1. Phylogeny  
   EPHA1 is a member of the Eph receptor family, which constitutes the largest subfamily of receptor tyrosine kinases (RTKs) in vertebrates. Within this family, Eph receptors are subdivided into two groups—the EphA and EphB classes—based on sequence homology and ligand-binding preferences. EPHA1 is classified in the EphA group, which generally interacts with glycosylphosphatidylinositol (GPI)‐anchored ephrin-A ligands. Orthologs of EPHA1 are found throughout jawed vertebrates, reflecting its conservation since the early evolutionary radiations of vertebrates, and its presence can be traced back to the gene duplication events (2R and 3R whole genome duplications) that expanded the RTK repertoire in the common ancestor of jawed vertebrates (Brunet2016wholegenomeduplications pages 6-7). Comparative analysis places EPHA1 in an evolutionary context alongside other EphA receptors (such as EPHA2, EPHA4, and EPHA8) that share similar domain organizations and signaling functions. The conserved amino acid sequences, particularly in the kinase and ligand-binding domains, emphasize that EPHA1 belongs to a group of RTKs critical for tissue patterning and cell–cell communication that have been maintained throughout vertebrate evolution (Arora2023ephreceptorsin pages 1-3). This conservation also suggests that the fundamental mechanisms by which EPHA1 mediates signal transduction are shared with its paralogs and orthologs in other species.
2. Reaction Catalyzed  
   EPHA1 functions as a classical receptor tyrosine kinase, catalyzing the transfer of a phosphate group from ATP to tyrosine residues on substrate proteins. The general chemical reaction catalyzed by EPHA1 can be summarized as follows:  
     ATP + [protein]–tyrosine → ADP + [protein]–phosphotyrosine + H⁺.  
   This reaction involves the binding of ATP to the kinase domain of EPHA1, followed by the transfer of the γ-phosphate to selected tyrosine residues on either the receptor itself (autophosphorylation) or on downstream effectors. This phosphorylation event alters the conformation and binding properties of the target protein, initiating or modulating intracellular signaling cascades (Madasu2024identificationofpotent pages 1-2).
3. Cofactor Requirements  
   The kinase activity of EPHA1, as with other receptor tyrosine kinases, is dependent on the presence of divalent cations. In particular, EPHA1 requires Mg²⁺ as a cofactor to coordinate the binding and proper positioning of ATP within its catalytic site. The magnesium ion interacts with the phosphate groups of ATP, stabilizing the negative charges during the transfer reaction and thereby facilitating the phosphoryl transfer process (Madasu2024identificationofpotent pages 1-2, Fagotto2014ephrinephsignalingin pages 17-18).
4. Substrate Specificity  
   High-throughput phosphoproteomic studies addressing the intrinsic substrate specificities of human tyrosine kinases have provided important insights into the substrate preferences of the Eph receptor family. Although a precise consensus sequence for EPHA1 has not been fully defined in isolation, data from large-scale kinase profiling indicate that Eph receptor kinases tend to phosphorylate tyrosine residues embedded within sequences that are enriched in acidic amino acids. In general, tyrosine kinases often exhibit a preference for substrates where acidic residues occur in the vicinity of the phosphorylation site, thereby contributing to recognition and binding affinity. For the Eph receptor family, including EPHA1, substrate phosphorylation is tightly connected to the formation of docking sites for SH2 domain‐containing proteins, which subsequently propagate downstream signals. The acidic residue bias may influence the positioning of these binding motifs within substrates, aiding in the recruitment of key adaptor proteins during forward signaling events (Sugiyama2019largescalediscoveryof pages 3-4, Xu2015identifyingthreedimensionalstructures pages 7-9). Although the exact amino acid pattern recognized by EPHA1 remains to be conclusively characterized, the overall intrinsic specificity profile resembles that of other Eph receptors and aligns with a preference for acidic residue–enriched motifs immediately adjacent to the tyrosine targeted for phosphorylation.
5. Structure  
   EPHA1 is a transmembrane receptor tyrosine kinase with a modular domain organization that underpins both its ligand recognition and its catalytic functions. The extracellular region of EPHA1 comprises several functionally distinct domains. At the N-terminus, a globular ligand-binding domain (LBD) mediates interaction with ephrin-A ligands, especially the high-affinity ligand EFNA1; this domain is followed by a cysteine-rich region that is thought to contribute to receptor dimerization and stability. In addition, EPHA1 contains two fibronectin type III (FN3) repeats that further support ligand binding and receptor architecture (Fagotto2014ephrinephsignalingin pages 17-18, Arora2023ephreceptorsin pages 1-3).

