## Phylogeny

According to the classification by Manning et al., BRAF is a member of the RAF family, which belongs to the TKL (Tyrosine Kinase-Like) kinase group (manning2002theproteinkinase pages 3-3, manning2002theproteinkinase pages 3-4, rauch2011thesecretlife pages 4-5). This group is a diverse collection of kinases that resemble both tyrosine and serine-threonine kinases (manning2002theproteinkinase pages 3-3). However, some classifications also place BRAF within the CMGC kinase group (manning2002theproteinkinase pages 1-2). Phylogenetic analyses indicate that single Raf homologs in invertebrates like *Caenorhabditis elegans* and *Drosophila melanogaster* are evolutionarily closer to B-Raf than to other human Raf isoforms (Raf-1 and A-Raf), suggesting B-Raf is the archetypal MEK kinase (rauch2011thesecretlife pages 9-11).

## Reaction Catalyzed

BRAF is a serine/threonine kinase that catalyzes the transfer of the gamma-phosphate group from ATP to the hydroxyl group of specific serine or threonine residues on a protein substrate, producing ADP and a phosphoprotein (vilacha2020makingnsclccrystal pages 23-25, rauch2011thesecretlife pages 4-5, unknownauthors2021impactoferk2 pages 7-12).

ATP + a protein substrate → ADP + a phosphoprotein with serine/threonine phosphorylation (vilacha2020makingnsclccrystal pages 23-25, rauch2011thesecretlife pages 4-5).

## Cofactor Requirements

The catalytic activity of BRAF requires an essential divalent cation cofactor, typically magnesium (Mg²⁺) (rauch2011thesecretlife pages 9-11, rauch2011thesecretlife pages 4-5, unknownauthors2021impactoferk2 pages 7-12). Mg²⁺ coordinates the phosphate groups of ATP, stabilizing its binding and facilitating the phosphoryl transfer during the kinase reaction (vilacha2020makingnsclccrystal pages 25-27, unknownauthors2021impactoferk2 pages 7-12).

## Substrate Specificity

BRAF is classified as a proline-directed kinase (johnson2023anatlasof pages 12-18). Its substrate consensus motif is characterized by a phosphorylatable serine or threonine residue with a strong, strict preference for a Proline (Pro) residue at the +1 position (johnson2023anatlasof pages 12-18, johnson2023anatlasof pages 2-3). The kinase also shows preferences for certain flanking residues at positions -3 to +3 relative to the phosphorylation site (johnson2023anatlasof pages 2-3).

## Structure

BRAF is a serine-threonine kinase composed of three conserved regions (CR1, CR2, and CR3) and a canonical bilobal kinase domain (vilacha2020makingnsclccrystal pages 23-25, vilacha2020makingnsclccrystal pages 3-5). \* **CR1 (N-terminus):** Contains a RAS-GTP binding domain (RBD) and a cysteine-rich domain that functions in autoinhibition (vilacha2020makingnsclccrystal pages 23-25, vilacha2020makingnsclccrystal pages 46-48). \* **CR2:** A flexible hinge region rich in serine/threonine residues that serves as a binding site for 14-3-3 proteins (vilacha2020makingnsclccrystal pages 23-25). \* **CR3 (C-terminus):** Houses the kinase domain, which contains the ATP binding site and catalytic loop (vilacha2020makingnsclccrystal pages 23-25).

The kinase domain has a smaller N-terminal lobe composed of a five-stranded β-sheet (β1–β5) and a larger C-terminal lobe rich in α-helices (vilacha2020makingnsclccrystal pages 3-5). Key structural and regulatory features within the kinase domain include: \* **P-loop:** A glycine-rich loop (residues G464-G469), also known as the G-loop, in the N-terminal lobe that is responsible for positioning ATP (vilacha2020makingnsclccrystal pages 23-25, vilacha2020makingnsclccrystal pages 3-5). ATP binding is stabilized by hydrogen bonds with hinge residues Q530 and C532, as well as interactions with K483 and E501 (vilacha2020makingnsclccrystal pages 23-25). \* **αC-helix:** A movable helix (residues T491-R506) in the N-lobe that toggles between an inactive ‘out’ conformation and an active ‘in’ conformation (vilacha2020makingnsclccrystal pages 23-25, vilacha2020makingnsclccrystal pages 25-27). \* **Activation Loop (A-loop):** A dynamic segment in the C-terminal lobe initiated by the conserved DFG motif (D594/F595/G596) and ending at the AxE motif (A621/P622/E623) (vilacha2020makingnsclccrystal pages 23-25). It contains key phosphorylation sites T599 and S602 that regulate kinase activation (unknownauthors2021impactoferk2 pages 7-12, vilacha2020makingnsclccrystal pages 3-5). The conformation of the DFG motif (DFG-in vs. DFG-out) is critical for catalytic activity (vilacha2020makingnsclccrystal pages 25-27).

