1. Phylogeny  
   Ephrin type‐B receptor 3 (EPHB3) is a member of the Eph receptor tyrosine kinase family, which is one of the largest and most evolutionarily conserved groups of receptor tyrosine kinases in metazoans. Within the Eph family, receptors are broadly divided into EphA and EphB subgroups based on sequence homology, ligand‐binding preferences, and structural features. EPHB3 belongs to the EphB subfamily, and sequence comparisons have revealed substantial conservation of its kinase domain and extracellular architectural motifs (stergiou2023ephephrinsignalingin pages 1-2, papadakos2023unravelingthesignificance pages 2-4). Orthologs of EPHB3 are present throughout vertebrate species, indicating that the roles played by this receptor—in axon guidance, synaptic patterning, angiogenesis, and cell positioning—are fundamental to vertebrate development. Furthermore, high amino acid similarity between EPHB3 and closely related receptors such as EPHB2 suggests that they arose from gene duplication events early in vertebrate evolution and have since maintained overlapping and, at times, redundant functions. Comparative phylogenetic studies based on the human kinome further place EPHB3 alongside other receptor tyrosine kinases that have evolved to mediate contact‐dependent signaling processes essential for tissue patterning (rasool2024masterregulatorsof pages 1-2, stergiou2023ephephrinsignalingin pages 1-2). This evolutionary relationship underscores an ancient and conserved mechanism by which cell–cell communication is coordinated during both embryogenesis and adult tissue homeostasis.
2. Reaction Catalyzed  
   EPHB3 functions as a receptor tyrosine kinase, and its intrinsic enzymatic activity catalyzes the transfer of a phosphate group from adenosine triphosphate (ATP) to specific tyrosine residues on substrate proteins. The general chemical reaction catalyzed by EPHB3 can be represented as:  
     ATP + [protein]–L-tyrosine → ADP + [protein]–L-tyrosine-phosphate + H⁺  
   This phosphorylation reaction is central to forward signaling, as it not only leads to receptor autophosphorylation but also creates docking sites for downstream signaling molecules containing Src homology 2 (SH2) domains (chatzikalil2024theclinicalrelevance pages 2-4, renuse2021tyrosinephosphoproteomicsof pages 7-9).
3. Cofactor Requirements  
   The catalytic activity of EPHB3 is dependent upon the binding of ATP and the presence of divalent cations, with Mg²⁺ serving as an essential cofactor. Mg²⁺ is required as it coordinates with the phosphate groups of ATP, correctly positioning the nucleotide in the active site of the kinase domain to facilitate the efficient transfer of the γ‐phosphate group onto tyrosine residues of substrates. This requirement is a common feature among receptor tyrosine kinases and is critical for the full catalytic activity of EPHB3 (chatzikalil2024theclinicalrelevance pages 2-4).
4. Substrate Specificity  
   EPHB3 preferentially phosphorylates tyrosine residues within substrate proteins. Its substrate specificity is determined by several factors: the intrinsic structure of the kinase domain that recognizes target tyrosine motifs, receptor clustering that shapes the local environment at the plasma membrane, and interactions mediated by its intracellular regulatory regions. In the context of forward signaling, autophosphorylation sites on EPHB3 itself function as high-affinity docking platforms for downstream effector proteins containing SH2 domains. Additionally, the recruitment of specific adaptor proteins through the PDZ-binding motif contributes to selective phosphorylation events on substrates that are part of signaling complexes. Overall, the substrate specificity of EPHB3 is reflective of the broader pattern observed in receptor tyrosine kinases, where the spatial conformation and clustering behavior drive selective phosphorylation of specific tyrosine residues on target proteins (renuse2021tyrosinephosphoproteomicsof pages 7-9, king2014ephbandephrinb pages 5-6, stergiou2023ephephrinsignalingin pages 22-23).
5. Structure  
   EPHB3 exhibits a canonical Eph receptor architecture characterized by several discrete domains that coordinate its functions. The protein is a single-pass transmembrane receptor composed of the following regions:

 • Extracellular Domain: The extracellular region is modular and consists of an N-terminal immunoglobulin-like ligand-binding domain that mediates interaction with transmembrane ephrin-B ligands found on adjacent cells. This is followed by a cysteine-rich region that provides structural stability and helps to facilitate proper folding. In addition, a series of fibronectin type III (FNIII) repeats are present, which further contribute to ligand-binding specificity and receptor dimerization (chatzikalil2024theclinicalrelevance pages 2-4, stergiou2023ephephrinsignalingin pages 2-4).

 • Transmembrane Domain: A single α-helical transmembrane segment anchors the receptor into the plasma membrane, thereby positioning the extracellular ligand-binding regions for intercellular communication and the intracellular catalytic machinery for signal transduction.

 • Intracellular Domain: The intracellular portion of EPHB3 includes a juxtamembrane region that is involved in autoinhibitory interactions. Upon ligand binding-induced receptor clustering, key tyrosine residues in this region become phosphorylated to relieve inhibition. The central tyrosine kinase domain is the catalytic core that transfers the phosphate group to target proteins. Downstream of the kinase domain, the receptor contains a sterile alpha motif (SAM), which is thought to play roles in receptor clustering and oligomerization. Finally, a short C-terminal PDZ-binding motif facilitates interactions with scaffold and adaptor proteins that are essential for downstream signaling complex assembly (chatzikalil2024theclinicalrelevance pages 2-4, papadakos2023unravelingthesignificance pages 2-4, scarini2024potentialroleof pages 8-9).  
In terms of three-dimensional organization, structural insights from related Eph receptors—supported by X-ray crystallography and computational models—suggest that EPHB3 adopts a compact extracellular fold that enables a high-affinity, homo-oligomerization interface upon ephrin binding. The kinase domain displays the characteristic bilobal structure with an N-terminal lobe primarily involved in ATP binding and a larger C-terminal lobe that contains the activation loop and substrate docking sites. These structural features are conserved among Eph receptors and underpin the receptor’s ability to mediate precise spatial and temporal signal transduction.