The receptor spans the plasma membrane through a single transmembrane helix that anchors the protein, ensuring proper orientation of the extracellular and intracellular domains. On the cytoplasmic side, EPHA1 possesses a juxtamembrane (JM) region that contains conserved tyrosine residues whose autophosphorylation is critical for the activation of the receptor’s kinase domain. The central kinase domain exhibits the canonical bilobal structure common to protein kinases; the smaller N-terminal lobe contains a glycine-rich loop that binds ATP, while the larger C-terminal lobe harbors the catalytic residues and an activation loop that undergoes conformational changes upon phosphorylation. Key structural features such as the C-helix in the N-lobe and the hydrophobic spines stabilize the active conformation of the kinase (Madasu2024identificationofpotent pages 12-12, Xu2015identifyingthreedimensionalstructures pages 7-9).

Following the kinase domain, EPHA1 contains a sterile alpha motif (SAM) domain that is implicated in mediating protein–protein interactions and receptor oligomerization. The SAM domain supports higher-order assembly of EPHA1 molecules, contributing to the propagation of signals by facilitating the formation of signaling clusters upon ligand binding. Finally, at the very C-terminus, a short PDZ-binding motif (PBM) serves to recruit additional adaptor proteins through PDZ domain interactions, thereby linking EPHA1 activation to downstream signaling cascades (Arora2023ephreceptorsin pages 1-3, Madasu2024identificationofpotent pages 12-12). Together, these domains confer a high degree of structural versatility, allowing EPHA1 to integrate extracellular cues with intracellular kinase activity and complex regulatory interactions.

1. Regulation  
   The activity of EPHA1 is tightly regulated by multiple mechanisms that ensure precise control over cellular signaling. A primary regulatory mechanism is ligand-induced receptor activation, wherein high-affinity binding of EFNA1 to the extracellular ligand-binding domain prompts receptor clustering and subsequent autophosphorylation of conserved tyrosine residues located in the juxtamembrane region and within the activation loop of the kinase domain. This autophosphorylation event releases inhibitory interactions within the receptor, thereby fully activating the catalytic function of the kinase (Madasu2024identificationofpotent pages 1-2, Fagotto2014ephrinephsignalingin pages 17-18).

Following ligand binding and autophosphorylation, EPHA1 serves as a docking platform for various intracellular proteins that contain phosphotyrosine-binding domains, such as SH2 domains. These interactions enable the recruitment of molecules including non‐receptor tyrosine kinases, adaptor proteins, and phosphatases that modulate downstream signaling activity. A key regulatory interaction involves integrin‐linked kinase (ILK), whose association with EPHA1 has been shown to modulate cytoskeletal dynamics and cell attachment. In this context, engagement of EFNA1 leads to receptor activation and a consequential inhibition of cell spreading and motility through the regulation of ILK and the downstream small GTPases RHOA and RAC (Madasu2024identificationofpotent pages 1-2, Offenhäuser2025epha2regulatesvascular pages 19-19).

The regulatory mechanisms of EPHA1 also involve dynamic changes in receptor conformation and protein–protein interactions mediated by its intracellular domains. Phosphorylation-dependent recruitment of proteins via PDZ-binding motifs and possible interactions that are facilitated by the SAM domain further modulate receptor stability, internalization, and downstream event propagation. In addition, dephosphorylation of EPHA1 by specific protein tyrosine phosphatases may serve as a negative feedback mechanism to attenuate signaling. Receptor internalization and subsequent degradation or recycling are also considered important post-translational regulatory processes that influence the duration and intensity of EPHA1-mediated signaling (Offenhauser2025epha2regulatesvascular pages 19-19, Fagotto2014ephrinephsignalingin pages 17-18). Overall, these regulatory layers allow EPHA1 to function as a context-dependent modulator of cell adhesion, migration, and proliferation without the need for additional external co-regulators beyond its ligand-binding events and intrinsic kinase activation.

