## Phylogeny

EPHB4 is a member of the EphB subfamily of Eph receptor tyrosine kinases, which includes five catalytically competent members (EphB1-EphB4) and the catalytically inactive EphB6 (overman2014completingthestructural pages 2-4). The kinase domain of EPHB4 shares 86% sequence identity with that of EphB3 (overman2014completingthestructural pages 2-4). According to the kinome classification by Manning et al., EPHB4 is assigned to the Eph receptor family within the tyrosine kinase (TK) group (overman2014completingthestructural pages 2-4, strozen2021theephb6receptor pages 4-7, choi2009discoveryandstructural pages 1-2).

## Reaction Catalyzed

ATP + [a protein-L-tyrosine] = ADP + [a protein-L-tyrosine phosphate] (unknownauthors2021identificationdenouvelles pages 46-47, overman2013stabilityandsolubility pages 2-3, unknownauthors2012ephreceptorclustering pages 34-36).

## Cofactor Requirements

The catalytic activity of EPHB4 requires divalent metal cations as cofactors (strozen2021theephb6receptor pages 4-7, choi2009discoveryandstructural pages 1-2). Both Mg²⁺ and Mn²⁺ can serve this role to stabilize ATP binding and facilitate the phosphotransfer reaction (overman2014completingthestructural pages 2-4, strozen2021theephb6receptor pages 4-7). The requirement for Mg²⁺ has been confirmed in kinase assays performed in buffers containing 10 mM MgCl₂ (overman2013stabilityandsolubility pages 3-4).

## Substrate Specificity

The intrinsic substrate specificity of EPHB4 has been determined experimentally using positional scanning peptide arrays (PSPA) (yaronbarir2024theintrinsicsubstrate pages 1-2). The consensus substrate motif for EPHB4 phosphorylation is defined by the preferred amino acid residues at positions flanking the central tyrosine, particularly at positions -1 to +3 (yaronbarir2024theintrinsicsubstrate pages 15-16). These preferences, captured in positional scoring matrices (PSSMs), allow for the identification of optimal substrate sequences for EPHB4 kinase activity (yaronbarir2024theintrinsicsubstrate pages 1-2, yaronbarir2024theintrinsicsubstrate pages 4-5).

## Structure

EPHB4 possesses a modular domain architecture with an extracellular region, a single transmembrane segment, and an intracellular region (chrencik2006structureandthermodynamic pages 1-2, overman2013stabilityandsolubility pages 5-5). The extracellular domain comprises an N-terminal ephrin-binding globular domain, a cysteine-rich region, and two fibronectin type III (FN III) repeats (chrencik2006structureandthermodynamic pages 1-2, overman2013stabilityandsolubility pages 5-5). The intracellular region contains a juxtamembrane (JM) region, a kinase catalytic domain (KD), a sterile alpha motif (SAM), and a C-terminal PDZ-binding motif (chrencik2006structureandthermodynamic pages 1-2, overman2013stabilityandsolubility pages 5-5).

The ephrin-binding domain adopts a jellyroll folding topology, forming a compact beta sandwich (chrencik2006structureandthermodynamic pages 2-3). The kinase domain has the traditional bi-lobed fold of tyrosine kinases (overman2014completingthestructural pages 2-4). Crystal structures are available for the EPHB4 ligand-binding domain (PDB: 2HLE) and the kinase domain in complex with the inhibitor staurosporine (PDB: 2VWU) (chrencik2006structuralandbiophysical pages 1-2, overman2014completingthestructural pages 7-9).

Key catalytic features include the activation loop, the C-helix, the VAIK motif for ATP interaction, a conserved glutamate in the αC-helix, and the DFG motif in the activation loop (chrencik2006structureandthermodynamic pages 1-2, strozen2021theephb6receptor pages 4-7). A unique structural feature is a three-residue insert (Pro-151, Gly-152, Ala-153) in the J-K loop of the ligand-binding domain, which contributes to ligand specificity (chrencik2006structuralandbiophysical pages 4-5). The D-E and J-K loops of this domain are flexible and undergo conformational changes to accommodate ligand binding (chrencik2006structureandthermodynamic pages 2-3).

