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Cell-Cell Signaling via Eph Receptors and Ephrins

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Summary

Eph receptors are the largest subfamily of receptor tyrosine kinases regulating cell shape, movements and attachment. The interactions of the Ephs with their ephrin ligands are restricted to the sites of cell–cell contact since both molecules are membrane attached. This review summarizes recent advances in our understanding of the molecular mechanisms underlining the diverse functions of the molecules during development and in the adult organism. The unique properties of this signaling system that are of highest interest and have been the focus of intense investigations are as follows: (i) the signal is often simultaneously transduced in both ligand- and receptor- expressing cells, (ii) signaling via the same molecules can generate opposing cellular reactions depending on the context, and (iii) the Ephs and the ephrins are divided in two subclasses with promiscuous intra-subclass interactions, but rarely observed inter-subclass interactions.

Introduction

The Eph receptors and their ephrin ligands control a diverse array of cell-cell interactions in the nervous and vascular systems, as well as in specialized epithelia [reviewed in 1–4]. Upon ephrin binding, the tyrosine kinase domain of the Ephs is activated, initiating “forward” signaling in the receptor-expressing cells. At the same time, signals are also induced in the ligand-expressing cells, a phenomenon referred to as “reverse” signaling [5] (Fig. 1). Both the Ephs and the ephrins are divided into two subclasses – A and B – based on their affinities for each other and on sequence conservation [6] (<http://eph-nomenclature.med.harvard.edu/>). In general, the A-subclass Eph receptors (EphA1–EphA9) bind promiscuously to the A-class ephrins (ephrin-A1 – ephrin-A6), while the EphB subclass receptors (EphB1 – EphB6) interact with the B-subclass ephrins (ephrin-B1–ephrin-B3) [1–4]. Since both Ephs and ephrins are membrane-bound, their interaction occurs only at sites of cell–cell contact (Fig. 1). It is thought that in the absence of cell-cell interactions, the molecules exist in loosely associated clusters (microdomains) within their respective plasma membranes, which become much more compact upon Eph/ephrin complex formation, generating clearly defined signaling centers at the cell-cell interfaces [7–8]. The past several years have brought significant advances in our understanding of the molecular mechanisms of action of Eph receptors and ephrins, and these are the subjects of this review.

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New insights into functions outside of the nervous system

The Ephs and ephrins were initially identified as axon guidance molecules mediating neuronal repulsion during CNS development [2–3], but it was soon discovered that they also regulate cell-cell communication in a variety of other tissues and cell types [1,9]. While their best-studied roles outside of the nervous system are those during development of the vascular system, recent studies reveal the importance of Ephs and ephrins in the immune system, bone, stem cells, epithelial cells and in the development and metastasis of many tumors.

Overwhelming evidence documents that the Eph receptors, in conjunction with their ligands, control blood vessel formation. Ephrin-A1, the first identified family member, was cloned from human umbilical vein endothelial cells (HUVECs) [10] and is implicated in regulating vascular morphogenesis and angiogenesis [11, 12]. EphB4, EphB2 and EphB3 and their ephrin-B2 and ephrin-B1 ligands were also shown to direct the formation of the circulatory system [12]. Most significantly, Ephrin-B2 is expressed in arteries, whereas its receptor, EphB4 – in veins, thus defining their boundaries during development [13]. In addition, EphB2 and ephrin-B2 mediate the interactions between the vessel endothelial cells and the adjacent mesenchymal cells [13,14]. An important new study [15] reveals that ephrin-B2 is also required for normal association between the blood-vessel endothelial cells and the supporting pericytes and vascular smooth muscle cells (mural cells). Defective mural-cell coverage is associated with the poorly organized and leaky vasculature seen in tumors or other human diseases. The authors further suggest that ephrin-B2 has some cell-cell-contact-independent functions [15] during these events.

Many Ephs and ephrins, including EphA1, EphA2, EphA3, EphA4, EphB2, EphB3, EphB4 and ephrin-A1 are overexpressed in a variety of cancers where they exhibit mostly, but not exclusively, tumor-promoting properties [14, 16]. Indeed, the founding Eph member, EphA1, was isolated from a hepatoma cell line [17] and is overexpressed in breast, liver, lung, and colon carcinomas. Many Ephs seem to be predominantly expressed in metastatic cell lines as compared to the primary tumor, and their expression levels often correlate with the grade of the tumor malignancy and invasiveness. EphB4 is expressed in all examined breast carcinoma cell lines [14] and its role in cancer is reviewed in [16]. EphB4 is particularly interesting because it has both tumor-suppressing and tumor-promoting activities, which are affected via different molecular mechanisms: the tumor suppression seems to be a result of EphB4-dependent downregulation of Crk that lead to inhibition of cell mobility and invasion, as well as facilitation of apoptosis; the tumor promoting properties seems to result mainly from the angiogenesis-promoting EphB4 activity [16].

