Ephrins in reverse, park and drive

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Eph receptors and their membrane-anchored ephrin ligands are thought to orchestrate cell movements by transducing bidirectional tyrosine-kinase-mediated signals into both cells expressing the receptors and cells expressing the ligands. Whether the resulting event is repulsion of an axonal growth cone, directing the orderly segmentation of hindbrain rhombomere cells or controlling angiogenic remodelling, such elaborate and diverse cell movements require intricate changes in the actin cytoskeleton, as well as precise regulation of cellular adhesion. Recent work by several groups has begun to link ephrin reverse signals to intracellular pathways that regulate actin dynamics and might help to explain how these ligands function as receptors to direct cell movement, adhesion and de-adhesion events.

The development of multicellular organisms requires an exquisite interplay of cell proliferation, differentiation, adherence and movement. Fundamental to normal development are many different cell-anchored receptors that communicate information into their cell after exposure to the appropriate ligand. Whether soluble and acting at a distance, or anchored to the membrane and acting on cell-cell contact, most ligand-receptor systems transduce information unidirectionally from the ligand-expressing cell into the receptor-expressing cell.

Presenting a contrast to this model are the large family of Eph receptor tyrosine kinases and their membrane-anchored ephrin ligands. On cell–cell contact, these molecules transduce important signals bidirectionally into both the receptor-expressing cell and the ligand-expressing cell in what is known as ‘forward’ and ‘reverse’ signaling, respectively [1] (Fig. 1). In this way, the Eph receptors can also function as ligands and the ephrin ligands can also function as receptors. Bidirectional signaling mediated by Ephs and ephrins is thought to communicate signals that regulate the cytoskeleton during axon pathfinding, cell migration, cell adhesion and vascular remodelling (reviewed in [2–5]) and is now also linked to the regulation of fluid production and ion homeostasis in nonmotile epithelial cells [6]. As the known range of biological functions performed by this family of multitalented receptors and ligands continues to expand, focus is shifting to determining how these molecules transduce such important and diverse signals into the cell.

Ephs and ephrins can be divided into two classes on the basis of their structural features and binding affinities. A-subclass ephrins (ephrin-A1 to ephrin-A5) are attached to the outer leaflet of the plasma membrane by a glycosylphosphatidylinositol (GPI) linkage. They bind to and activate the A-subclass Eph receptors
(EphA1 to EphA8). The three B-subclass ephrins (ephrin-B1 to ephrin-B3) are attached to the plasma membrane by a single hydrophobic transmembrane domain and have a short, highly conserved cytoplasmic tail. They bind to and activate B-subclass Eph receptors (EphB1 to EphB6).

With the exception of EphA4, which can bind both A- and B-subclass ephrins, there seems to be very limited crosstalk between the A and B subclasses.

Whereas Eph receptors transduce forward signals into the cell through activation of their intracellular catalytic tyrosine kinase domain (reviewed in [7,8]), ephrins do not have an intracellular catalytic domain and must therefore transduce their reverse signals in an altogether different way. Evidence shows that the GPI-anchored A-ephrins transduce reverse signals in C. elegans development [9,10], axon pathfinding [11] and cell adhesion [12–14]. A-ephrins are thought to localize to lipid rafts in the plasma membrane and seem to reverse signal by activating cytoplasmic proteins, such as Src family tyrosine kinases, which are located on the intracellular surface of these specialized microdomains [12–14].

The three B-ephrins use a different mechanism to transduce reverse signals. Their cytoplasmic tails contain several conserved tyrosines that become phosphorylated after the cognate EphB receptors are engaged [15,16]. Like cytokine receptor signaling (reviewed in [17]), the phosphorylation of B-ephrin cytoplasmic tyrosines is thought to be important in the transduction of reverse signals (see below). B-ephrin reverse signaling has been implicated in numerous migration and adhesion events, including repulsive axon guidance [1,18,19], hindbrain segmentation [20,21] and cerebellar granule cell migration [22]. Recent studies have begun to shed light on the molecular mechanisms by which the B-ephrin tail directs signals that influence the cytoskeleton. Here, we review these new developments and highlight the molecular components that might function downstream of the ephrins and help to transduce reverse signals.