## Regulation

EPHB4 activity is regulated by post-translational modifications, primarily phosphorylation at tyrosine residues, which is modulated by ligand binding (piffko2022ephrinb2–ephb4signalingin pages 13-14). Activation involves phosphorylation of juxtamembrane tyrosine residues (Tyr590, Tyr596) and an activation loop tyrosine (Tyr774), which relieves autoinhibition by the JM segment (overman2013stabilityandsolubility pages 5-5, rutkowski2016newrolesfor pages 29-32). Signaling by some EPHB4 mutants, such as A742V, occurs without evident tyrosine phosphorylation, suggesting that dimerization or other modifications like serine/threonine phosphorylation are also critical regulatory mechanisms (ferguson2015novelephb4receptor pages 12-13).

## Function

EPHB4 is expressed at low levels in multiple organs but not in the brain and is found on venous endothelial cells (rutkowski2016newrolesfor pages 29-32, unknownauthors2018thefunctionaland pages 70-75). Its signaling is initiated by binding to its ligand, ephrin-B2 (EFNB2) (ferguson2015novelephb4receptor pages 12-13).

EPHB4 signaling modulates the Ras/MEK/ERK, PI3K/Akt, PI3K/mTORC1, and JAK/STAT pathways (piffko2022ephrinb2–ephb4signalingin pages 13-14, unknownauthors2018thefunctionaland pages 70-75, zeng2019ephrinb2ephb4rasa1signalingin pages 4-6). Downstream substrates include other receptor tyrosine kinases, such as EPHA2, PDGFRß, Ret, and VEGFR2 (ferguson2015novelephb4receptor pages 12-13). EPHB4 interacts with various proteins, including the adaptor protein Crk, EphB6, PI3K, PTEN, VEGF, Rac1, MMP2, PECAM1, EpoR, Fer, Lat, and STAT5A (piffko2022ephrinb2–ephb4signalingin pages 13-14, ferguson2015novelephb4receptor pages 8-10, rutkowski2016newrolesfor pages 32-36).

Its biological functions include the regulation of cell adhesion, migration, angiogenesis, lymphangiogenesis, and the segregation of arterial and venous lineages during embryonic development (piffko2022ephrinb2–ephb4signalingin pages 13-14, unknownauthors2018thefunctionaland pages 70-75).

## Inhibitors

Known inhibitors of EPHB4 include the small molecule NVP-BHG712, antibody-based blockers, and peptide antagonists that mimic ephrin-binding (piffko2022ephrinb2–ephb4signalingin pages 13-14). Soluble EPHB4 (sEPHB4) also functions as an inhibitor, and the kinase can be targeted by the non-specific inhibitor staurosporine (ferguson2015novelephb4receptor pages 8-10, overman2014completingthestructural pages 2-4).

## Other Comments

EPHB4 dysregulation is associated with numerous cancers, including lung, prostate, ovarian, colorectal, and melanoma, where its overexpression often correlates with tumor progression (piffko2022ephrinb2–ephb4signalingin pages 13-14). It is also implicated in congenital vascular disorders such as central conducting lymphatic anomaly (CCLA) and capillary malformation-arteriovenous malformation (CM-AVM) (zeng2019ephrinb2ephb4rasa1signalingin pages 4-6).

Mutations in EPHB4 identified in lung cancer (e.g., G723S, A742V, P881S) can confer gain-of-function effects, promoting cell proliferation and motility (ferguson2015novelephb4receptor pages 12-13, ferguson2015novelephb4receptor pages 5-8). The A742V and P881S mutants show markedly reduced phosphorylation but still enhance proliferation (ferguson2015novelephb4receptor pages 8-10). Mutations in the kinase domain can also cause a loss of phosphorylation and disrupt ephrinB2-dependent activation, leading to lymphatic dysfunction (zeng2019ephrinb2ephb4rasa1signalingin pages 4-6). The G723S mutation confers partial resistance to paclitaxel and sEPHB4 (ferguson2015novelephb4receptor pages 8-10).

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