Bi-directional A-class Eph/ephrin interactions were recently shown to regulate insulin secretion [18] by directing the communications between the insulin secreting β cells of the pancreas. These cells are aggregated in pancreatic islets, where cell-cell contacts inhibit basal insulin secretion but enhance glucose-stimulated insulin secretion, thus contributing to glucose homeostasis during fasting and feeding. It is now clear that EphA forward signaling inhibits insulin secretion, whereas ephrin-A reverse signaling stimulates it.

A recent study also shows that B-class Ephs and ephrins mediate the activities of osteoclasts, which degrade bone, and osteoblasts, which form bone, to maintain bone homeostasis [19]. Indeed the osteoclasts express ephrin-B2, while osteoblasts express the corresponding EphB4 receptor. Reverse signaling through ephrin-B2 into osteoclast precursors suppresses osteoclast differentiation, while forward signaling through EphB4 into osteoblasts enhances it – thereby maintaining bone homeostasis [19].

Structures studies of the Eph/ephrin interactions

Biochemical and X-ray crystallographic investigations have generated abundant structural information about the interacting domains of the Ephs, ephrins, and their complexes [4]. The first crystals structure of an Eph/ephrin complex [20] indicated that the proteins form a tetrameric, ring-like assembly in which two receptor and two ligand molecules interact via two distinct interfaces (Fig. 2). Such an architectural arrangement would explain how cell-cell contact results in the rearrangement of both ligands and receptors - initiating signals in both interacting cells. As illustrated on Fig. 2, one of the Eph/ephrin interfaces is very extensive and is responsible for the high-affinity ligand-receptor dimerization, whereas the second interface is smaller and is responsible for the assembly of the EphB-ephrin-B dimers into the circular tetramers [4, 20].

Interestingly while the lower-affinity tetramerization interface showed a clear structural basis for subclass discrimination, the high-affinity dimerization interface did not provide such clear view into the Eph subclass binding preferences. This promoted a closer examination of the possibility for overlooked cross-subclass interactions resulting in the very intriguing finding that two of the most widely studied molecules in the field, ephrin-A5 and EphB2, interact both *in vivo* and *in vitro* inducing receptor forward signaling [21*]. The overall conclusion of this study is that the total bidirectional signals transduced into opposing cells are a combination of all Eph-ephrin interactions—both intra- and inter-subclass. Thus, even interactions that are of lower affinity could significantly affect cell behavior depending on the number/density of interacting molecules.

In the past year the first structure of uncomplexed A-class ligand (ephrin-A5) was reported [22], as well as the structures of ephrin-B1 [23] and, most importantly, that of a third ligand/receptor complex - EphB4/ephrin-B2 [24]. These studies further illuminate the molecular determinants of the unique binding-partner specificities of Ephs and ephrins. The EphB4/ephrin-B2 structure, in particular, suggests that a single amino acid (Leu-95) plays a central role in defining ligand selectivity of the B-class receptors, and this selectivity can be altered via structure-based protein engineering.

Both the EphB2/ephrin-A5 and the EphB4/ephrin-B2 complexes are heterodimers in the crystals, architecturally distinct from the tetrameric EphB2/ephrin-B2 structure. Since the EphB4/ephrin-B2 tetramers are generated via crystal-packing interactions and their biological significance was not addressed in the initial study, random-mutagenesis experiments using yeast expression libraries were recently performed aimed at further defining the Eph and ephrin molecular surfaces that are important for complex formation and signaling initiation. The first study [25] identified three distinct EphA3 surface areas that are essential for the EphA3/ephrin-A5 interaction. Two of these mapped to the crystallographically-observed dimerization and tetramerization interfaces, confirming their physiological importance. Interestingly, the third essential interface falls outside of the structurally characterized interaction domains. The other random-mutagenesis study [26], identified ephrin-A5 residues essential for the assembly of high affinity signaling complexes with EphA3. Again residues at both the proposed dimerization and tetramerization interfaces were identified, in addition to a cluster of 10 residues, defining a new (third) surface region required for oligomerization and activation of EphA3 signaling (green on Fig. 2). These two studies, taken together, suggest that the positioning of the Ephs and ephrins in the signaling complexes is important and is maintained not only by the interactions between the minimal ligand/receptor binding modules, but also by additional domains and interfaces. Indeed, both the cysteine-rich [27] and the sterile alpha motif (SAM) [28] Eph domains have been implicated in ligand-independent receptor-receptor interactions.