**Eph–ephrin binding and activation of reverse signaling**

Both A- and B-subclass ephrins are found in glycosphingolipid-enriched lipid rafts in the plasmalemmal membrane. Crystallographic studies indicate that, on binding to Eph receptors, the ephrin–ephrin homodimers [23] are disrupted and a circular tetrameric structure comprising two receptor molecules and two ligand molecules is formed [24]. The changes induced on Eph–ephrin binding and formation of this circular tetrameric structure are likely to lead to conformational alterations in the B-ephrin transmembrane and cytoplasmic domains. This could result in a more permissive conformation that allows phosphorylation of the ephrin cytoplasmic tail by adjacent Src family tyrosine kinases (SFKs) that are also localized to lipid rafts [25]. Reverse signaling might be enhanced further by the ability of the circular Eph–ephrin tetramers to aggregate into higher-order clusters within the lipid rafts, which leads to the formation of discrete signaling centres (Fig. 2).

**Fig. 1.** Bidirectional signaling of B-subclass Eph receptor tyrosine kinases and ephrin ligands. When cells expressing EphB receptors contact cells expressing B-ephrins, the Eph receptors direct forward signals into a cell through their intrinsic catalytic tyrosine kinase activity and the ephrins direct reverse signals into an adjacent cell through tyrosine phosphorylation of their cytoplasmic tails by Src family tyrosine kinases.

**Fig. 2.** B-ephrin reverse signaling. BHK cells transfected with ephrin-B1 were serum-starved and either left unstimulated (a) or exposed to aggregated EphB2 extracellular domains for 1 hour (b), 2 hours (c) or 4 hours (d), and then fixed and processed for immunofluorescence using antibodies against ephrin-B1 (red) and against CAP (green). After the stimulation of reverse signaling, ephrin-B1 becomes clustered to spots on the membrane and conserved tyrosine residues in its cytoplasmic tail are phosphorylated. In turn, this leads to the recruitment of Grb4 (through its SH2 domain) to the B-ephrin tail and the co-recruitment of its SH3-binding partners (in this case CAP) to the same spots on the membrane (yellow). Note that the B-ephrin–Grb4–CAP signaling centres increase in size with longer periods of stimulation (arrowheads).
Partitioning-defective protein 3 (PAR-3) was originally identified in C. elegans as a molecule that is needed for the correct localization of cellular proteins during asymmetric cell divisions in early development [30]. PAR-3 and its Drosophila and mammalian orthologs, Bazooka and ASIP/mPAR-3, have three PDZ domains and form a ternary complex with atypical protein kinase C (aPKC) and another PDZ domain adaptor protein, PAR-6 (reviewed in [31]). These proteins seem to form an evolutionarily conserved molecular cassette that functions in asymmetric cell divisions and in establishing and maintaining epithelial cell polarity and cytoskeletal dynamics.

**PDZ Interactions**

Many PDZ domain proteins associate with the B-ephrins, including GRIP1, GRIP2, Pick1, mPAR-3, PDZ-RGS3, Syntenin and PTP-BL/FAP-1 [22,25–29] (Fig. 3). Although several of these have been discussed elsewhere (reviewed in Refs [4,5]), four proteins – mPAR-3, PDZ-RGS3, Syntenin and PTP-BL – warrant further consideration because they emphasize an emerging theme, namely, the control of cytoskeletal structures by B-ephrin reverse signaling.

