERK MAPK cascade. Following RAS activation at the plasma membrane, RAF1 phosphorylates MEK1/2, triggering ERK1/2 activation and regulating cell proliferation, differentiation and survival (García-Alonso et al., 2022; Leicht et al., 2007; Roskoski, 2010). RAF1 also modulates apoptosis: it can inactivate the pro-apoptotic protein BAD either directly (Ser112) or indirectly as a scaffold for PKCθ, and possesses kinase-independent anti-apoptotic scaffolding functions (Bahar et al., 2023; García-Alonso et al., 2022; Mukherjee et al., 2024; Unknown Authors, 2014; Unknown Authors, 2023).

## Inhibitors

ATP-competitive RAF inhibitors include BAY 43-9006 (sorafenib) and SB-590885 (Leicht et al., 2007; Roskoski, 2010). Some inhibitors produce “paradoxical activation” by inducing RAF dimerisation and enhancing ERK signalling in RAS-active, B-RAF-WT cells (Roskoski, 2010).

## Other Comments

Germline gain-of-function mutations in RAF1 disrupt autoinhibition and cause Noonan syndrome, often affecting residues near Ser259 or within the activation segment (Kobayashi et al., 2010; Razzaque et al., 2007; Tartaglia et al., 2010). Somatic RAF1 mutations are uncommon in cancer, but RAF1 contributes to tumorigenesis via kinase-independent survival functions, prompting interest in RAF1 degradation strategies (García-Alonso et al., 2022).

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