Activation of the Eph kinase domain

The activity of all RTKs is tightly regulated and their activation follows some general rules [29, 30]. Ligand binding brings together two or more catalytically repressed kinase domains and holds them in an orientation favoring phosphorylation in trans. Stimulation of the kinase activity nearly always involves the phosphorylation of the kinase activation loop, which in its non-phosphorylated form blocks the active site [31]. In some RTKs, including the Eph, Kit, Flt, platelet-derived growth-factor beta (PDGF β) and TrkB receptors, the juxtamembrane region is also involved in regulation of the kinase activity [31, 32]. The crystal structure of the intracellular region of EphB2 [33] revealed that the unphosphorylated juxtamembrane region forms a well-ordered structure interacting with the N-terminal lobe of the kinase, presumably causing the distortion of a key α -helix and leading to kinase inactivation. Phosphorylation of the juxtamembrane tyrosine residues would push this region away via electrostatic repulsion, relieving the structural constraints that distort the active site. In addition, upon phosphorylation, the solvent-exposed juxtamembrane segment becomes available for interactions with downstream signaling proteins [32]. In the past year the same group performed a much more detailed study of Eph kinase activation utilizing a combination of mutational analysis, X-ray crystallography and NMR spectroscopy on the auto-inhibited and active forms of EphB2 and EphA4 [34*]. They showed that constitutively active mutants induce disorder of the juxtamembrane region and its dissociation from the kinase domain, interestingly occurring without major conformational changes in the kinase. These results suggest that a change in inter-lobe dynamics, rather than a transition to a static active conformation, underlies Eph RTK activation.

Architectural role of Eph/ephrin assemblies

The ability of Ephs and ephrins to form ordered multimeric assemblies suggest a potential architectural role that could either be separate or linked to their direct signaling role [4]. Specifically, the interacting molecules could create stable membrane-associated platforms for the architectural organization of various cellular structures at the sites of cell-cell contact. The best-studied example so far is their role in the formation, organization and function of excitatory CNS synapses [35]. Indeed, EphB2 associates with NMDA receptors, controlling both their density in postsynaptic clusters and the number of postsynaptic release sites [35, 36]. Interestingly, based on the known Eph/ephrin complex structure and assuming that the other extracellular regions adopt relatively extended conformations, the distance between the membranes of two interacting cells can be estimated at ~20 nm – consistent with the size of the synaptic cleft. In a recent extension of these studies Dalva and colleagues show that B-class ligand/receptor binding induces a direct interaction at the synapses between EphB and NMDA-type glutamate receptors involving only the extracellular regions of the molecules and independent of the receptor kinase activity [37]. These results confirm that EphB activation promotes Eph/NMDA-R assemblies that are essential for synapse development or function.

Targeting the Eph/ephrin interactions with peptides and small molecule inhibitors

The Ephs and ephrins are involved not only in early developmental processes, but also in the function of the adult organism and the ability to modulate their signaling could have important medical applications. The Eph/ephrin crystal structures [20, 21*, 24] document that the ligand/receptor interface is dominated by the insertion of an ephrin loop into a hydrophobic channel on the Eph surface and that, unlike most other protein-protein interactions, the Eph/ephrin binding could be disrupted by small molecules or peptides targeting this channel. Indeed, the group of Dr. Elena Pasquale used phage display

technology to identify a series of peptides that specifically bind EphB2, EphB2, EphA2 and EphA4 [38–40*], often effectively competing with the cognate ephrin ligands. The crystal structure of one of these antagonist peptides was determined in complex with its target, EphB4 [41*]. As expected, the peptide bound in the hydrophobic ligand-binding channel of EphB4. Interestingly, isothermal titration calorimetry revealed a thermodynamic discrepancy between ephrin-B2 binding, which is an entropically driven process, and peptide binding, which is an enthalpically driven process [24, 41*].