**PAR-3**

Partitioning-defective protein 3 (PAR-3) was originally identified in C. elegans as a molecule that is needed for the correct localization of cellular proteins during asymmetric cell divisions in early development [30]. PAR-3 and its Drosophila and mammalian orthologs, Bazooka and ASIP/mPAR-3, have three PDZ domains and form a ternary complex with atypical protein kinase C (aPKC) and another PDZ domain adaptor protein, PAR-6 (reviewed in [31]). These proteins seem to form an evolutionarily conserved molecular cassette that functions in asymmetric cell divisions and in establishing and maintaining epithelial cell polarity (reviewed in [32]). Another potential participant in this complex are B-ephrins, as Pawson and coworkers [27] have shown that the second or third PDZ domain of mPAR-3 can bind the carboxy-terminal tails of B-ephrins.

Recent studies have also found that the GTPases Cdc42 and Rac1, both of which are involved in maintaining the apical–basolateral polarity of epithelial cells, can each bind the PAR-6–PAR-3–aPKC protein complex [33,34]. Interestingly, B-ephrins can also show strikingly restricted apical–basal localization patterns in the cell [6,35]. Albeit rather speculative, together these data suggest that B-ephrins might help to organize cytoskeletal complexes that are involved in establishing or maintaining cell polarity.

**PDZ-RGS3**

As with PAR-3, most of the identified PDZ domain proteins that interact with the B-ephrins either contain several PDZ domains or are capable of oligomerization and could therefore also promote the formation of large, multimeric complexes. But most of these proteins do not contain catalytic domains that could signal directly downstream of the B-ephrins. In contrast, Flanagan and coworkers [22] have recently described a B-ephrin interacting protein, PDZ-RGS3, that contains a PDZ domain and a catalytic RGS domain that is capable of regulating heterotrimeric G protein signaling. RGS domains act as GTPase-activating proteins (GAPs) for the α subunits of trimeric G proteins, catalyzing the hydrolysis of GTP to GDP and thus inactivating signaling. In response to EphB receptor binding to ephrin-B1, chemotaxis mediated by the G-protein-coupled chemokine receptor CXCR4 was inhibited in cerebellar granule cells, presumably through the activity of PDZ-RGS3 on Gαi signaling downstream of this chemokine receptor [22]. Because the association between PDZ-RGS3 and B-ephrins is constitutive, it is likely that the clustering and/or relocalization of PDZ-RGS3 after the binding of ephrin to EphB receptors is pivotal to this process. The ligand for CXCR4, stromal-cell-derived factor 1 (SDF-1), has been shown to function as a chemoattractant and, in the context of cerebellar granule cell migration, SDF-1 is thought to promote outward migration towards the fine vascular (pial) membrane covering the brain [36].

Flanagan and coworkers [22] propose that B-ephrin reverse signaling mediated in vivo by PDZ-RGS3 might act to neutralize CXCR4 attraction to SDF-1, thereby allowing the subsequent inward migration of cerebellar granule cells. If this notion is correct, then B-ephrins may perform similar functions downstream of CXCR4 in other cell types, for example, in migratory cell populations in the developing heart, lungs and kidneys where the expression of B-ephrin and CXCR4 overlaps. Moreover, B-ephrin reverse signaling through

![Fig. 3. PDZ-domain-containing proteins that interact with B-ephrins. All three of the B-ephrin cytoplasmic tails end in a consensus PDZ domain interaction motif (Tyr-Tyr-Lys-Val) that binds to each of the proteins shown here. All proteins are represented approximately according to scale.](http://tcb.trends.com/issue/2002/07/03/f3.png)
Syntenin was first reported as a transcript (termed Mda-9) that is downregulated during melanoma differentiation and was shown subsequently to interact via its two PDZ domains with the carboxyl termini of several proteins including syndecans, B-ephrins, EphA7, pro-transforming growth factor-α, neurexins, neurofascin and the anion exchanger AE2 [6,26,27,29,37–40]. Syntenin is expressed widely and can be found in most tissues. Subcellular expression studies have shown that Syntenin localizes to sites of cell adhesion, microfilaments, actin stress fibres and the nucleus [41,42]. Notably, Syntenin can form homodimers and might function as a scaffolding protein to assemble multiprotein signaling complexes [39]. Syntenin has a well-established connection to neuronal syndecans, which are expressed on both pre- and postsynaptic specializations and are key regulators of synaptogenesis [29,38,43–46].

