## Phylogeny

• Member of the protein-tyrosine kinase (PTK) superfamily, Eph family, EphB sub-family, which in humans comprises the catalytically active EPHB1-4 and the kinase-dead paralogue EPHB6 (overman2014completingthestructural pages 2-4).  
• Catalytic domains of EPHB1-4 share 83–89 % sequence identity, indicating recent gene duplication within the vertebrate lineage (overman2014completingthestructural pages 6-7).  
• Orthologous EphB1 genes are conserved across vertebrates, including teleost fish, amphibians, birds and mammals, reflecting retention after whole-genome duplications in gnathostomes (arcas2020theevolutionaryhistory pages 15-16).  
• Eph receptor genes pre-date the metazoan ancestor; diversification occurred through neofunctionalisation during multicellular evolution (arcas2020theevolutionaryhistory pages 2-3).

## Reaction Catalyzed

ATP + protein-L-tyrosine ⇌ ADP + protein-L-tyrosine-phosphate (overman2013biochemicalandbiophysical pages 1-2).

## Cofactor Requirements

Catalytic turnover requires divalent Mg²⁺, demonstrated by crystal structures solved with MgCl₂ and the ATP analogue ADPNP (overman2014completingthestructural pages 9-10).

## Substrate Specificity

• In vitro peptide screening defined an optimised substrate (“EPHOPT”) that accepts small residues at the −1 position and mandates an exposed tyrosine at position 0, but no broad consensus motif has yet been reported for EPHB1 (overman2013biochemicalandbiophysical pages 11-14).  
• Intramolecular binding of the partially ordered activation loop to the substrate groove observed in the homologous EPHB3 structure implies a preference for extended, unstructured tyrosine sites (overman2014completingthestructural pages 7-9).

## Structure

• Modular architecture: extracellular ligand-binding domain ➔ cysteine-rich region ➔ two fibronectin type III repeats ➔ single transmembrane helix ➔ juxtamembrane segment ➔ protein-tyrosine kinase domain ➔ sterile α-motif (SAM) ➔ C-terminal PDZ-binding motif (arcas2020theevolutionaryhistory pages 1-2).  
• Isolated kinase domain (residues 602–896) crystallises at 2.5 Å resolution with the canonical bilobed fold (overman2014completingthestructural pages 1-2).  
– Key catalytic elements: Lys665 (β3), the DFG759–761 motif and the HRD motif constitute the catalytic and regulatory spines (overman2014completingthestructural pages 7-9).  
– Hinge residues Gly699 and Ala700 enlarge the adenine pocket and govern ATP-competitive inhibitor binding (overman2013biochemicalandbiophysical pages 7-8).  
– Activation loop Tyr594, Tyr600 and Tyr604 are situated for autophosphorylation-dependent activation (kundu2023recurringephb1mutations pages 14-15).  
– Apo crystals display an open αC-helix, whereas staurosporine binding induces a closed, active-like conformation (overman2014completingthestructural pages 2-4).  
– The activation loop can occupy the substrate groove in cis, a conformation analogous to that captured for EPHB3, suggesting an intrinsic autoinhibitory arrangement (overman2014completingthestructural pages 7-9).

## Regulation

• Ligand-driven receptor clustering triggers trans-autophosphorylation of juxtamembrane and activation-loop tyrosines, relieving autoinhibition and creating SH2 docking sites (overman2013biochemicalandbiophysical pages 10-11).  
• Autophosphorylation at Y594, Y600 and Y604 is essential for full kinase activation; cancer-associated variant R351L markedly reduces phosphorylation at these sites (kundu2023recurringephb1mutations pages 14-15).  
• The protein-tyrosine phosphatase PTP1B reverses EphB autophosphorylation and was co-expressed to obtain a low-phosphorylation state for structural studies (overman2013biochemicalandbiophysical pages 1-2).  
• Juxtamembrane tyrosines impose intramolecular inhibition that is lifted upon their phosphorylation (overman2013biochemicalandbiophysical pages 10-11).  
• Assembly with the kinase-defective receptor EPHB6 provides additional regulatory complexity through hetero-oligomeric signalling (strozen2021theephb6receptor pages 13-14).

## Function

• Binds transmembrane ephrin-B ligands EFNB1, EFNB2 and EFNB3 on adjacent cells, initiating forward signalling in the Eph-expressing cell (overman2014completingthestructural pages 1-2).  
• Downstream interactors include SH2/SH3 adaptors, Src family kinases, PI3K, MAP kinases, Rho-family GTPases, guanine-nucleotide exchange factors and phosphatases, coordinating cell repulsion and adhesion responses (overman2014completingthestructural pages 1-2).  
• Guides ventro-temporal retinal ganglion cell axons at the optic chiasm during development, acting redundantly with other EphB receptors (arcas2020theevolutionaryhistory pages 1-2).  
• Together with EFNB3, controls chemotaxis, proliferation and polarity of adult hippocampal neural progenitors (arcas2020theevolutionaryhistory pages 1-2).  
• Participates in dendritic-spine maturation and synaptogenesis in concert with other EphB receptors (arcas2020theevolutionaryhistory pages 1-2).  
• Aberrant EphB signalling, including EPHB1, is linked to tumour development and progression (overman2014completingthestructural pages 1-2).  
• EphB1 binds ephrin-B ligands promiscuously, whereas EphB4 is selective for ephrin-B2, illustrating functional divergence within the sub-family (chrencik2006structuralandbiophysical pages 1-2).

## Inhibitors

• Broad-spectrum kinase inhibitor staurosporine binds the active site in co-crystal structures (overman2014completingthestructural pages 9-10).  
• Anilinopyrimidine inhibitors:  
– CMPD1 IC₅₀ = 0.091 µM (overman2013biochemicalandbiophysical pages 7-8)  
– CMPD2 IC₅₀ = 0.057 µM (overman2013biochemicalandbiophysical pages 7-8)  
– CMPD3 IC₅₀ = 0.160 µM (overman2013biochemicalandbiophysical pages 7-8)  
• Gly699 in the hinge region is critical for high-affinity binding; replacement by Cys in EPHB3 reduces potency, underscoring a selectivity determinant (overman2014completingthestructural pages 7-9).

## Other Comments

• Cancer-associated missense mutations in the kinase domain (e.g., R351L, D762N, R743W) disrupt autophosphorylation or signalling without uniformly impairing cell-compartmentalisation functions (kundu2023recurringephb1mutations pages 14-15).  
• Altered EPHB1 expression has been documented in gastric, colorectal and prostate cancers, supporting its context-dependent tumour-suppressor or oncogenic roles (overman2013biochemicalandbiophysical pages 10-11).

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