The available structural, biophysical and biochemical data suggests that small-molecules would also be able to target the Eph surface channel and disrupt ligand binding. Such inhibitors should be more specific than the broad-specificity kinase inhibitors binding in the enzyme's ATP/substrate pocket. Small-molecule inhibitors of several other cancer-related protein-protein interactions, such as p53-MDM2, were recently identified and are currently evaluated as potential anti-tumor drugs [42]. Moreover, while the other targeted protein-protein interactions occur inside the cell – the Eph/ephrin interactions are extracellular, eliminating the need for internalization and increasing the available chemical-structure space of pharmacologically useful hits.

Regarding the development of screens for small-molecule Eph/ephrin inhibitors, it should be noted that the canonical (intra-class) Eph/ephrin dimerization interfaces (EphB2/ephrin-B2 [20] and EphB4/ephrinB2 [24]) include two distinct regions, only one of which is observed in the inter-class EphB2-ephrin-A5 complex [21*]. Specifically, in addition to the Eph-channel/ephrin-loop interactions (purple on Fig. 3), a second, structurally separate contact area encompasses the ephrin-docking site along the surface of the receptor (yellow on Fig. 3). Since the latter area includes some subclass-specific interactions, the corresponding surfaces do not interact in the EphB2/ephrin-A5 complex - resulting in the smaller overall contact interface. Indeed, EphB2/ephrin-A5 complex formation buries only ~1300 Å², as compared to ~2100 Å² buried in the EphB4/ephrinB2 complex and ~2300 Å² buried in the EphB2/ephrinB2 complex. Therefore, it would be beneficial to design a HTS-suitable surrogate binding assay using ligand and receptor constructs interacting solely via the Eph-channel/ephrin-loop interface - the intended target of the small-molecule compounds (see Fig. 3 – bottom panels). Indeed, a similar Homologous Time-Resolved Fluorescence (HTRF) assay was recently described [22], based on Fluorescence Resonance Energy Transfer (FRET) between a Eu³⁺ cryptate labeled EphB2 and XL665-labeled ephrin-A5.

Eph/ephrin interactions on the surface of the same cell

It was initially assumed that functional Eph/ephrin interactions occur only between molecules expressed on the surface of opposing cells [2]. Surprisingly though, different Eph receptors and ephrins are often co-expressed by the same cells raising the question about potential interactions in *cis* (within the same cell) affecting the functional signaling in *trans* (between the opposing cells). The in-*cis*-interaction theory gained momentum after several reports indicated that the responsiveness of EphA expressing retinal axons is negatively modulated by A-class ligands expressed on the same cells [43, 44]. Interestingly, a seemingly contradictory conclusion was published by Marquardt, Pfaff and colleagues [45], who documented that at least in spinal motor neurons coexpressed Ephs and ephrins segregate laterally into distinct membrane domains from which they induce opposing effects: EphAs direct growth cone collapse/repulsion, while ephrin-As signal motor axon growth/attraction. This subcellular segregation enables the utilization of both Ephs and ephrins as functional guidance receptors within the same neuronal growth cone. Such an arrangement would also explain the fact that the ratio of A-class Ephs/ephrins expressed within a given cell regulates its responsiveness to external signals [9].

In a recent study [46**], the hypothesis of functional Eph/ephrin interactions within the same membrane was revitalized, identifying two types of Eph/ephrin interactions in *cis*: (i) “masking” interactions involving the ligand binding domain of the receptor [44], and (ii) novel inhibitory interactions (abolishing EphA activation) involving other receptor domains. Importantly the authors suggest that the formation of in-*cis* complexes transforms the uniform expression of EphAs in the nasal part of the retina into a gradient of functional EphAs and has a key role in controlling retinotectal mapping [46**, 47]. It should be noted that the study showing the segregation of the Eph and ephrins into separate microdomains and the study documenting the functional interaction in *cis* were performed in different neuronal types. In the general case, both microdomain segregation and interactions in *cis*, could play a role in fine-tuning the response of Eph and ephrin - expressing cells to external signaling cues.