In an additional link to synaptogenesis, Syntenin associates with neurexins, which have been shown to mediate presynaptic maturation (reviewed in [47]). B-ephrins are also found in many presynaptic terminals and, when one considers the previously reported functions of Eph receptors and syndecans in postsynaptic maturation [43], it is tempting to speculate that Syntenin might assemble a larger supramolecular complex comprising B-ephrins, syndecans and neurexins that functions during synaptogenesis to regulate the maturation of presynaptic specializations. Notably, recent reports indicate that B-ephrin reverse signaling might be important in synaptic plasticity within the hippocampus [48–50].

Taken together, these results suggest that B-ephrin reverse signaling might not only help to guide growth cones to their targets, but might also have important roles in establishing and maintaining functional synapses by physically associating with other synaptic proteins including Syntenin.

PTP-BL
PTP-BL (protein tyrosine phosphatase BAS-like), which is also known as FAP-1, PTP-RIP, PTP-BAS, PTP-L1 and PTP1E, is a 250-kDa cytosolic PTPase that contains an amino-terminal Band 4.1 domain, five PDZ domains and a carboxy-terminal PTP domain. PTP-BL is expressed in many tissues and is particularly prominent in kidney and lung epithelial cells, as well as in the developing peripheral nervous system. Previously reported interacting proteins such as APC, RhoGAP-1, RIL and the zyxin-related protein ZRP-1 implicate PTP-BL in dynamic changes of the actin cytoskeleton (see Ref. [51] and references therein). In addition, PTP-BL interacts with the cytoplasmic domain of ephrin-B1 via its fourth PDZ domain [27].

A recent report from Klein and coworkers [25] suggests that PTP-BL might function as a negative regulator of B-ephrin tyrosine phosphorylation. In this study, SFKs were found to act as positive regulators of B-ephrin reverse signaling by phosphorylating specific tyrosine residues in the cytoplasmic domain of ephrin-B1 [25]. They found that overexpressed PTP-BL is recruited with delayed kinetics to sites of activated B-ephrins, where it seems to act as a negative regulator of reverse signaling by dephosphorylating tyrosine residues in the ephrin cytoplasmic domain and the major autophosphorylation site of Src. The presence of both B-ephrins and PTP-BL in the growth cones of dissociated dorsal root ganglia neurons suggests further that this phosphatase has an endogenous role in the negative regulation of B-ephrin reverse signaling.

SH2 interactions
Recently we found that the Grb4 adaptor protein also transduces B-ephrin reverse signals [52]. Grb4, which is also known as Nck-2 or Nckβ, is a 380-amino-acid-protein comprised of three amino-terminal Src-homology 3 (SH3) domains and a carboxy-terminal SH2 domain. The closest known relatives of Grb4 are another mammalian adaptor protein, Nck and a protein identified in Drosophila called Dreadlocks or Dock. All three proteins share the same domain structure and, although the Dock protein has been reported as the Drosophila homolog of Nck, an alignment shows that Dock is related equally to Nck (44% identical and 65% similar) and Grb4 (45% identical and 64% similar). Consistent with having a possible role in cytoskeletal reorganization, Dock is essential for axon pathfinding in the fly, and the mammalian Grb4 and Nck proteins regulate actin dynamics (reviewed in [53]).