1. Regulation  
   Regulation of EPHB3 activity is achieved through multiple, tightly interwoven mechanisms designed to ensure precise control over its signaling outputs. One of the primary regulatory events is ligand-induced receptor clustering. Binding of transmembrane ephrin-B ligands on adjacent cells triggers receptor oligomerization, leading to conformational changes that facilitate autophosphorylation of key tyrosine residues within the juxtamembrane domain. This autophosphorylation acts to relieve intrinsic autoinhibition of the kinase domain, thereby enabling full catalytic activity (chatzikalil2024theclinicalrelevance pages 22-23, stergiou2023ephephrinsignalingin pages 21-22).

Once activated, the phosphorylated tyrosine residues on EPHB3 serve as docking sites for SH2 domain-containing adaptor proteins, which further propagate signaling cascades involved in cytoskeletal reorganization and cellular migration. The C-terminal PDZ-binding motif contributes to this process by recruiting additional scaffold proteins that help establish multi-protein signaling complexes at the plasma membrane.

Moreover, the duration and amplitude of EPHB3 signaling are modulated by counteracting processes. Protein tyrosine phosphatases (PTPs) can dephosphorylate EPHB3, thereby attenuating signaling. In some cellular contexts, proteolytic cleavage by metalloproteases leads to receptor ectodomain shedding, which is followed by receptor internalization and degradation, thus serving as an effective mechanism for signal termination (king2014ephbandephrinb pages 5-6, stergiou2023ephephrinsignalingin pages 21-22).

These regulatory mechanisms—comprising ligand-induced clustering, tyrosine autophosphorylation, adaptor recruitment, phosphatase activity, and receptor internalization—work in concert to modulate EPHB3 activity in a manner that is both cell type and context dependent.

1. Function  
   EPHB3 plays a central role in mediating contact-dependent bidirectional signaling between cells. In forward signaling, ligand engagement leads to receptor activation and subsequent phosphorylation of downstream effectors involved in the regulation of diverse biological processes. One of the critical functions of EPHB3 is in the developing nervous system, where it contributes significantly to axon guidance. By modulating the cytoskeleton and regulating cell adhesion, EPHB3 is instrumental in directing axonal projections and ensuring proper formation of major interhemispheric connections, including the corpus callosum and anterior commissure (scarini2024potentialroleof pages 8-9, rasool2024masterregulatorsof pages 1-2).

In addition to its role in axon guidance, EPHB3 influences the maturation of dendritic spines and the formation of excitatory synapses. These processes are fundamental for establishing synaptic circuits that underlie neural plasticity and proper cognitive function. Forward signaling through EPHB3 activates intracellular pathways that control actin cytoskeleton dynamics, thereby promoting dendritic spine morphogenesis and synaptogenesis.

Beyond its well‐characterized roles in neural development, EPHB3 also regulates cell positioning and migration in various tissues. For example, in the gastrointestinal tract, EPHB3 helps to compartmentalize cells by enhancing cell–cell contacts and regulating cell motility. Its tumor suppressor function has been documented in colorectal cancer models, where elevated levels of EPHB3 expression have been correlated with improved clinical outcomes, likely due to its ability to inhibit tumor cell invasion and metastasis (scarini2024potentialroleof pages 8-9, stergiou2023ephephrinsignalingin pages 23-25). Furthermore, EPHB3 plays a role in angiogenesis through its regulation of endothelial cell behavior, thereby contributing to blood vessel formation and vascular remodeling during development and tissue regeneration.

Collectively, the multifunctional roles of EPHB3 in axon guidance, synapse formation, cell migration, and angiogenesis underscore its importance as a modulator of development and tissue homeostasis.

1. Other Comments  
   Experimental approaches aimed at modulating Eph/ephrin signaling have significant therapeutic implications. Although specific small-molecule inhibitors exclusively targeting EPHB3 have not been fully established in clinical settings, several preclinical studies have explored the use of soluble decoy receptors, peptide mimetics, and kinase inhibitors to disrupt Eph receptor–ephrin interactions. Such strategies have been investigated as a means to mitigate aberrant cell migration and angiogenesis associated with various malignancies, including colorectal cancer, where EPHB3 expression levels serve as a prognostic marker (scarini2024potentialroleof pages 8-9, masaoutis2021ephrinreceptors(ephs) pages 11-12).  
   In addition, the ability of EPHB3 to mediate bidirectional signaling makes it an attractive target for modulating both forward and reverse signaling pathways. The reverse signaling mediated by ephrin-B ligands, although not directly catalyzed by EPHB3, similarly contributes to cellular responses and is of interest in the development of therapeutic interventions aimed at rebalancing intercellular communication in pathological conditions.  
   Given its established role in neural circuit formation and tumor suppression, further research into the precise mechanisms governing EPHB3 activity may assist in the design of novel therapeutics that selectively alter its signaling. This could lead to new interventions in neurodevelopmental disorders, cancers, and diseases characterized by dysregulated cell adhesion and migration (king2014ephbandephrinb pages 5-6, stergiou2023ephephrinsignalingin pages 22-23, masaoutis2021ephrinreceptors(ephs) pages 11-12).
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