## Regulation

BRAF activity is regulated by autoinhibition, dimerization, post-translational modifications, and conformational changes (vilacha2020makingnsclccrystal pages 25-27, vilacha2020makingnsclccrystal pages 46-48). \* **Autoinhibition:** The inactive state is maintained by autoinhibitory interactions between the N-terminal regulatory region (specifically the cysteine-rich domain in CR1) and the C-terminal kinase domain (vilacha2020makingnsclccrystal pages 23-25, vilacha2020makingnsclccrystal pages 25-27, vilacha2020makingnsclccrystal pages 46-48). \* **Dimerization:** Activation is induced by RAS-GTP, which promotes the formation of BRAF homo- or heterodimers (e.g., with CRAF) (vilacha2020makingnsclccrystal pages 23-25, rauch2011thesecretlife pages 9-11). Dimerization occurs via a side-to-side interface where the αC-helix of each monomer packs against the other (vilacha2020makingnsclccrystal pages 25-27, rauch2011thesecretlife pages 9-11). \* **Post-Translational Modification:** Phosphorylation of key residues in the activation loop, specifically threonine 599 (T599) and serine 602 (S602), is critical for modulating kinase activity and inducing conformational changes required for activation (unknownauthors2021impactoferk2 pages 7-12, vilacha2020makingnsclccrystal pages 46-48). Unlike ARAF and CRAF, BRAF does not require phosphorylation in its N-terminal region for activation (vilacha2020makingnsclccrystal pages 25-27). \* **Conformational Regulation:** Upon activation, the αC-helix moves from an ‘out’ to an ‘in’ position, enabling the formation of a critical salt bridge between K483 (on the β3 strand) and E501 (on the αC-helix). This change is coupled with the repositioning of the activation segment into a “DFG-in” conformation, which is necessary for catalysis (vilacha2020makingnsclccrystal pages 25-27).

## Function

BRAF is a serine/threonine kinase that functions as a core component of the mitogen-activated protein kinase (MAPK/ERK) signaling pathway (vilacha2020makingnsclccrystal pages 23-25, rauch2011thesecretlife pages 23-23). The kinase transduces mitogenic signals from the cell membrane to the nucleus to regulate fundamental cellular processes, including proliferation, differentiation, survival, and apoptosis (vilacha2020makingnsclccrystal pages 23-25, rauch2011thesecretlife pages 9-11). \* **Upstream/Downstream Partners:** BRAF is activated downstream of RAS GTPases (unknownauthors2021impactoferk2 pages 7-12). Activated BRAF phosphorylates and activates its primary substrates, the downstream kinases MEK1 and MEK2, which in turn activate ERK (rauch2011thesecretlife pages 9-11, vilacha2020makingnsclccrystal pages 23-25). \* **Interacting Partners:** BRAF forms homo- and heterodimers with other Raf isoforms, such as CRAF (Raf-1), and its signaling is modulated by interactions with regulatory proteins like 14-3-3 and scaffolding proteins like KSR (Kinase Suppressor of Ras) (vilacha2020makingnsclccrystal pages 23-25, rauch2011thesecretlife pages 9-11).

## Inhibitors

Known inhibitors of BRAF include first- and second-generation small molecules that target the kinase domain (vilacha2020makingnsclccrystal pages 27-29). \* **First-generation:** Includes sorafenib, which binds to and stabilizes the inactive DFG-out conformation but lacks specificity for mutant BRAF (vilacha2020makingnsclccrystal pages 27-29). \* **Second-generation:** Includes mutant-selective, ATP-competitive inhibitors such as vemurafenib, dabrafenib, and encorafenib (vilacha2020makingnsclccrystal pages 27-29, vilacha2020makingnsclccrystal pages 46-48). These are classified as type I 1/2 inhibitors that primarily bind to an inactive αC-out/DFG-in conformation (vilacha2020makingnsclccrystal pages 27-29). Vemurafenib weakly induces dimerization, while dabrafenib is a strong inducer (vilacha2020makingnsclccrystal pages 27-29). \* **Other Inhibitors:** LY3009120 is also a known RAF inhibitor (vilacha2020makingnsclccrystal pages 48-49).

## Other Comments

BRAF is frequently mutated in human cancers, including melanoma, non-small cell lung cancer (NSCLC), and hairy-cell leukemia (vilacha2020makingnsclccrystal pages 25-27, vilacha2020makingnsclccrystal pages 46-48). \* **V600E Mutation:** The most prevalent oncogenic mutation (accounting for ~92% of BRAF mutations) is a substitution of valine with glutamic acid at position 600 (V600E), located near the DFG motif in the activation loop (vilacha2020makingnsclccrystal pages 27-29). This mutation acts as a phosphomimetic, disrupting the inactive conformation and locking the kinase in a constitutively active state independent of upstream RAS signaling (vilacha2020makingnsclccrystal pages 27-29, vilacha2020makingnsclccrystal pages 46-48). The V600E mutation leads to a ~500-fold increase in basal kinase activity and promotes an active monomeric state, although it also enhances dimerization potential (vilacha2020makingnsclccrystal pages 25-27). \* **Mutation Classes:** Oncogenic BRAF mutations are categorized into three functional classes: class 1 are RAS-independent active monomers (e.g., V600E), class 2 are active as RAS-independent dimers, and class 3 are RAS-dependent with impaired kinase activity but can allosterically activate CRAF (vilacha2020makingnsclccrystal pages 25-27, rauch2011thesecretlife pages 9-11). \* **Inhibitor Resistance:** Resistance to BRAF inhibitors can arise from secondary mutations in downstream pathway components like NRAS or MEK, or through mutations at the gatekeeper residue (T529), which hinders drug binding (vilacha2020makingnsclccrystal pages 29-31, vilacha2020makingnsclccrystal pages 46-48).

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