To examine directly the function of Grb4 in B-ephrin reverse signaling, we carried out a series of cell-based experiments [52]. Stimulating ephrin-B1-expressing cells with a soluble aggregated form of the EphB2 receptor extracellular domain leads to the formation of discrete signaling centres that contain multimerized clusters of tyrosine-phosphorylated ephrin molecules (Fig. 2). Grb4 is recruited specifically to these signaling centres through its SH2 domain, which binds to tyrosine-phosphorylated B-ephrin tails. In response to B-ephrin reverse signaling, cells increase focal adhesion kinase (FAK) catalytic activity, lose adhesive fod, round up and disassemble actin stress fibres [52]. Such cytoskeletal responses are consistent with the purported repellent activities that reverse signaling has on cells and axonal growth cones. These cytoskeletal events can be also...
Expanding the B-ephrin reverse signaling network: Grb4 SH3 binding proteins

As an adaptor protein, Grb4 is thought to link cell-surface receptors through its SH2 domain to downstream effectors through its three SH3 domains. SH3 domains bind proline-rich regions of target proteins that contain the general Pro-Xaa-Xaa-Pro consensus motif. A unique set of proteins that are implicated in cytoskeletal regulation, including Abi-1, Axin, Cbl-associated protein (CAP), Dynamin, hnRNPK and Pak1, bind the Grb4 SH3 domains [52]. In addition, the SH3 domains of Grb4 interact with the Abl tyrosine kinase, the LIM-domain-only protein (CAP), Dynamin, hnRNPK and Pak1, binds the Grb4 SH3 domains [52]. In addition, the SH3 domains of Grb4 interact with the Abl tyrosine kinase, the LIM-domain-only protein (CAP), Dynamin, hnRNPK and Pak1, binds the Grb4 SH3 domains [52].

As demonstrated with CAP ([52]; Fig. 2), it is likely that all of the binding partners of the Grb4 SH3 domains are recruited to the plasma membrane after the clustering of B-ephrins and the activation of reverse signaling. Strikingly, almost all of the reported Grb4-interacting proteins seem to have roles in regulating cellular adhesion and actin polymerization. Several have well-described functions, and we briefly highlight them here because they also might participate in propagating B-ephrin reverse signals.

Abl and Abi-1

As demonstrated with CAP ([52]; Fig. 2), it is likely that all of the binding partners of the Grb4 SH3 domains are recruited to the plasma membrane after the clustering of B-ephrins and the activation of reverse signaling. Strikingly, almost all of the reported Grb4-interacting proteins seem to have roles in regulating cellular adhesion and actin polymerization. Several have well-described functions, and we briefly highlight them here because they also might participate in propagating B-ephrin reverse signals.

An attractive potential effector of B-ephrin–Grb4 reverse signaling is the Abl tyrosine kinase. Grb4 binds to Abl with either its amino-terminal or middle SH3 domain [54]. In addition to its tyrosine kinase catalytic domain, Abl has several protein–protein interaction motifs including SH2 and SH3 domains, nuclear localization signals and DNA- and actin-binding domains. Abl is expressed in many cell types, is found in many cellular compartments, can associate physically with a broad range of targets and seems to function in several signaling pathways (e.g. those involving Cadherins, Catenins, Ena/VASP, Dab, Trio, Profilin and Mena; see [57] for a review). Evidence has slowly accumulated that shows that Abl might act as a key regulator of axon pathfinding and neuronal migration [58]. Importantly, Abl seems to integrate signals that control actin polymerization and cell adhesion in elaborating growth cones. In an additional connection to Abl, the Abl-interacting protein 1 (Abi-1) is also a potential binding partner for the Grb4 SH3 domains. The two members of the Abi family, Abi-1 and Abi-2, are highly homologous and, as their name implies, were identified originally as binding partners of Abl [59,60]. Both contain an amino-terminal homeobox-like domain, proline-rich regions and a carboxy-terminal SH3 domain. Overexpression studies have shown that Abi-1 inhibits the ability of Abl to activate essential signal transduction pathways involved in transformation of cells without directly diminishing Abl tyrosine kinase activity [59,60]. In addition to binding Abl, Abi-1 associates with the Ras guanine nucleotide exchange factor Son of Sevenless (SOS) and the epidermal growth factor receptor substrate Eps8 in a trimolecular complex that exhibits exchange activity for Rac [61]. Abi-1 is localized to sites of actin polymerization at the tips of lamellipodia and filopodia [62], and depletion of Abi-1 by microinjecting neutralizing antibodies into fibroblasts blocks membrane ruffling in response to platelet-derived growth factor [61]. These observations suggest that Abi-1 is involved directly in controlling actin polymerization in protrusion events and implicates Abi-1 as a potential regulator of cell motility downstream of B-ephrin reverse signaling.