Repulsion v.s. adhesion; enzymatic cleavage v.s. transendocytosis

The Ephs and ephrins were first described as modulators of neuronal repulsion [2, 3] and only later was discovered that they can also promote cell adhesion [48]. Interestingly, forward signaling seems to be more often repulsive, while reverse signaling via A-class ephrins is mostly attractive/adhesive [48]. It is becoming clear that whether the Eph/ephrin interactions result in cell adhesion or cell repulsion depends to a large extent on the mechanism of signal termination and specifically on whether the two interacting cells can easily separate following cell-cell contact. Cell separation is not a trivial issue since the Eph/ephrin interactions are very high affinity and these molecules are very abundant at the cell surface. Thus, if the cell-cell contacts that they mediate are to be repulsive, the Eph/ephrin interactions need to be broken to allow the cells to move apart. Two general mechanisms have been proposed for this: the first one involves regulated cleavage of either ephrins or Ephs by transmembrane proteases, while the second one involves rapid trans-endocytosis of whole Eph/ephrin complexes during the retraction of the interacting cells or neuronal growth cones.

The first evidence for proteolytic ephrin regulation identified ephrin-B3 as a substrate for the rhomboid transmembrane protease RHBDL2 [49]. For A-type ephrins, Flanagan and colleagues documented in an elegant study that ephrin-A2 is functionally cleaved by the metalloprotease ADAM10, a process essential for disrupting Eph/ephrin cell contacts *in vivo* [50**]. Subsequent structure-function studies of the ADAM10/EphA3/ephrin-A5(A2) interactions [51**] suggested a simple mechanism, which ensures that only Eph-bound ephrins are recognized and cleaved to allow their internalization into the Eph-expressing cell (Fig. 4): (1) prior to cell-cell contact, ADAM10 is constitutively associated with EphA3 via an yet unknown interface providing close proximity to potential substrates; (2) cell-contact dependent EphA3/ephrin-A5(A2) complexes present a new ADAM recognition surface, which binds the substrate-recognition pocket in the ADAM10 Cys-rich domain. (3) The disintegrin domain, forming a continuous, rigid structure with the cysteine-rich domain, positions the N-terminal protease domain for effective cleavage of the ephrin stem region in *trans* (on the surface of the opposing cell). (4) The cleavage breaks the molecular tethers between the opposing cell surfaces, allowing for signal termination and internalization of the Eph/ephrin complexes. This model is supported by recent structural studies of the full-length VAP1 molecule, a snake venom homolog of the mammalian ADAMs [52]. The structure reveals a C-shaped scaffold with the protease active site sitting at one end and a hyper-variable region (HVR) in the cysteine-rich domain sitting at the other. The HVR is a functional homologue of the Eph/ephrin-recognition pocket in cysteine-rich domain of ADAM10. While it has only been reported that ADAMs cleave A-class ephrins, it could be reasonably expected that they might also cleave B-class ephrins, as well as Eph receptors.

Interestingly, B-class ephrins are also cleaved by a presenilin γ -secretase activity during Eph/ephrin bi-directional signaling [53*, 54], which could provide a novel mechanism for ephrin reverse signaling. The emerging model involves initial cleavage of B-ephrins by an α -secretase (an ADAM protease) triggered by Eph/ephrin complex formation. The remaining membrane-attached ephrin fragment is further processed by a γ -secretase to release an intracellular signaling peptide, which is proposed to bind Src, preventing its association with the inhibitory kinase Csk. The activated Src in turn phosphorylates ephrin-B2, in a feedback mechanism, rendering it resistant to further cleavage by the γ -secretase. Growing evidence also implicates regulated intramembrane cleavage in other signaling events in neurons. The myelin-associated inhibitors MAG, Omgp, and Nogo, for example, act through the common neuronal receptor NgR, which uses p75 as a signaling co-receptor. It was recently shown that MAG binding to NgR induces cleavage of p75 by the combined action of an α - and a γ -secretase activities, resulting in the release of an intracellular signaling peptide, which induces Rho GTPase activation, inhibiting the axonal growth [55].

As an alternative approach to proteolytic processing, EphB-ephrinB complexes could also be removed from the cell surface by trans-endocytosis [9]. It was shown that during cell-cell contact B-class Ephs and ephrins co-cluster and trigger the simultaneous endocytosis of full-length proteins into both opposing cells in a manner that depends on the intracellular domains of the molecules [56*] and on Rac signaling [57*]. The factors influencing this alternate mechanism of signal termination are not well characterized [9, 59], though actin polymerization and depolymerization [58] may affect the process.