1. Function  
   EPHA1 acts as a critical mediator of contact-dependent bidirectional signaling between adjacent cells due to its ability to engage with membrane-bound ephrin-A ligands. In the forward signaling pathway, the binding of high-affinity ligand EFNA1 to EPHA1 triggers receptor oligomerization and autophosphorylation, thereby initiating intracellular signaling cascades. This forward signaling regulates multiple cellular processes including the modulation of cell adhesion to the extracellular matrix, control of cell spreading and motility, and regulation of cytoskeletal dynamics mediated by small GTPases such as RHOA and RAC. Through these downstream effects, EPHA1 plays an essential role in maintaining cell–cell interactions and tissue integrity (Madasu2024identificationofpotent pages 1-2, Fagotto2014ephrinephsignalingin pages 17-18).

Furthermore, EPHA1 signaling has been implicated in angiogenesis, where it contributes to vascular remodeling and the formation of new blood vessels. By modulating cell adhesion and migration, EPHA1 influences the behavior of endothelial cells during the process of neovascularization. In addition to its roles in cell adhesion and migration, EPHA1 is involved in the regulation of cell proliferation. Activation of EPHA1 has been associated with the inhibition of cell spreading accompanied by enhanced attachment to the extracellular matrix; such modulation is thought to occur through the regulation of integrin-linked kinase (ILK) activity and subsequent alteration of the RHOA and RAC signaling pathways (Offenhauser2025epha2regulatesvascular pages 19-19, Madasu2024identificationofpotent pages 1-2).

EPHA1 may additionally participate in the control of apoptotic pathways. Although the precise mechanisms remain to be fully delineated, there is evidence that signaling via EPHA1 can influence programmed cell death, potentially by affecting intracellular mediators that converge on apoptotic machinery. Expression patterns of EPHA1, which have been documented in epithelial tissues and in certain pathological contexts such as cancer and neurodegenerative disorders, further support its multifaceted role in development and homeostasis. For instance, altered EPHA1 expression has been linked to disease risk in Alzheimer’s disease, as noted in gene expression studies (Karch2012expressionofnovel pages 9-9). Thus, EPHA1 serves as an important regulator in cellular processes that determine tissue architecture, vascular integrity, and cell survival.

1. Other Comments  
   In addition to its well-established roles in mediating forward signaling upon binding ephrin-A ligands, EPHA1 has attracted attention as a potential therapeutic target in several disease contexts. Although the development of EPHA1-specific inhibitors has not reached the same level of advancement as for some other Eph receptors, pan-Eph receptor inhibitors identified using DNA-encoded chemical library screening approaches have demonstrated potent inhibitory activity against this family of kinases (Madasu2024identificationofpotent pages 9-10). These inhibitors operate by targeting the conserved ATP-binding pocket or by disrupting receptor–ligand interactions, thereby attenuating downstream signaling pathways.

Disease associations for EPHA1 include roles in cancer progression and angiogenesis. Aberrant expression or dysregulation of EPHA1 signaling has been observed in several tumor types, where it may contribute to altered cell adhesion, enhanced migratory potential, and changes in vascular permeability. For example, studies in related Eph receptors, such as EPHA2, have shown that dysregulated signaling can promote metastatic behavior in prostate cancer (Offenhauser2025epha2regulatesvascular pages 19-19). Genetic studies have also implicated EPHA1 as a risk factor in Alzheimer’s disease, suggesting that alterations in its signaling pathway could have important consequences for neurodegenerative processes (Karch2012expressionofnovel pages 9-9).

Because EPHA1 modulates integrin-linked kinase activity and consequently affects the balance between RHOA-mediated stress fiber formation and RAC-mediated lamellipodia formation, it represents a critical nodal point in the regulation of cellular motility and adhesion. These regulatory functions are of particular interest as potential intervention points for pharmacological modulation and therapeutic development. Inhibitors that disrupt EPHA1 activity may therefore offer therapeutic benefits not only in oncology but also in diseases characterized by aberrant angiogenesis and inflammatory dysregulation. Moreover, the ability of EPHA1 to participate in bidirectional signaling also implies that therapeutic strategies could be designed to modulate both forward signaling in receptor-expressing cells and reverse signaling in ephrin-expressing neighboring cells, thereby providing a broader spectrum of intervention options (Arora2023ephreceptorsin pages 1-3, Madasu2024identificationofpotent pages 9-10).

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Each reference contributes to the comprehensive understanding of EPHA1’s evolutionary conservation, catalytic mechanism, regulatory control, structural organization, substrate specificity, and its roles in cell signaling pathways relevant to development, vascular regulation, and disease.