Axin

Axin was first identified as the product of the mouse fused gene and contains an amino-terminal RGS-like domain and a carboxy-terminal Dishevelled homology (DIX) domain. Although possible roles...
downstream of Grb4-mediated ephrin reverse signaling remain to be explored, Axin is known to function in the canonical Wnt signaling pathway as a scaffolding molecule. Axin promotes the formation of a complex comprising itself, glycogen synthase kinase-3β (GSK-3β), adenomatous polyposis coli (APC) and β-catenin, which in turn enhances the phosphorylation of β-catenin by GSK-3β and consequently induces β-catenin degradation (reviewed in [63]).

β-Catenin is important in cell adhesion and also binds to transcription factors of the lymphoid enhancer factor/T-cell factor family, thereby modulating expression of Wnt-responsive genes (reviewed in [64,65]). Accumulation of β-catenin arising from either mutations in APC and/or mutations that activate β-catenin results in the formation of tumors (reviewed in [66,67]). Thus, B-ephrin reverse signaling through Axin might affect cell adhesion, remodeling of the actin cytoskeleton, gene transcription and tumorigenesis through regulation of β-catenin.

CAP
Murine CAP (also known as Ponsin or SH3P12) has 12 isoforms, all of which contain two Sorbin homology domains in their amino terminus and three SH3 domains in their carboxyl terminus [68]. Between the second and third SH3 domains of CAP is a Pro-Gln-Gln-Pro motif that mediates the interaction with Grb4 [52]. The widely expressed, multifunctional CAP protein lies at the interface between the actin cytoskeleton and sites of cell adhesion [69]. Through direct interactions with the actin and α-catenin-binding protein vinculin, and the PDZ-domain-containing and actin-binding protein afadin (also known as AF-6), CAP localizes to adherens junctions and seems to form a bridge between cadherins, nectins and the actin cytoskeleton [69,70]. In addition, CAP seems to have a role in regulating stress fibre and focal adhesion formation [68,71], perhaps through its ability to associate with different signaling proteins including SOS, FAK and Cbl. Through these interactions, a staggering number of pathways and proteins can be connected to CAP (reviewed in [72,73]) all of which might act downstream of Grb4-mediated B-ephrin reverse signaling.

Pak1
The p21-activated kinase (Pak) family of serine/threonine kinases were first identified as targets for active Rac or Cdc42 to the Pak1 amino terminus causes a conformational change that displaces the autoregulatory domain of the kinase and induces autophosphorylation and an increase in catalytic activity [74].

Recruitment of Pak1 to the membrane by Nck in response to the activation of growth factor tyrosine kinases or by attaching a membrane-targeting Cys-Ala-Ala-Xaa box results in activation of the Pak1 kinase domain [75,76]. Once activated, Pak1 is essential for cytoskeletal changes that are associated with cellular motility and adhesion (reviewed in [77]). For example, Pak1 kinase activity can cause disassembly of actin stress fibres and focal adhesions [78] and has roles in axon pathfinding [79]. Conversely, kinase-defective Pak1 promotes the formation of lamellipodia and causes membrane ruffling [78]. Activated Pak1 has several substrates, including both light and heavy chain myosin kinases, LIM kinase-1, and Mek1 (reviewed in [77]).

Pak1 has also been reported to associate with a family of guanine-nucleotide-exchange factors – αPIX/Cool-2 and βPIX/Cool-1 (reviewed in [77]). αPIX/Cool-2 seems to increase the amounts of GTP-bound Cdc42, whereas βPIX/Cool-1 increases the amounts of GTP-bound Rac. Thus, B-ephrin reverse signaling through Pak1 has many potential ways to influence cytoskeletal dynamics.