Conclusion and future directions

The central roles of Ephs and ephrins in the development and function of many organs and systems have placed them in the center of intensive genetic, biochemical and structural studies that have greatly advanced our understanding of this signaling system. Of particular importance are insights into the structural determinants defining the unique binding-partner preferences of the molecules and insights into the mechanisms of kinase-domain activation, as well as signal termination via proteolytic cleavage and/or trans-endocytosis. These relate directly to understanding how interactions between the same ligands and receptors can induce very different, and often completely opposite, effects such as cell attraction and repulsion, cell adhesion and mobility.

Future studies should further address the issue of trans-endocytosis v.s. cleavage, as well as Eph/ephrin interactions in *cis* v.s. microdomain segregation, in different cell and neuronal types during different developmental stages. The potential roles of other cell-surface and secreted proteases (e.g. ADAM17 and MMPs [60]) in both Eph and ephrin cleavage should also be investigated. The novel reverse signaling mechanism, based on intracellular release of the ephrin tails is very interesting and poses the question of whether the intracellular domains of the Eph receptors could also be released following similar processing by α - and γ -secretases. Surprisingly, the identities of the postulated co-receptors mediating ephrin reverse signaling still remain elusive although several candidate molecules have been reported at scientific meetings. As far as structural studies are concerned, it is essential to determine the structures of the Eph/ephrin/ADAM complex to fully understand the regulation of the ADAM-mediated ephrin cleavage. Structures of the complete extracellular Eph regions are also lacking and the precise roles of the domains outside of the minimal interaction regions are still unknown. Finally, the Holy Grail of the ephrin (and, in general, the receptor-tyrosine kinase) signaling field is the structure determination of a full-length transmembrane Eph receptor alone, and in complex with its activating ligand.

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* of special interest

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Figure 1.

Activation of bi-directional Eph/ephrin signaling. The current model of signaling initiation involves an initial 1:1 high-affinity interaction between ligands and receptors, followed by tetramerization, oligomerization and clustering of the molecules at the sites of cell-cell contact [4]. This brings about phosphorylation of the cytoplasmic Eph (as well as B-class ephrin) domains and downstream signaling initiation [3]. Kinase activation is controlled by the phosphorylation of residues in the activation loop and the juxtamembrane segment, which affect the inter-lobe(subdomain) dynamics of the kinase domain [31–35]. The minimal ligand-binding domain of the receptor is in blue and the ephrin receptor-binding domain is in pink. The kinase and SAM (sterile α -motif) domain is in green and the phosphate groups are in orange.

Figure 2. Schematic representation of the heterotetrameric complex formed between the Eph/ephrin interaction domains. Three interaction interfaces are indicated: dimerization and tetramerization - identified by crystallography [4] and confirmed by mutagenesis [25, 26], as well as a potential oligomerization interface - identified by a random mutagenesis approach [26].

Figure 3.

Schematic representation and a comparison of the interaction surfaces in the canonical intra-subclass EphB2/ephrin-B2 [20] and EphB4/ephrin-B2 [24] complexes with the unusual inter-subclass EphB2/ephrin-A5 complex [21*]. While ephrin-B2 has several interacting surface areas with the B-class receptors, the interaction of ephrin-A5 with EphB2 is mostly confined to the hydrophobic receptor surface channel. Peptides “pep” [39–41*] and small-molecule compounds “C” can also bind the channel, potentially inhibiting the Eph/ephrin interactions. The distinct architecture of the EphB2/ephrin-A5 complex and the lower affinity of the interaction can be used for the development of interaction assays, including FRET-based ones [22], suitable for high-throughput screening of compound libraries for small-molecule Eph signaling inhibitors “C”.

Figure 4.

Cleavage of the Eph-bound ephrins allows for signaling termination and cell separation/repulsion. ADAM10 associates constitutively with the Eph receptors and ephrin binding generates a new ADAM-recognition motif at the Eph/ephrin interface allowing for productive protease positioning and efficient ephrin cleavage in *trans*. It seems likely that in certain cases cleavage in *cis* could also occur and that the Eph receptors could also potentially be cleaved off the cell surface. Further cleavage by a γ -secretase internalizes the cytoplasmic tails of the B-class ephrins initiating reverse signaling while the Eph/ephrin complexes are internalized in endosomes. The α - (ADAM10) and γ -secretases are likely retained at the cell surface to participate in another cleavage cycle. The interaction domains of the receptors are in blue and those of the ligands - in red. The protease domain of ADAM10 is in pink, the disintegrin domain - in yellow, and the Cys-rich domain - in green. The γ -secretase is drawn in purple.