Ephrin signaling: shifting cells into reverse, park and even drive?
Precise control of cell movement and adhesion is required to coordinate the growth of organs, limbs and tissues, and is also fundamental to the guidance of axons and wiring of the neural network. Although the molecular mechanisms that govern the movement and adherence of cells are just beginning to be understood, one emerging theme is that, in response to specific extracellular cues, cell-surface receptors transmit biochemical signals that produce changes in the cytoskeleton, which in turn regulate cellular motility and adhesion. The studies reviewed here implicate ephrin reverse signaling along with several potential downstream partners in the control of cytoskeletal dynamics. Moreover, many data suggest that ephrin reverse signals direct cell movements that range from repulsion to adhesion to attraction. These cell movements can be thought of as ‘going in reverse’, ‘parking’ and ‘driving’.

For example, B-ephrin reverse signaling in axonal growth cones during intraretinal and anterior commissure pathfinding has been shown to repulse axons away from regions of EphB receptor expression [18,19]. Likewise, during the partitioning of rhombomeric compartments in the developing hindbrain, ephrin reverse signaling in even-numbered rhombomere cells functions to...
restrict intermingling with Eph-receptor-expressing odd-numbered rhombomere cells [20, 21]. These ephrin activities could be thought of as shifting cells into reverse gear, which ultimately causes them to move away from cells that express Eph receptors.

Interestingly, accumulating evidence suggests that reverse signaling of both A- and B-subclass ephrins also mediates cellular adhesion. The B-ephrins seem to regulate adhesive events during midline fusion of the palate and genitalia (80); and N. Yokoyama and M. Henkemeyer, unpublished) and perhaps during synaptic plasticity [48–50], whereas the A-ephrins have been shown to increase integrin-mediated cell adhesion in vitro by activating Fyn tyrosine kinases [12–14]. These ephrin-mediated activities could once again be thought of as shifting cellular gears but, rather than into reverse, cells are put into park, which causes them to adhere and stay where they are.

Finally, ephrin reverse signaling might not only mediate cellular adhesion but could also attract axons to regions of increased Eph receptor expression. Evidence for this activity comes from studies of venderal axon pathfinding in the accessory olfactory bulb, where axons expressing large amounts of A-ephrins specifically project to mitral cell dendrites that express large amounts of EphA receptors [11]. In a similar situation, B-ephrin reverse signaling mediated by EphA4 is implicated in the attraction of anterior commissure axons [81]. In these instances, ephrin activity could be thought of as shifting cells into yet a third gear, drive, which causes them to migrate towards regions with Eph receptor expression.

The principal difference between park and drive is that the former causes cells to adhere or stick preferentially to one another and stay where they are, whereas the latter allows cells or migrating growth cones to invade and move through presumably adhesive/permissive territory. Although both are adhesive in nature, a parked cell stops moving, whereas a cell or growth cone in drive continues to move. Thus, ephrin reverse signaling might cause migrating cells to shift from reverse to park and perhaps even to drive.

Clearly this analogy is imperfect, as ephrins seem to have roles in cellular processes such as proliferation and ion homeostasis that might have little to do with cellular motility. Currently, it is also unclear how ephrins could cause cells to shift from reverse to park or to drive. In certain instances, the ability of B-ephrin reverse signaling to affect cell movement might not be characterized readily as repulsion, adhesion or attraction. For example, B-ephrin reverse signaling through PDZ-RGS3 might best be viewed as neutralizing an attractive response [22]. Nonetheless, our analogy and the studies reviewed here illustrate the point that ephrin reverse signals are important in regulating cytoskeletal dynamics in many different cell movements. Consequently, future studies are likely to focus on how ephrins direct seemingly disparate repulsive versus adhesive and attractive responses and the biochemical mechanisms that they use to transduce these signals. Resolving these issues and others promises to be an exciting, albeit complex, period of discovery in the field.

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